

Exploring Chemical Analysis

Fifth Edition

Daniel C.
Harris

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BOOKS**



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Periodic Table

1	H
20 14 0.0888 ^d	Hydrogen 1.007 94 ±7
3	Li
1615 454 0.53	Lithium 6.941 2±1
4	Be
2745 1560 1.85	Beryllium 9.012 182 ±3
11	Na
1156 371 0.97	Sodium 22.989 769 28 ±2
12	Mg
1363 922 1.74	Magnesium 24.305 0 ±6

Atomic Number Boiling point (K) Melting point (K) Density at 300 K (g/cm ³) (Densities marked with ^a are at 273K and 1 bar and the units are g/L)	22 3562 1943 4.50 Titanium 47.867 ±1	+4,3 Common oxidation states Atomic mass with uncertainty in last digit Example: Ti = 47.867 ± 0.001 Numbers in parentheses are longest-lived isotope
-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------

[Atomic masses from *Pure Appl. Chem.* 2009, 81, 2131]

3	4	5	6	7	8	9
19	20	21	22	23	24	25
K 1032 336 0.86 Potassium 39.098 3 ±1	Ca 1757 1112 1.55 Calcium 40.078 ±4	Sc 3104 1812 3.0 Scandium 44.955 912 ±6	Ti 3562 1943 4.50 Titanium 47.867 ±1	V 3682 2175 5.8 Vanadium 50.941 5 ±1	Cr 2945 2130 7.19 Chromium 51.996 1 ±6	Mn 2335 1517 7.43 Manganese 54.938 045 ±5
37	38	39	40	41	42	43
Rb 961 313 1.53 Rubidium 85.467 8 ±3	Sr 1650 1041 2.6 Strontium 87.62 ±1	Y 3611 1799 4.5 Yttrium 88.905 85 ±2	Zr 4682 2125 6.49 Zirconium 91.224 ±2	Nb 5017 2740 8.55 Niobium 92.906 38 ±2	Mo 4912 2890 10.2 Molybdenum 95.96 ±2	Tc 4538 2473 11.5 Technetium (98)
55	56	57	72	73	74	75
Cs 944 302 1.87 Cesium 132.905 451 9 ±2	Ba 2171 1002 3.5 Barium 137.327 ±7	La 3730 1193 6.7 Lanthanum 138.905 47 ±7	Hf 4876 2500 13.1 Hafnium 178.49 ±2	Ta 5731 3287 16.6 Tantalum 180.947 88 ±2	W 5828 3680 19.3 Tungsten 183.84 ±1	Re 5869 3453 21.0 Rhenium 186.207 ±1
87	88	89	104	105	106	107
Fr 950 300 — Francium (223)	Ra 1809 973 5 Radium (226)	Ac 3473 1323 10.07 Actinium (227)	Rf — — Rutherfordium (267)	Db — — Dubnium (268)	Sg — — Seaborgium (271)	Bh — — Bohrium (270)
58	59	60	61	62	63	
Ce 3699 1071 6.78 Cerium 140.116 ±1	Pr 3785 1204 6.77 Praseodymium 140.907 65 ±2	Nd 3341 1289 7.00 Neodymium 144.242 ±3	Pm 3785 1204 6.48 Promethium (145)	Sm 2064 1345 7.54 Samarium 150.36 ±2	Eu 1870 1090 5.26 Europium 151.964 ±1	
90	91	92	93	94	95	
Th 5061 2028 11.7 Thorium 232.038 06 ±2	Pa — — Protactinium 231.035 88 ±2	U 4407 1405 18.9 Uranium 238.028 91 ±3	Np — — Neptunium (237)	Pu 3503 913 19.8 Plutonium (244)	Am 2880 1268 13.6 Americium (243)	

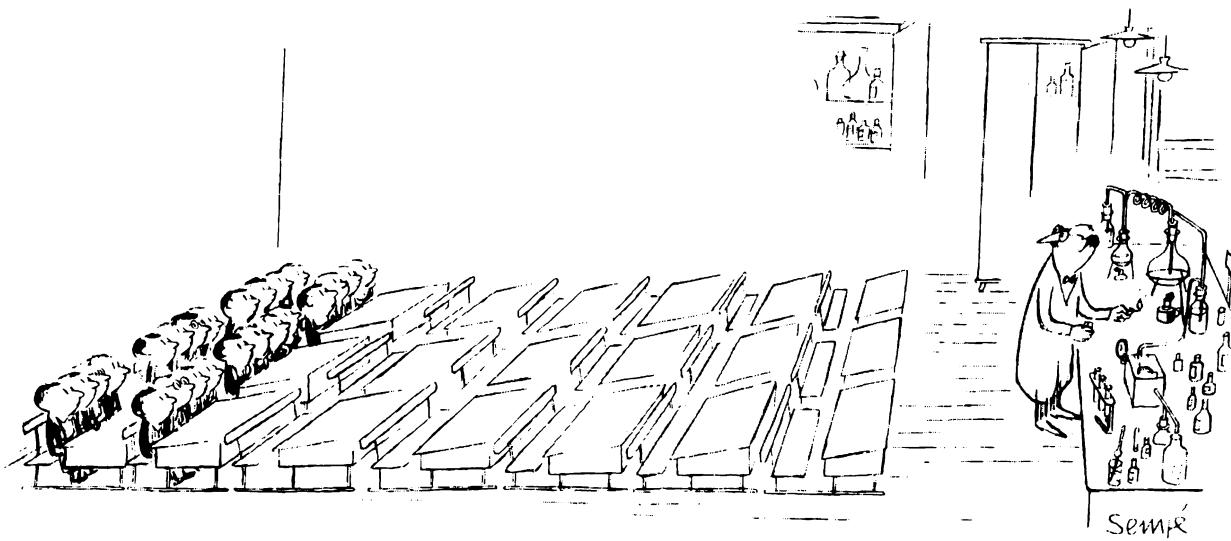
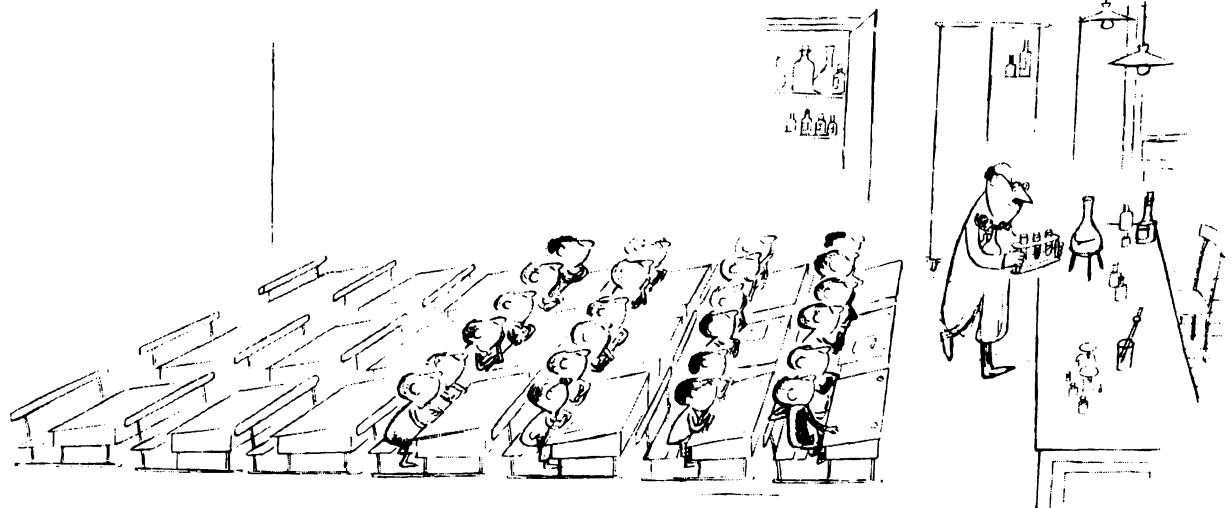
[†]Commercial lithium compounds are artificially depleted of ⁶Li. The atomic mass of commercial Li is in the range 6.939 to 6.996. If a more accurate value is required, it must be determined for the specific material.

58	59	60	61	62	63
Ce 3699 1071 6.78 Cerium 140.116 ±1	Pr 3785 1204 6.77 Praseodymium 140.907 65 ±2	Nd 3341 1289 7.00 Neodymium 144.242 ±3	Pm 3785 1204 6.48 Promethium (145)	Sm 2064 1345 7.54 Samarium 150.36 ±2	Eu 1870 1090 5.26 Europium 151.964 ±1
90	91	92	93	94	95
Th 5061 2028 11.7 Thorium 232.038 06 ±2	Pa — — Protactinium 231.035 88 ±2	U 4407 1405 18.9 Uranium 238.028 91 ±3	Np — — Neptunium (237)	Pu 3503 913 19.8 Plutonium (244)	Am 2880 1268 13.6 Americium (243)

of the Elements

	13	14	15	16	17	
	5 +3 4275 2300 2.34 Boron 10.811 ± 7	6 ±4,2 4470 4100 2.62 Carbon 12.010 ± 8	7 ±3,5,4,2 77 63 1.234 ^a Nitrogen 14.006 ± 2	8 -2 90 50 1.410 ^a Oxygen 15.999 ± 3	9 -1 85 53 1.674 ^a Fluorine 18.998 ± 5	10 27 25 0.889 ^a Neon 20.179 ± 6
10	11	12				18 87 84 ^a 1.760 ^a Argon 39.948 ± 1
28 3187 1726 8.90 Nickel 58.693 ± 4	29 +2,3 2836 1358 8.96 Copper 63.546 ± 3	30 +2 1180 693 7.14 Zinc 65.38 ± 2	31 +3 2478 303 5.91 Gallium 69.723 ± 1	32 +4 3107 1210 5.32 Germanium 72.64 ± 1	33 ±3,5 876 — 5.72 Arsenic 74.921 ± 2	34 -2,4,6 958 494 4.80 Selenium 78.96 ± 3
46 3237 1825 12.0 Palladium 106.42 ± 1	47 +2,4 2436 1234 10.5 Silver 107.868 ± 2	48 +2 1040 594 8.65 Cadmium 112.411 ± 8	49 +3 2346 430 7.31 Indium 114.818 ± 3	50 +4,2 2876 505 7.30 Tin 118.710 ± 7	51 ±3,5 1860 904 6.68 Antimony 121.760 ± 1	52 -2,4,6 1261 723 6.24 Tellurium 127.60 ± 3
78 4100 2045 21.4 Platinum 195.084 ± 9	79 +2,4 3130 1338 19.3 Gold 196.966 ± 4	80 +2,1 630 234 13.5 Mercury 200.59 ± 2	81 +3,1 1746 577 11.85 Thallium 204.383 ± 2	82 +4,2 2023 601 11.4 Lead 207.2 ± 1	83 +3,5 1837 545 9.8 Bismuth 208.980 ± 1	84 +4,2 1235 527 9.4 Polonium (209)
110 — Darmstadtium (281)	111 — Roentgenium (280)	112 — Copernicium (285)	113 (284)	114 (289)	115 (288)	116 (293)
						117 (294)
						118 (294)

64 3539 1585 7.89 Gadolinium 157.25 ± 3	+3 3496 1630 8.27 Terbium 158.925 ± 35	65 2835 1682 8.54 Dysprosium 162.500 ± 1	+3.4 2968 1743 8.80 Holmium 164.930 ± 32	66 3136 1795 9.05 Erbium 167.259 ± 3	+3 2220 1818 9.33 Thulium 168.934 ± 21	67 3136 1795 9.05 Erbium 167.259 ± 3	+3.2 2220 1818 9.33 Thulium 168.934 ± 21	68 3136 1795 9.05 Erbium 167.259 ± 3	+3.2 2220 1818 9.33 Thulium 168.934 ± 21	69 3136 1795 9.05 Erbium 167.259 ± 3	+3.2 2220 1818 9.33 Thulium 168.934 ± 21	70 3136 1795 9.05 Erbium 167.259 ± 3	+3.2 2220 1818 9.33 Thulium 168.934 ± 21	71 3668 1936 9.84 Lutetium 174.966 ± 8
96 — 1340 13.5 Curium (247)	+3 — 97 Berkelium (247)	97 +4.3 — Bk 900	98 +3 — Cf 900	99 — — Es Einsteinium (252)	100 — — Fm Fermium (257)	101 — — Md Mendelevium (260)	102 — — No Nobelium (259)	103 — — Lr Lawrencium (262)						



[“The Experiment” by Sempé. Copyright C. Charillon, Paris.]

Exploring Chemical Analysis

FIFTH EDITION

Daniel C. Harris

**Michelson Laboratory
China Lake, California**



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Rosalyn Yalow (1921–2011)
[Veterans Administration undated photo
courtesy Benjamin Yalow.]



Analytical chemistry is essential to a wide range of scientific fields. Rosalyn Yalow,* coinventor with Solomon Berson of the sensitive radioimmunoassay analytical procedure used in medicine and biology, passed away during the writing of this book. In 1977, she became only the second woman to receive the Nobel Prize for Physiology or Medicine. Berson should have shared that prize, but he died in 1972 and the prize is not awarded posthumously. Radioimmunoassay is an example of a tremendously important contribution to chemical analysis made by someone other than an “analytical chemist.”

Yalow received a Ph.D. in nuclear physics from the University of Illinois in 1945 and went on to a career in medical research at the Bronx Veterans Hospital in New York. Despite graduating with high honors as the first female physics major at Hunter College in New York at the age of 19, she was not accepted to graduate school because universities believed that no job would be open to a Jewish woman, no matter how capable she was. Only the exigencies of World War II allowed her to enroll in graduate school. Even so, when men came home from the war, there were no jobs for Yalow.

She taught temporarily at Hunter College, where she was a mentor to young women, including Mildred Dresselhaus, who went on to become Institute Professor at M.I.T. and a National Medal of Science recipient. Yalow “could talk to anyone, and no matter what their background, she could explain the most complicated concept in simple terms so they would understand.” While teaching physics in the daytime, she volunteered at night as a researcher at the Veterans Hospital for three years until she was finally hired. This unpretentious “young woman physicist who stuck out and brought a quantitative approach to everything” was driven from within to excel. At age 8, she knew she would be a scientist. “I, as a small child, made up my mind that people and institutions were going to need me.” She overcame almost impenetrable barriers to women in her career, but she did not believe that the remedy was to provide special advantages to women. She believed that women should have the same opportunities as men and would “have to get there because they’re good.”

*E. Straus, *Rosalyn Yalow, Nobel Laureate: Her Life and Work in Medicine* (New York: Basic Books, 1999); S. B. McGrawe, *Nobel Prize Women in Science*, 2nd ed. (Washington, DC: Joseph Henry Press, 1998). Quotations come from these sources.

Preface

This book is intended to provide a *short, interesting, elementary* introduction to analytical chemistry for students of chemistry and students whose primary interests might lie outside of chemistry. I selected topics that I think should be covered in a single exposure to analytical chemistry and tried to refrain from going into more depth than necessary.

What's New?

The *F* test for comparison of variance is introduced early in the chapter on statistics and applied to the *t* test for comparison of means. The meaning of statistical hypothesis testing is explained with an example from epidemiology. Two cases of standard addition with variable volume or constant volume are distinguished more clearly. Propagation of uncertainty for pH is described. A better diagram showing the working of an electronic balance and a photograph of the optical train of an ultraviolet-visible spectrophotometer are included. The direction of electron flow in a galvanic cell is described in terms of electrons moving toward more positive potential. There is a brief discussion of high-resolution mass spectrometry. New topics in liquid chromatography include ultra-performance liquid chromatography, superficially porous particles, hydrophilic interaction chromatography, a waveguide absorbance detector, and an illustration of the charged aerosol detector. Instructions for Excel® spreadsheets were updated to Excel 2007.

New applications include solid-phase extraction for the measurement of caffeine, measuring the common cold virus with an imprinted polymer on a quartz crystal microbalance, a precipitation titration conducted on the *Phoenix Mars Lander*, updated classroom data from a saltwater aquarium, microdialysis in biological sampling, measuring pH of oceans and rivers by spectrophotometry with indicators, continued highlighting of the effects of increasing carbon dioxide in the air and ocean, a description of the lithium-ion battery, how perchlorate was discovered on Mars with ion-selective electrodes, protein immunosensing with solid-state ion-selective electrodes, X-ray photoemission from the peeling of tape, how a home pregnancy test works, laser-ablation atomic emission on Mars, lead isotopes in archaeology, bisphenol A in food containers, measuring *trans* fat in food with an ionic liquid gas chromatography stationary phase, chromated copper arsenate preservative in wood, preconcentration of trace elements from seawater, simultaneous separation of anions and cations, detecting contaminated heparin, and DNA profiling with a lab-on-a-chip.

Problem Solving

The two most important ways to master this course are to work problems and to gain experience in the laboratory. **Worked Examples** are a principal pedagogic tool designed to teach problem solving and to illustrate how to apply what you have just read. At the end of each worked example is a similar **Test Yourself** question and answer. I recommend that you answer the Test Yourself question right after reading

the example. **Ask Yourself** questions at the end of each section should be tackled as you work your way through this book. Problems are broken down into elementary steps in these questions. **Solutions** to Ask Yourself questions can be found at the back of the book. **Problems** at the end of each chapter cover the entire chapter. **Short answers** to problems are at the back of the book and complete solutions appear in a separate **Solutions Manual**. **How Would You Do It?** problems at the end of most chapters are more open ended and might have many good answers.

Features

Chapter Openers show the relevance of analytical chemistry to the real world and to other disciplines of science. **Boxes** discuss interesting topics related to what you are currently studying, or they amplify points from the text. **Demonstration** boxes describe classroom demonstrations, and **Color Plates** near the center of the book illustrate demonstrations or other points. **Marginal Notes** amplify what is in the text. **Spreadsheets** are introduced in Chapter 3, and applications appear throughout the book. You can study this book without ever using a spreadsheet, but your experience will be enriched by spreadsheets and they will serve you well outside of chemistry. Problems intended to be worked on a spreadsheet are marked by an icon. You might choose to work more problems with a spreadsheet than those that are marked.

Essential vocabulary in the text is highlighted in **bold** and listed in **Important Terms** at the end of each chapter. Other unfamiliar terms are usually italicized. The **Glossary** at the end of the book defines all bold terms and many italicized terms. **Key Equations** are highlighted and collected at the end of the chapter. **Appendices** contain tables of chemical information and a discussion of balancing redox equations. The **inside covers** contain your trusty periodic table, physical constants, and other useful information.

Media Supplements

The **Book Companion Website**, www.whfreeman.com/exploringchem5e, has instructions for laboratory experiments, a list of analytical chemistry experiments from the *Journal of Chemical Education*, and chapter quizzes. All the artwork from the book is available in PowerPoint slides from the password-protected Instructor's Web site.

The People

My wife Sally works on every aspect of this book. She contributes mightily to whatever clarity and accuracy we have achieved.

My editors Brittany Murphy and Jessica Fiorillo at W. H. Freeman and Company played key roles in helping to set the direction of this revision by conducting user reviews and through their suggestions and advice. Jodi Simpson questioned content and English in her insightful copyediting. Nothing gets past Jodi. Georgia Lee Hadler shepherded the manuscript through editing and production and is most responsible for the pleasing appearance of the book. Diana Blume created the design. Ted Szccepanski conducted photo research. Solutions to problems were checked by Heather Audesirk and Thomas Avila, two senior chemistry students at Harvey Mudd College.

I truly appreciate comments, criticism, and suggestions from students and teachers. You can reach me at the Chemistry Division, Mail Stop 6303, 1900 N. Knox Road, China Lake, CA 93555.

Acknowledgments

Sam Kounaves of Tufts University graciously provided information and graphics concerning the mission of the *Phoenix Mars Lander*. Hal Van Ryswyk of Harvey Mudd College introduced me to the students who worked as problem checkers for this edition, and Hal provided analytical data from the saltwater aquarium in his classroom. Yong Cai and Lucy Yehiayan of Florida International University were extremely patient with my questions concerning the chromatograms and procedure from their study of chromated copper arsenate wood preservative. Cedric Hurth, Frederic Zenhausern, and Amol Surve of the University of Arizona were extremely helpful in supplying graphics to illustrate DNA profiling by their ingenious lab-on-a-chip. Benjamin Yalow provided the photograph of his mother that appears on page xii. Corrections to the previous edition were offered by Jeffrey Smith of Carleton University in Ottawa, Barbara Belmont of California State University, Dominguez Hills, and Jeffrey Jankowski of North Central College, Naperville, IL.

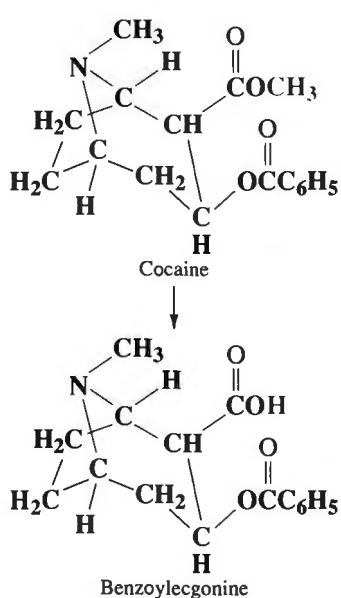
Reviewers of the fourth edition who helped provide direction for the fifth edition were Zhigang Liu (New York University), Binyomin Abrams (Boston University), Barbara Belmont (California State University, Dominguez Hills), Grady Hanrahan (California Lutheran University), Chu-Ngi Ho (East Tennessee State University), William R. Lammela (Nazareth College), Gerald Morine (Bemidji State University), Daniel C. Robie (City University of New York–York College), Daniel J. Swart (Minnesota State University, Mankato), Kevin L. Braun (Beloit College), and Maureen Ronau (Connecticut College).

People who reviewed parts of the manuscript for the fifth edition were Darrin L. Smith (Eastern Kentucky University), Gary Long (Virginia Tech University), Stephen S. Lawrence (Saginaw Valley State University), Mary Sohn (Florida Institute of Technology), John T. Williams (Waynesburg College), Richard H. Hanson (University of Arkansas, Little Rock), Samuel Melaku Abegaz (Columbus State University), Karl Bishop (Husson College), Thomas X. Carroll (Keuka College), Zhan Chen (University of Michigan), Salim M. Diab (Lewis University), Diego J. Diaz (University of Central Florida), Susan Godbey (Eastern Kentucky University), Isabelle G. Haithcox (College of Notre Dame), Kazi Javed (Kentucky State University), Christopher C. Mulligan (Illinois State University), Diep Nguyen (Illinois Institute of Technology), Aisling M. O'Connor (Fitchburg State College), Peng Sun (East Tennessee State University), David O'Dell (Glenville State College), Timothy T. Ehler (Buena Vista University), Mark Dietz (University of Wisconsin, Milwaukee), Heather L. Holmes (Eastern Michigan University), John Richardson (Shippensburg University of Pennsylvania), Shyam S. Shukla (Lamar University), Nancy Breen (Roger Williams University), Erin M. Gross (Creighton University), Lisa M. Reilly (Bethany College), Kevin Cantrell (University of Portland), Paul A. Flowers (University of North Carolina, Pembroke), Marta Maurer (Pennsylvania State University, Altoona), Donald Mencer (Wilkes University), and Seong S. Seo (Albany State University). I thank you all for your comments and suggestions.

Cocaine Use? Ask the River



Map of Italy, showing where Po River was sampled to measure cocaine metabolite. [The reference for this cocaine study is E. Zuccato, C. Chiabrandi, S. Castiglioni, D. Calamari, R. Bagnati, S. Schiaretti, and R. Fanelli, *Environ. Health* 2005, 4, 14. The notation refers to the journal *Environmental Health* published in the year 2005, volume 4, page 14, available at <http://www.ehjournal.net/content/4/1/14>.]



How honest do you expect people to be when questioned about illegal drug use? In Italy in 2001, 1.1% of people aged 15 to 34 years old acknowledged using cocaine “at least once in the preceding month.” Researchers studying the occurrence of therapeutic drugs in sewage realized that they had a tool to measure illegal drug use.

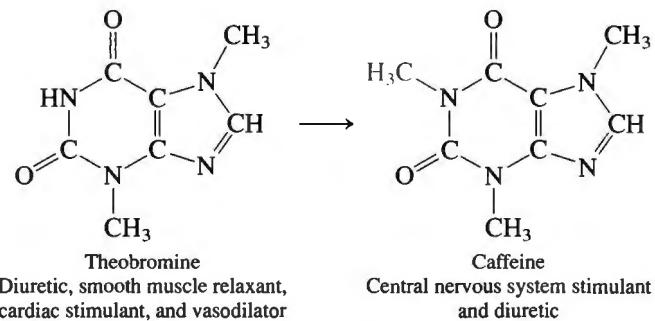
After ingestion, cocaine is largely converted to benzoylecgonine before being excreted in urine. Scientists collected representative composite samples of water from the Po River and samples of wastewater entering treatment plants serving four Italian cities. They concentrated minute quantities of benzoylecgonine from large volumes of water by solid-phase extraction, which is described in Chapter 22. Extracted chemicals were washed from the solid phase by a small quantity of solvent, separated by liquid chromatography, and measured by mass spectrometry. Cocaine use was estimated from the concentration of benzoylecgonine, the volume of water flowing in the river, and the fact that 5.4 million people live upstream of the collection site.

Benzoylecgonine in the Po River corresponded to 27 ± 5 100-mg doses of cocaine per 1 000 people per day by the 15- to 34-year-old population. Similar results were observed in water from four treatment plants. Cocaine use is much higher than people admit in a survey.

The Analytical Process

C

Chocolate has been the savior of many a student on the long night before a major assignment was due. My favorite chocolate bar, jammed with 33% fat and 47% sugar, propelled me over mountains in California's Sierra Nevada. In addition to its high energy content, chocolate packs an extra punch from the stimulant caffeine and its biochemical precursor, theobromine.



Too much caffeine is harmful for many people, and even small amounts cannot be tolerated by some unlucky persons. How much caffeine is in a chocolate bar? How does that amount compare with the quantity in coffee or soft drinks? At Bates College in Maine, Professor Tom Wenzel teaches his students chemical problem solving through questions such as these.¹ How *do* you measure the caffeine content of a chocolate bar?

0-1 The Analytical Chemist's Job

Two students, Denby and Scott, began their quest at the library with a computer search for analytical methods. Searching through *Chemical Abstracts* and using “caffeine” and “chocolate” as key words, they uncovered numerous articles in chemistry journals. The articles, “High Pressure Liquid Chromatographic Determination of Theobromine and Caffeine in Cocoa and Chocolate Products”² described a procedure suitable for the equipment available in their laboratory.

Sampling

The first step in any chemical analysis is procuring a representative, small sample to measure—a process called **sampling**. Is all chocolate the same? Of course not. Denby and Scott chose to buy chocolate in the neighborhood store and analyze pieces of it. If you wanted to make universal statements about “caffeine in chocolate,”



Chocolate is great to eat but not so easy to analyze. [Salina Hainzl/Stock. xchng.]

A *diuretic* makes you urinate.
A *vasodilator* enlarges blood vessels.

Chemical Abstracts is the most comprehensive database of the chemical literature. It is commonly accessed online through *Scifinder*.

Bold terms should be learned. They are listed at the end of the chapter and in the Glossary at the back of the book. *Italicized* terms are also important and many can be found in the Glossary.

Homogeneous: same throughout
Heterogeneous: differs from region to region

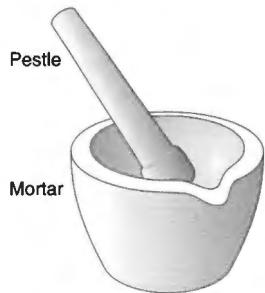


Figure 0-1 Ceramic mortar and pestle used to grind solids into fine powders.

you would need to analyze a variety of chocolates from different manufacturers. You would also need to measure multiple samples of each type to determine the range of caffeine content in each kind of chocolate from the same manufacturer.

A pure chocolate bar is probably fairly **homogeneous**, which means that composition is the same everywhere. A piece from one end probably has the same caffeine content as a piece from the other end. Chocolate with a macadamia nut in the middle is **heterogeneous**, which means that composition differs from place to place because the nut is different from the chocolate. If you were sampling a heterogeneous material, you would need to use a strategy different from that used to sample a homogeneous material.

Sample Preparation

Denby and Scott analyzed a piece of chocolate from one bar. The first step in the procedure calls for weighing a quantity of chocolate and extracting fat from it by dissolving the fat in a hydrocarbon solvent. Fat needs to be removed because it would interfere with chromatography later in the analysis. Unfortunately, shaking a chunk of chocolate with solvent does not extract much fat because the solvent has no access to the inside of the chocolate. So, our resourceful students sliced the chocolate into fine pieces and placed the pieces into a mortar and pestle (Figure 0-1), thinking they would grind the solid into small particles.

Imagine trying to grind chocolate! The solid is too soft to grind. So Denby and Scott froze the mortar and pestle with its load of sliced chocolate. Once the chocolate was cold, it was brittle enough to grind. Then small pieces were placed in a preweighed 15-milliliter (mL) centrifuge tube, and the mass of chocolate was noted.

Figure 0-2 outlines the next part of the procedure. A 10-mL portion of the organic solvent, petroleum ether, was added to the tube, and the top was capped with a stopper. The tube was shaken vigorously to dissolve fat from the solid chocolate into the solvent. Caffeine and theobromine are insoluble in this solvent. The mixture of liquid and fine particles was then spun in a centrifuge to pack the chocolate at the bottom of the tube. The clear liquid, containing dissolved fat, could now be **decanted** (poured off) and discarded. Extraction with fresh portions of solvent was repeated twice more to ensure complete removal of fat from the chocolate. Residual solvent in the chocolate was finally removed by heating the uncapped centrifuge tube in a

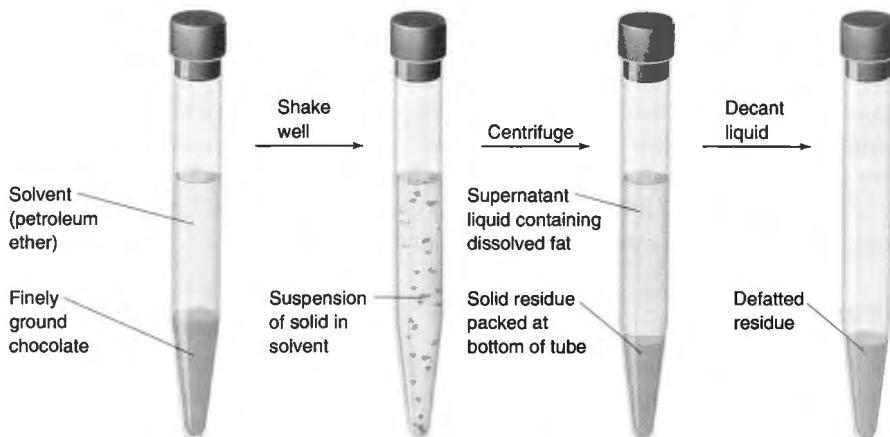


Figure 0-2 Extracting fat from chocolate to leave defatted solid residue for analysis.

beaker of boiling water. By weighing the centrifuge tube plus its content of defatted chocolate residue and subtracting the known mass of the empty tube, Denby and Scott could calculate the mass of chocolate residue.

Substances being measured—caffeine and theobromine in this case—are called **analytes**. The next step in the sample preparation procedure is to make a **quantitative transfer** (a complete transfer) of the fat-free chocolate residue to an Erlenmeyer flask and to dissolve the analytes in water for the chemical analysis. If any residue were not transferred from the tube to the flask, then the final analysis would be in error because not all of the analyte would be present. To perform the quantitative transfer, Denby and Scott added a few milliliters of pure water to the centrifuge tube and used stirring and heating to dissolve or suspend as much of the chocolate as possible. The **slurry** (a suspension of solid in a liquid) was then poured from the tube into a 50-mL flask. They repeated the procedure several times with fresh portions of water to ensure that every last bit of chocolate was transferred from the centrifuge tube into the flask.

To complete the dissolution of analytes, Denby and Scott added water to bring the volume up to about 30 mL. They heated the flask in a boiling water bath to extract all the caffeine and theobromine from the chocolate into the water. To compute the quantity of analyte later, the total mass of solvent (water) must be accurately known. Denby and Scott knew the mass of chocolate residue in the centrifuge tube and they knew the mass of the empty Erlenmeyer flask. So they put the flask on a balance and added water drop by drop until there were exactly 33.3 g of water in the flask. Later, they would compare known solutions of pure analyte in water with the unknown solution containing 33.3 g of water.

Before Denby and Scott could inject the unknown solution into a chromatograph for chemical analysis, they had to “clean up” (purify) the unknown even further (Figure 0-3). The slurry of chocolate residue in water contained tiny solid particles that would surely clog their expensive chromatography column and ruin it. So they transferred a portion of the slurry to a centrifuge tube and centrifuged the mixture to pack as much of the solid as possible at the bottom of the tube. The cloudy, tan, **supernatant liquid** (liquid above the packed solid) was then filtered in a further attempt to remove tiny particles of solid from the liquid.

A solution of anything in water is called an **aqueous** solution.

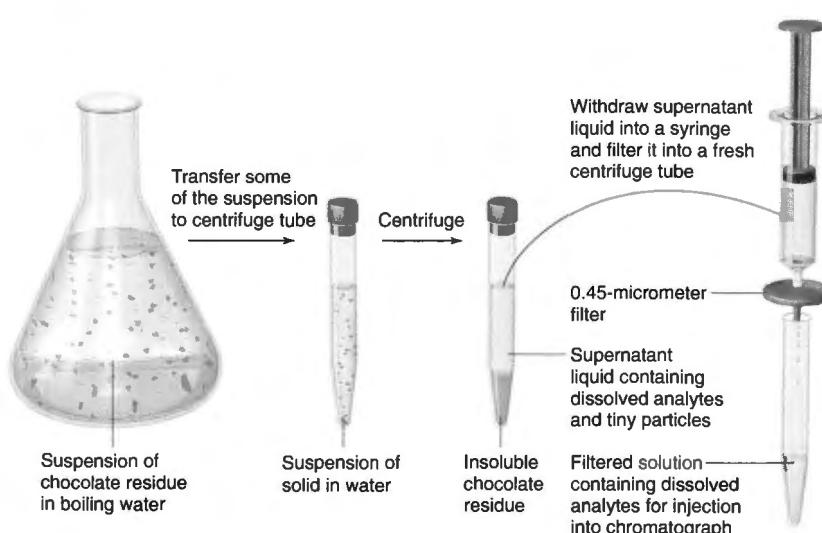


Figure 0-3 Centrifugation and filtration separate solid residue from the aqueous solution of analytes.

It is critical to avoid injecting solids into the chromatography column; but the tan liquid still looked cloudy. So Denby and Scott took turns between their classes to repeat the centrifugation and filtration steps five times. After each cycle in which supernatant liquid was filtered and centrifuged, it became a little cleaner. But the liquid was never completely clear. Given enough time, more solid always seemed to precipitate from the filtered solution.

The tedious procedure described so far is called **sample preparation**—transforming sample into a state that is suitable for analysis. In this case, fat had to be removed from the chocolate, analytes had to be extracted into water, and residual solid had to be separated from the water.

Analytical chemists are always developing better tools. Denby and Scott measured caffeine in chocolate in the mid-1990s by a *protocol* (a procedure) developed in the 1970s. Today, we would use *solid-phase extraction*, to replace solvent extraction, centrifugation, and filtration by a quick, simple procedure (Figure 22-33).

The Chemical Analysis (At Last!)

Compromising with reality, Denby and Scott decided that the solution of analytes was as clean as they could make it in the time available. The next step was to inject solution into a *chromatography* column, which separates the analytes and measures their quantity. The column in Figure 0-4a is packed with tiny particles of silica (SiO_2) to which

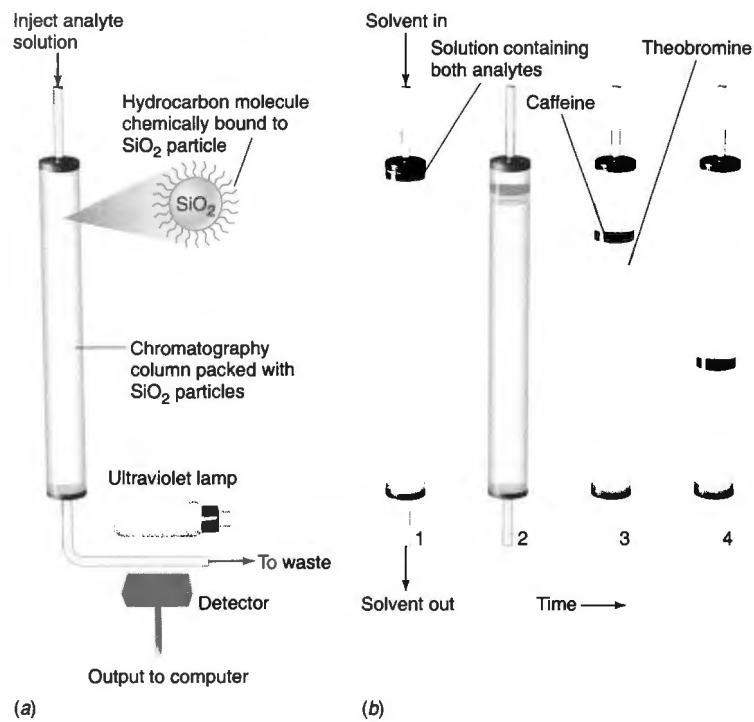


Figure 0-4 Principle of liquid chromatography. (a) Chromatography apparatus with an ultraviolet absorbance monitor to detect analytes at the column outlet. (b) Separation of caffeine and theobromine by chromatography. Caffeine is more soluble than theobromine in the hydrocarbon layer on the particles in the column. Therefore caffeine is retained more strongly and moves through the column more slowly than theobromine.

are attached long hydrocarbon molecules. Twenty microliters (20.0×10^{-6} liters) of the solution of chocolate extract were injected into the column and washed through with a solvent made by mixing 79 mL of pure water, 20 mL of methanol, and 1 mL of acetic acid. Caffeine is more soluble than theobromine in the hydrocarbon on the silica surface. Therefore caffeine “sticks” to the coated silica particles in the column more strongly than theobromine does. When both analytes are flushed through the column by solvent, theobromine reaches the outlet before caffeine (Figure 0-4b).

Analytes are detected at the outlet by their ability to absorb ultraviolet radiation. As compounds emerge from the column, they absorb radiation emitted from the lamp in Figure 0-4a and less radiation reaches the detector. The graph of detector response versus time in Figure 0-5 is called a *chromatogram*. Theobromine and caffeine are the major peaks in the chromatogram. The small peaks are other components of the aqueous extract from chocolate.

The chromatogram alone does not tell us what compounds are in an unknown. If you do not know beforehand what to expect, you would need to identify the peaks. One way to identify individual peaks is to add an authentic sample of either caffeine or theobromine to the unknown and see whether one of the peaks grows in magnitude. Another way is to record the *mass spectrum* (Chapter 21) of each peak as it emerges from the column.

Identifying *what* is in an unknown is called **qualitative analysis**. Identifying *how much* is present is called **quantitative analysis**. The vast majority of this book deals with quantitative analysis.

In Figure 0-5, the *area* under each peak is proportional to the quantity of that component passing through the detector. The best way to measure the area is with a computer that monitors the detector output during chromatography. Denby and Scott did not have a computer on their chromatograph, so they measured the *height* of each peak instead.

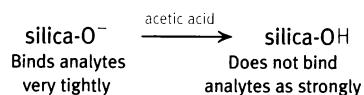
Calibration Curves

In general, analytes with equal concentrations give different detector responses. Therefore, the response must be measured for known concentrations of each analyte. A graph showing detector response as a function of analyte concentration is called a **calibration curve** or a *standard curve*. To construct a calibration curve, **standard solutions** containing known concentrations of pure theobromine or caffeine were prepared and injected into the column, and the resulting peak heights were measured. Figure 0-6 is a chromatogram of one of the standard solutions, and Figure 0-7 shows calibration curves made by injecting solutions containing 10.0, 25.0, 50.0, or 100.0 micrograms of each analyte per gram of solution.

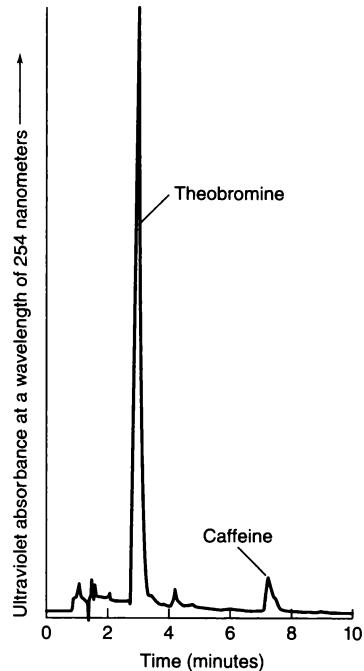
Straight lines drawn through the calibration points could then be used to find the concentrations of theobromine and caffeine in an unknown. Figure 0-7 shows that, if the observed peak height of theobromine from an unknown solution is 15.0 cm, then the concentration is 76.9 micrograms per gram of solution.

Figure 0-5 Chromatogram of 20.0 microliters of dark chocolate extract. A 4.6-mm-diameter \times 150-mm-long column, packed with 5-micrometer particles of Hypersil ODS, was eluted (washed) with water:methanol:acetic acid (79:20:1 by volume) at a rate of 1.0 mL per minute.

Chromatography solvent is selected by a systematic trial-and-error process. Acetic acid neutralizes charged oxygen atoms on the silica surface that would strongly retain a small fraction of caffeine and theobromine.



Only substances that absorb ultraviolet radiation at a wavelength of 254 nanometers are observed in Figure 0-5. By far, the major components in the aqueous extract are sugars, but they are not detected in this experiment.



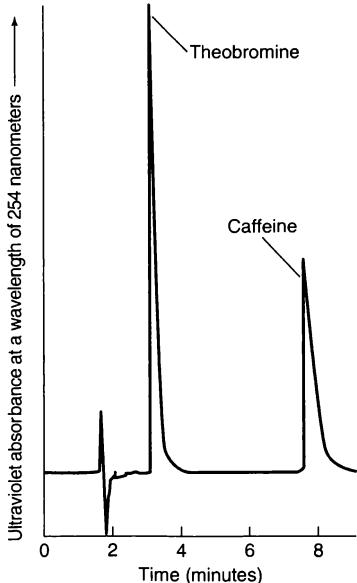


Figure 0-6 Chromatogram of 20.0 microliters of a standard solution containing 50.0 micrograms of theobromine and 50.0 micrograms of caffeine per gram of solution.

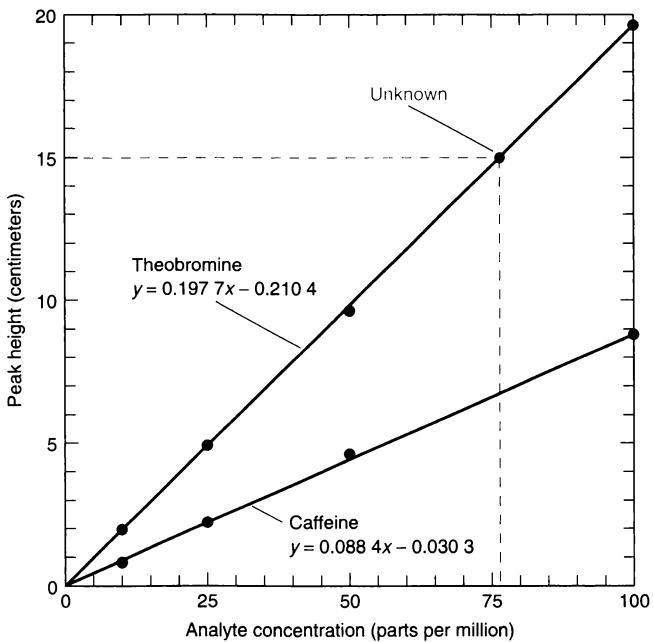


Figure 0-7 Calibration curves, showing observed peak heights for known concentrations of pure compounds. One part per million is 1 microgram of analyte per gram of solution. Equations of the straight lines drawn through the experimental data points were determined by the *method of least squares*; described in Chapter 4.

Interpreting the Results

Knowing how much analyte is in the aqueous extract of the chocolate, Denby and Scott could calculate how much theobromine and caffeine were in the original chocolate. Results for dark and white chocolates are shown in Table 0-1. The quantities found in white chocolate are only about 2% as great as the quantities in dark chocolate.

Table 0-1 also reports the *standard deviation* of three replicate measurements for each sample. (*Replicate* means repeated measurements of the same quantity.) Standard deviation, which is discussed in Chapter 4, is a measure of the reproducibility of the results. If three samples give identical results, the standard deviation would be 0. If the standard deviation is very large, then the results are not very reproducible. For theobromine in dark chocolate, the standard deviation (0.002) is less than 1% of the average (0.392); so we say the measurement is very reproducible. For theobromine in white chocolate, the standard deviation (0.007) is nearly as great as the average (0.010), so the measurement is not very reproducible.

Table 0-1 Analyses of dark and white chocolate

Analyte	Grams of analyte per 100 grams of chocolate	
	Dark chocolate	White chocolate
Theobromine	0.392 ± 0.002	0.010 ± 0.007
Caffeine	0.050 ± 0.003	0.0009 ± 0.0014

Uncertainties are the *standard deviation* of three replicate injections of each extract.

The arduous path to reliable analytical results is not the end of the story. The purpose of the analysis is to reach some interpretation or decision. The questions posed at the beginning of this chapter were “How much caffeine is in a chocolate bar?” and “How does it compare with the quantity in coffee or soft drinks?” After all this work, Denby and Scott discovered how much caffeine is in *one* particular chocolate bar. It would take a great deal more work to sample many chocolate bars of the same type and many different types of chocolate to gain a more universal view. Table 0-2 compares results from different kinds of analyses of different sources of caffeine. A can of soft drink or a cup of tea contains about one-quarter to one-half of the caffeine in a small cup of coffee. Chocolate contains even less caffeine, but a hungry backpacker eating enough baking chocolate can get a pretty good jolt!

Table 0-2 Caffeine content of beverages and foods^a

Source	Caffeine (milligrams per serving)	Serving size ^b (ounces)
Regular coffee	106–164	5
Decaffeinated coffee	2–5	5
Tea	21–50	5
Cocoa beverage	2–8	6
Baking chocolate	35	1
Sweet chocolate	20	1
Milk chocolate	6	1
Caffeinated soft drinks	5–74	12
IGA Cola	5	12
Sam’s Cola (WalMart)	13	12
Coca Cola	34	12
Pepsi	39	12
Dr. Pepper	43	12
Mountain Dew	55	12
Vault Zero	74	12
Red Bull	80	8.2

a. Data from http://www.holymtn.com/tea/caffeine_content.htm. Carbonated beverages from K.-H. Chou and L. N. Bell, *J. Food Sci.* **2007**, 72, C337. Red Bull from <http://wilstar.com/caffeine.htm>.

b. 1 ounce = 28.35 grams.

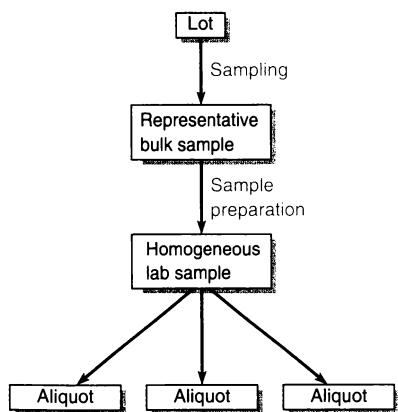
Quality Assurance

How could Denby and Scott be sure that their analytical results are reliable? Professional analysts follow a set of practices, called **quality assurance**, intended to give themselves and their customers confidence in the quality of their results. One way that Denby and Scott could assess the reliability of their analytical method might be to melt some chocolate, add a known quantity of caffeine to the melt, mix it as well as possible, and freeze the chocolate. The added caffeine is called a *spike*. When the spiked chocolate is analyzed, they should find a quantity of caffeine equal to that in the original chocolate plus the amount in the spike. If they find the expected quantity, they can have some confidence that their method extracts all the caffeine that was present and measures it accurately.

Chapter 5 discusses quality assurance.

Box 0-1 Constructing a Representative Sample

The diagram below shows steps in transforming a complex substance into individual samples that can be analyzed. A *lot* is the total material (for example, a railroad car full of grain or a carton of macadamia chocolates) from which samples are taken. A *bulk sample* (also called a *gross sample*) is taken from the lot for analysis and *archiving* (storing for future reference). The bulk sample must be representative of the lot or the analysis will be meaningless. From the representative bulk sample, a smaller, homogeneous *laboratory sample* is formed that must have the same composition as the bulk sample. For example, you might obtain a laboratory sample by grinding an entire solid bulk sample to a fine powder, mixing thoroughly, and keeping one bottle of powder for testing. Small test portions (called *aliquots*) of the laboratory sample are used for individual analyses.

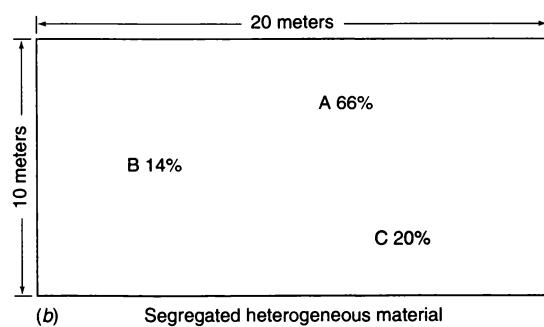
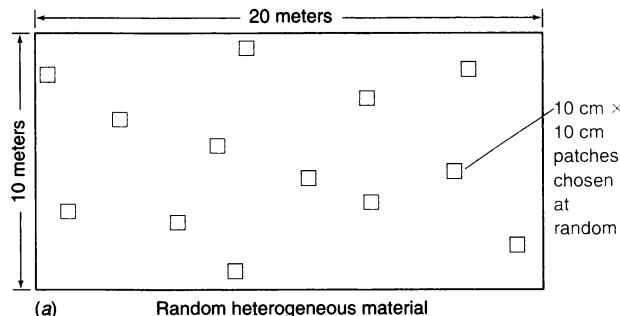


Sampling is the process of selecting a representative bulk sample from the lot. *Sample preparation* converts a bulk sample into a homogeneous laboratory sample. Sample preparation also refers to steps that eliminate interfering species or that concentrate the analyte.

In a **random heterogeneous material**, differences in composition are random and on a fine scale. When you collect a portion of the material for analysis, you obtain some of each of the different compositions. To construct a representative sample from a heterogeneous material, you can first visually divide the material into many small regions. For example, if you want to measure the magnesium content of the grass in the 10-meter \times 20-meter field in panel *a*, you could divide

the field into 20 000 small patches that are 10 centimeters on a side. A **random sample** is collected by taking portions from the desired number of regions chosen at random. Assign a number to each small patch and use a computer to generate 100 numbers at random from 1 to 20 000. Then harvest and combine the grass from each of the selected 100 patches to construct a representative bulk sample for analysis.

In a **segregated heterogeneous material**, large regions have obviously different compositions. To obtain a representative specimen of such a material, you need to construct a **composite sample**. For example, the field in panel *b* has three different types of grass in regions A, B, and C. You could draw a map of the field on graph paper and measure the area in each region. In this case, 66% of the area lies in region A, 14% lies in region B, and 20% lies in region C. To construct a representative bulk sample from this segregated material, take 66 small patches from region A, 14 from region B, and 20 from region C. You could do so by drawing random numbers from 1 to 20 000 to select patches until you have the desired number from each region.



0-2 General Steps in a Chemical Analysis

The analytical process often begins with a question such as “Is this water safe to drink?” or “Does emission testing of automobiles reduce air pollution?” A scientist translates such questions into the need for particular measurements. An analytical chemist then chooses or invents a procedure to carry out those measurements.

When the analysis is complete, the analyst must translate the results into terms that can be understood by others. A most important feature of any result is its limitations. What is the statistical uncertainty in reported results? If you took samples in a different manner, would you obtain the same results? Is a tiny amount (a *trace*) of analyte found in a sample really there or is it contamination? Once all interested parties understand the results and their limitations, then people can draw conclusions and reach decisions.

We can now summarize general steps in the analytical process:

Formulating the question	Translate general questions into specific questions to be answered through chemical measurements.
Selecting analytical procedures	Search the chemical literature to find appropriate procedures or, if necessary, devise new procedures to make the measurements.
Sampling	Select representative material to analyze, as described in Box 0-1. If you begin with a poorly chosen sample or if the sample changes between the time it is collected and the time it is analyzed, results are meaningless. “Garbage in—garbage out!”
Sample preparation	Converting a representative sample into a form suitable for analysis is called <i>sample preparation</i> , which usually means dissolving the sample. Samples with a low concentration of analyte may need to be concentrated. It may be necessary to remove or <i>mask</i> species that interfere with the chemical analysis. For a chocolate bar, sample preparation consisted of removing fat and dissolving the desired analytes. Fat was removed because it would interfere with chromatography.
Analysis	Measure the concentration of analyte in several identical aliquots (portions). The purpose of <i>replicate measurements</i> (repeated measurements) is to assess the variability (uncertainty) in the analysis and to guard against a gross error in the analysis of a single aliquot. <i>The uncertainty of a measurement is as important as the measurement itself</i> , because it tells us how reliable the measurement is. If necessary, use different analytical methods on similar samples to make sure that all methods give the same result and that the choice of analytical method is not biasing the result. You may also wish to construct and analyze several different bulk samples to see what variations arise from your sampling procedure. Steps taken to demonstrate the reliability of the analysis are called <i>quality assurance</i> .

Chemists use the term **species** to refer to any chemical of interest. Species is both singular and plural.

Interference occurs when a species other than analyte increases or decreases the response of the analytical method and makes it appear that there is more or less analyte than is actually present.

Masking transforms an interfering species into a form that is not detected. For example, Ca^{2+} in lake water can be measured with a reagent called EDTA. Al^{3+} interferes with this analysis because it also reacts with EDTA. Al^{3+} can be masked by treating the sample with excess F^- to form AlF_6^{3-} , which does not react with EDTA.

Reporting and interpretation

Deliver a clearly written, complete report of your results, highlighting any special limitations that you attach to them. Your report might be written to be read only by a specialist (such as your instructor), or it might be written for a general audience (perhaps your mother). Be sure the report is appropriate for its intended audience.

Drawing conclusions

Once a report is written, the analyst might not further participate in what is done with the information, such as modifying the raw material supply for a factory or creating new laws to regulate food additives. The more clearly a report is written, the less likely it is to be misinterpreted.

Most of this book deals with measuring chemical concentrations in homogeneous aliquots of an unknown. The analysis is meaningless unless you have collected the sample properly, you have taken measures to ensure the reliability of the analytical method, and you communicate your results clearly and completely. The chemical analysis is only the middle part of a process that begins with a question and ends with a conclusion.

Ask Yourself

Answers to Ask Yourself questions are at the back of the book.

0-A. After reading Box 0-1, answer the following questions:

- (a) What is the difference between a *heterogeneous* and a *homogeneous* material?
- (b) What is the difference between a *random* heterogeneous material and a *segregated* heterogeneous material?
- (c) What is the difference between a *random* sample and a *composite* sample? When would each be used?

Important Terms*

aliquot	homogeneous	random heterogeneous material	slurry
analyte	interference	material	species
aqueous	masking	random sample	standard solution
calibration curve	qualitative analysis	sample preparation	supernatant liquid
composite sample	quality assurance	sampling	
decant	quantitative analysis	segregated heterogeneous material	
heterogeneous	quantitative transfer		

Problems

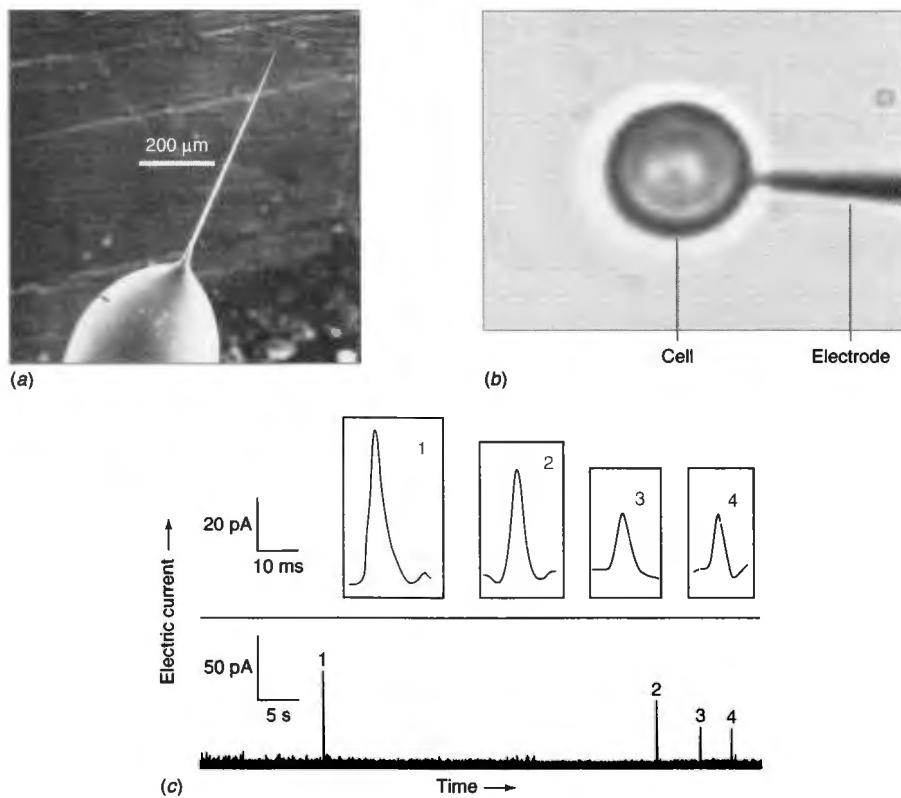
- 0-1. What is the difference between *qualitative* and *quantitative* analysis?
- 0-2. List the steps in a chemical analysis.
- 0-3. What does it mean to *mask* an interfering species?
- 0-4. What is the purpose of a calibration curve?

*Terms are introduced in **bold** type in the chapter and are also defined in the Glossary.

Notes and References

1. T. J. Wenzel, *Anal. Chem.* **1995**, *67*, 470A.
2. W. R. Kreiser and R. A. Martin, Jr., *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 591.
Off. Anal. Chem. **1978**, *61*, 1424; W. R. Kreiser

Biochemical Measurements with a Nanoelectrode



(a) Carbon fiber electrode with a 100-nanometer-diameter (100×10^{-9} meter) tip extending from glass capillary. The marker bar is 200 micrometers (200×10^{-6} meter). [From W.-H. Huang, D.-W. Pang, H. Tong, Z.-L. Wang, and J.-K. Cheng, *Anal. Chem.* **2001**, *73*, 1048.] (b) Electrode positioned adjacent to a cell detects release of the neurotransmitter dopamine from the cell. A nearby, larger counterelectrode is not shown. (c) Bursts of electric current detected when dopamine is released. Insets are enlargements. [From W.-Z. Wu, W.-H. Huang, W. Wang, Z.-L. Wang, J.-K. Cheng, T. Xu, R.-Y. Zhang, Y. Chen, and J. Liu, *J. Am. Chem. Soc.* **2005**, *127*, 8914.]

An electrode whose tip is smaller than a single cell allows us to measure neurotransmitter molecules released by a nerve cell in response to a chemical stimulus. We call the electrode a *nanoelectrode* because its active region has dimensions of nanometers (10^{-9} meters). Neurotransmitter molecules released from one *vesicle* (a small compartment) of a nerve cell diffuse to the electrode where they donate or accept electrons, generating an electric current measured in picoamperes (10^{-12} amperes) for a period of milliseconds (10^{-3} seconds). This chapter discusses units that describe chemical and physical measurements of objects ranging in size from atoms to galaxies.

Chemical Measurements

Most people who practice analytical chemistry do not identify themselves as analytical chemists. For example, chemical analysis is an essential tool used by biologists to understand how organisms function and by doctors to diagnose disease and monitor the response of a patient to treatment. Environmental scientists measure chemical changes in the atmosphere, water, and soil that occur in response to the activities of both man and nature. Forensic scientists identify and sometimes measure drugs, combustion residues, and fibers from crime scenes. You are taking this course because you might make chemical measurements yourself or you will need to understand analytical results reported by others. This chapter provides basic working knowledge of measurements and equilibrium.

1-1 SI Units and Prefixes

SI units of measurement derive their name from the French *Système International d'Unités*. **Fundamental units** (base units) from which all others are derived are defined in Table 1-1. Standards of length, mass, and time are the *meter* (m), *kilogram* (kg) (Figure 1-1), and *second* (s), respectively. Temperature is measured in *kelvins* (K), amount of substance in *moles* (mol), and electric current in *amperes* (A). Table 1-2 lists derived quantities that are defined in terms of the fundamental quantities. For example, force is measured in *newtons* (N), pressure is measured in *pascals* (Pa), and energy is measured in *joules* (J), each of which can be expressed in terms of the more fundamental units of length, time, and mass.

It is convenient to use the prefixes in Table 1-3 to express quantities that vary over many **orders of magnitude** (powers of ten). For example, the pressure of dissolved oxygen in arterial blood is approximately 1.3×10^4 Pa. Table 1-3 tells us that 10^3 is assigned the prefix k for “kilo.” We can express the pressure in multiples of 10^3 as follows:

$$1.3 \times 10^4 \text{ Pa} \times \frac{1 \text{ kPa}}{10^3 \text{ (Pa)}} = 1.3 \times 10^1 \text{ kPa} = 13 \text{ kPa}$$

The unit kPa is read “kilopascals.” Write units beside each number in a calculation and cancel identical units in the numerator and denominator. This practice ensures that you know the units for your answer.



Figure 1-1 Of the fundamental SI units in Table 1-1, only the kilogram is defined by an artifact, rather than by a reproducible physical measurement. The international kilogram in France, made from a Pt-Ir alloy in 1885, has been removed from its protective enclosure to be weighed against working copies only in 1890, 1948, and 1992. Its mass could change from reaction with the atmosphere or from wear, so there is ongoing research to define a standard for mass based on measurements that should not change over time. [Bureau International des Poids et Mesures.]

Pressure is force per unit area.

1 pascal (Pa) = 1 N/m².

The pressure of the atmosphere is about 100 000 Pa.

Question In the past, medical technicians referred to 1 microliter (μL) as 1 lambda (λ). How many microliters are in 1 milliliter? (Answer: 1 000)

Table 1-1 Fundamental SI units

Quantity	Unit (symbol)	Definition
Length	meter (m)	One meter is the distance light travels in a vacuum during $\frac{1}{299\,792\,458}$ of a second.
Mass	kilogram (kg)	One kilogram is the mass of the prototype kilogram kept at Sèvres, France.
Time	second (s)	One second is the duration of 9 192 631 770 periods of the radiation corresponding to a certain atomic transition of ^{133}Cs .
Electric current	ampere (A)	One ampere of current produces a force of 2×10^{-7} newtons per meter of length when maintained in two straight, parallel conductors of infinite length and negligible cross section, separated by 1 meter in a vacuum.
Temperature	kelvin (K)	Temperature is defined such that the triple point of water (at which solid, liquid, and gaseous water are in equilibrium) is 273.16 K, and the temperature of absolute zero is 0 K.
Luminous intensity	candela (cd)	Candela is a measure of luminous intensity visible to the human eye. One cd is the luminous intensity in a given direction of a source that emits monochromatic radiation of frequency 540×10^{12} hertz and of which the radiant intensity in that direction is $\frac{1}{683}$ watt per steradian.
Amount of substance	mole (mol)	One mole is the number of atoms in exactly 0.012 kg of ^{12}C (approximately 6.022×10^{23}).
Plane angle	radian (rad)	There are 2π radians in a circle.
Solid angle	steradian (sr)	There are 4π steradians in a sphere.

Table 1-2 SI-derived units with special names

Quantity	Unit	Abbreviation	Expression in terms of other units	Expression in terms of SI base units
Frequency	hertz	Hz		1/s
Force	newton	N		$\text{m} \cdot \text{kg}/\text{s}^2$
Pressure	pascal	Pa	N/m^2	$\text{kg}/(\text{m} \cdot \text{s}^2)$
Energy, work, quantity of heat	joule	J	$\text{N} \cdot \text{m}$	$\text{m}^2 \cdot \text{kg}/\text{s}^2$
Power, radiant flux	watt	W	J/s	$\text{m}^2 \cdot \text{kg}/\text{s}^3$
Quantity of electricity, electric charge	coulomb	C		$\text{s} \cdot \text{A}$
Electric potential, potential difference, electromotive force	volt	V	W/A	$\text{m}^2 \cdot \text{kg}/(\text{s}^3 \cdot \text{A})$
Electric resistance	ohm	Ω	V/A	$\text{m}^2 \cdot \text{kg}/(\text{s}^3 \cdot \text{A}^2)$

Frequency is the number of cycles per unit time for a repetitive event. *Force* is the product mass \times acceleration. *Pressure* is force per unit area. *Energy* or *work* is force \times distance = mass \times acceleration \times distance. *Power* is energy per unit time. The *electric potential difference* between two points is the work required to move a unit of positive charge between the two points. *Electric resistance* is the potential difference required to move one unit of charge per unit time between two points.

Table 1-3 Prefixes

Prefix	Abbreviation	Factor	Prefix	Abbreviation	Factor
yotta	Y	10^{24}	deci	d	10^{-1}
zetta	Z	10^{21}	centi	c	10^{-2}
exa	E	10^{18}	milli	m	10^{-3}
peta	P	10^{15}	micro	μ	10^{-6}
tera	T	10^{12}	nano	n	10^{-9}
giga	G	10^9	pico	p	10^{-12}
mega	M	10^6	femto	f	10^{-15}
kilo	k	10^3	atto	a	10^{-18}
hecto	h	10^2	zepto	z	10^{-21}
deca	da	10^1	yocto	y	10^{-24}

Example Counting Neurotransmitter Molecules with an Electrode

Box 1-1 describes the process by which neurotransmitters are released from a nerve cell in discrete bursts. The neurotransmitter measured by the electrode at the opening of this chapter is dopamine. Each dopamine molecule that diffuses to the electrode releases two electrons. The charge transferred to the electrode by burst 1 in panel *c* of the chapter opener is 0.27 pC (picocoulombs, 10^{-12} C). One coulomb of charge corresponds to 6.24×10^{18} electrons. How many molecules are released in burst 1?

SOLUTION Table 1-3 tells us that 1 pC equals 10^{-12} C. Therefore 0.27 pC corresponds to

$$0.27 \text{ pC} \times \left(\frac{10^{-12} \text{ C}}{\text{pC}} \right) = 2.7 \times 10^{-13} \text{ C}$$

The key to converting between units is to write a conversion factor such as 10^{-12} C/pC, carry out the multiplication, cancel the same units that appear in the numerator and denominator, and show that the answer has the correct units. The number of electrons in 0.27 pC is

$$(2.7 \times 10^{-13} \text{ C}) \times \left(\frac{6.24 \times 10^{18} \text{ electrons}}{\text{C}} \right) = 1.68 \times 10^6 \text{ electrons}$$

Each molecule releases two electrons, so the number of molecules in one burst is

$$(1.68 \times 10^6 \text{ electrons}) \times \left(\frac{1 \text{ molecule}}{2 \text{ electrons}} \right) = 8.4 \times 10^5 \text{ molecules}$$

 **Test Yourself** How many molecules of dopamine are in burst 4, whose total charge is 0.13 pC? (Answer: 4.1×10^5 molecules)

Ask Yourself

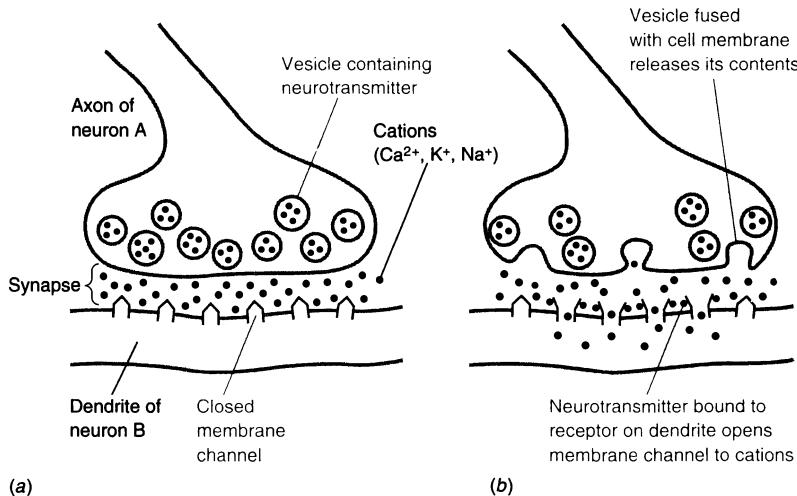
1-A. (a) What are the names and abbreviations for each of the prefixes from 10^{-24} to 10^{24} ? Which abbreviations are capitalized?

(b) A representative concentration of ozone (O_3) in the stratosphere is 8×10^{-7} liters of O_3 per liter of air. Express this concentration as nL O_3/L and $\mu\text{L O}_3/\text{L}$.

Box 1-1 Exocytosis of Neurotransmitters

Nerve signals are transmitted from the *axon* of one nerve cell (a *neuron*) to the *dendrite* of a neighboring neuron across a junction called a *synapse*. A change in electric potential at the axon causes tiny chemical-containing packages called *vesicles* to fuse with active zones at the cell membrane. This process, called *exocytosis*, releases neurotransmitter molecules stored in the vesicles. When neurotransmitters bind to receptors on the dendrite, gates open up to allow cations to cross the dendrite membrane. Cations diffusing into the dendrite change its electric potential, thereby transmitting the nerve impulse into the second neuron.

Release of the neurotransmitter dopamine from a single active zone on a cell surface can be monitored by placing a nanoelectrode next to the cell, as shown at the opening of this chapter. When stimulated by K^+ ion injected near the cell, vesicles release dopamine by exocytosis. Each dopamine molecule that diffuses to the nanoelectrode gives up two electrons. Panel c at the beginning of the chapter shows four pulses measured over a period of 1 minute near one active zone. Each pulse lasts ~ 10 milliseconds and has a peak current of $\sim 10\text{--}50$ picoamperes. The number of electrons in a pulse tells us how many dopamine molecules were released from one vesicle.



Action of neurotransmitters at synapse. (a) Before release of neurotransmitter. (b) Neurotransmitters are released by *exocytosis* when vesicles merge with the outer cell membrane. Only certain active zones of the outer membrane are capable of exocytosis. (Cells can take molecules in by the reverse process, called *endocytosis*.)

1-2 Conversion Between Units

1 *calorie* (cal) of energy will heat 1 gram of water from 14.5° to 15.5°C .
1 000 *joules* will raise the temperature of a cup of water by about 1°C .
 $1 \text{ cal} = 4.184 \text{ J}$.

Although SI is the internationally accepted system of measurement in science, other units are encountered. Conversion factors are found in Table 1-4. For example, common non-SI units for energy are the *calorie* (cal) and the *Calorie* (with a capital C, which represents 1 000 calories, or 1 kcal). Table 1-4 states that 1 cal is exactly 4.184 J (joules).

You require approximately 46 Calories per hour (h) per 100 pounds (lb) of body mass to carry out basic functions required for life, such as breathing, pumping blood, and maintaining body temperature. This minimum energy requirement for a conscious person at rest is called the *basal metabolism*. A person walking at 2 miles per

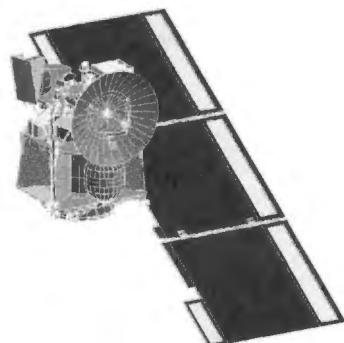
Table 1-4 Conversion factors

Quantity	Unit	Symbol	SI equivalent ^a
Volume	liter	L	* 10^{-3} m^3
	milliliter	mL	* 10^{-6} m^3
Length	angstrom	Å	* 10^{-10} m
	inch	in.	*0.025 4 m
Mass	pound	lb	*0.453 592 37 kg
	metric ton		*1 000 kg
Force	dyne	dyn	* 10^{-5} N
Pressure	bar	bar	* 10^5 Pa
	atmosphere	atm	*760 mm Hg
	atmosphere	atm	*1.013 25 bar
	atmosphere	atm	*101 325 Pa
	torr (= 1 mm Hg)	Torr	133.322 Pa
Energy	pound/in. ²	psi	6 894.76 Pa
	erg	erg	* 10^{-7} J
	electron volt	eV	1.602 176 487 $\times 10^{-19} \text{ J}$
	calorie, thermochemical	cal	*4.184 J
	Calorie (with a capital C)	Cal	*1 000 cal = 4.184 kJ
Power	British thermal unit	Btu	1 055.06 J
	horsepower		745.700 W
Temperature	centigrade (= Celsius)	°C	*K – 273.15
	Fahrenheit	°F	*1.8(K – 273.15) + 32

a. An asterisk (*) indicates that the conversion is exact (by definition).

hour on a level path requires approximately 45 Calories per hour per 100 pounds of body mass beyond basal metabolism. The same person swimming at 2 miles per hour consumes 360 Calories per hour per 100 pounds beyond basal metabolism.

In 1999, the \$125 million Mars Climate Orbiter spacecraft was lost when it entered the Martian atmosphere 100 km lower than planned. The navigation error would have been prevented if people had labeled their units of measurement. Engineers who built the spacecraft calculated thrust in the English unit, pounds of force. Jet Propulsion Laboratory engineers thought they were receiving the information in the metric unit, newtons. Nobody caught the error. [Image courtesy of JPL/NASA.]



$$\begin{aligned}1 \text{ pound (mass)} &\approx 0.453 6 \text{ kg} \\1 \text{ mile} &\approx 1.609 \text{ km}\end{aligned}$$

Example Unit Conversions

Express the rate of energy use by a walking woman (46 + 45 = 91 Calories per hour per 100 pounds of body mass) in kilojoules per hour per kilogram of body mass.

SOLUTION We will convert each non-SI unit separately. First, note that 91 Calories equals 91 kcal. Table 1-4 states that 1 cal = 4.184 J, or 1 kcal = 4.184 kJ, so

$$91 \text{ kcal} \times \frac{4.184 \text{ kJ}}{1 \text{ kcal}} = 381 \text{ kJ}$$

Table 1-4 also says that 1 lb is 0.453 6 kg; so 100 lb = 45.36 kg. The rate of energy consumption is, therefore,

$$\frac{91 \text{ kcal/h}}{100 \text{ lb}} = \frac{381 \text{ kJ/h}}{45.36 \text{ kg}} = 8.4 \frac{\text{kJ/h}}{\text{kg}}$$

You could have written this as one calculation with appropriate unit cancellations:

$$\text{rate} = \frac{91 \text{ kcal/h}}{100 \text{ lb}} \times \frac{4.184 \text{ kJ}}{1 \text{ kcal}} \times \frac{1 \text{ lb}}{0.453 6 \text{ kg}} = 8.4 \frac{\text{kJ/h}}{\text{kg}}$$

Don't panic about the number of significant digits in problems in this chapter. We will take up significant figures in Chapter 3.



Test Yourself A person who is swimming at 2 miles per hour requires 360 + 46 Calories per hour per 100 pounds of body mass. Express the energy use in kJ/h per kg of body mass. (Answer: 37 kJ/h per kg)

Example

Watts Measure Power (Energy per Second)

$$1 \text{ W} = 1 \text{ J/s}$$

The complex unit joules per hour per kilogram (J/h/kg) is the same as the expression

$$\frac{\text{J}}{\text{h}\cdot\text{kg}}$$

One watt is 1 joule per second. The woman in the preceding example expends 8.4 kilojoules per hour per kilogram of body mass while walking. (a) How many watts per kilogram does she use? (b) If her mass is 50 kg, how many watts does she expend?

SOLUTION (a) She expends $8.4 \times 10^3 \text{ J per h per kg}$. We can write the units as J/h/kg , which is equivalent to writing $\text{J}(\text{h}\cdot\text{kg})$. Because an hour contains $60 \text{ s/min} \times 60 \text{ min/h} = 3600 \text{ s}$, the required power is

$$8.4 \times 10^3 \frac{\text{J}}{\text{h}\cdot\text{kg}} \times \frac{3600 \text{ s}}{1 \text{ h}}$$

Oops! The units didn't cancel out. I guess we need to use the inverse conversion factor:

$$8.4 \times 10^3 \frac{\text{J}}{\text{h}\cdot\text{kg}} \times \frac{1 \text{ h}}{3600 \text{ s}} = 2.33 \frac{\text{J}}{\text{s}\cdot\text{kg}} = 2.33 \frac{\text{J/s}}{\text{kg}} = 2.33 \frac{\text{W}}{\text{kg}}$$

(b) Our intrepid walker has a mass of 50 kg. Therefore her power requirement is

$$2.33 \frac{\text{W}}{\text{kg}} \times 50 \text{ kg} = 116 \text{ W}$$



Ask Yourself With the conversion factor in Table 1-4, express the woman's energy use in horsepower. (Answer: 0.156 horsepower)

Ask Yourself

1-B. A 120-pound woman working in an office expends about $2.2 \times 10^3 \text{ kcal/day}$, whereas the same woman climbing a mountain needs $3.4 \times 10^3 \text{ kcal/day}$.

- How many joules per day does the woman expend in each activity?
- How many seconds are in 1 day?
- How many joules per second (= watts) does the woman expend in each activity?
- Which consumes more power (watts), the office worker or a 100-W light bulb?

1-3 Chemical Concentrations

The minor species in a solution is called the **solute**, and the major species is the **solvent**. In this text, most discussions concern *aqueous* solutions, in which the solvent is water. **Concentration** refers to how much solute is contained in a given volume or mass.

Molarity and Molality

Molarity (M) is the number of moles of a substance per liter of solution. A **mole** is Avogadro's number of atoms or molecules or ions ($6.022 \times 10^{23} \text{ mol}^{-1}$). A **liter** (L)

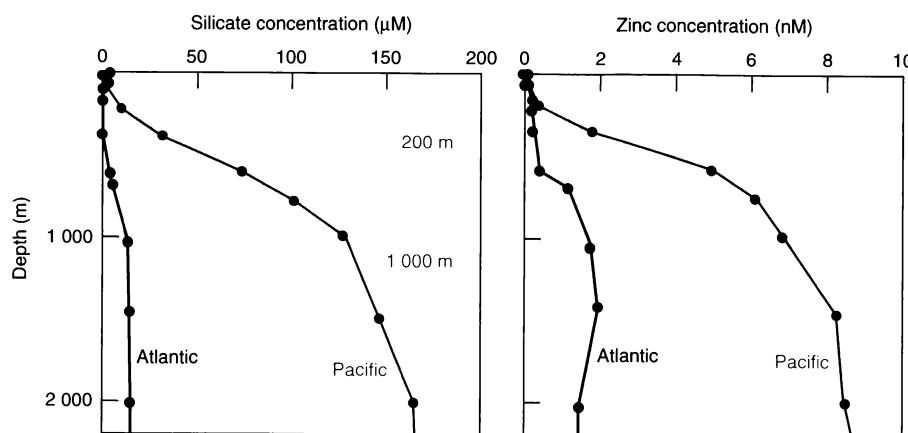


Figure 1-2 Concentration profiles of dissolved silicate and zinc in the northern Atlantic and northern Pacific oceans. Seawater is *heterogeneous*: Samples collected at depths of 200 or 1 000 m do not have the same concentrations of each species. Living organisms near the ocean surface deplete seawater of both silicate and zinc. [Data from K. S. Johnson, K. H. Coale, and H. W. Jannasch, *Anal. Chem.* **1992**, *64*, 1065A.]

is the volume of a cube that is 10 cm on each edge. Because 10 cm = 0.1 m, $1 \text{ L} = (0.1 \text{ m})^3 = (10^{-1})^3 \text{ m}^3 = 10^{-3} \text{ m}^3$. In Figure 1-2, chemical concentrations in the ocean are expressed in micromoles per liter ($10^{-6} \text{ mol/L} = \mu\text{M}$) and nanomoles per liter ($10^{-9} \text{ mol/L} = \text{nM}$). The molarity of a species is usually designated by square brackets, as in $[\text{Cl}^-]$.

The **atomic mass** of an element is the number of grams containing Avogadro's number of atoms. The **molecular mass** of a compound is the sum of atomic masses of the atoms in the molecule. It is the number of grams containing Avogadro's number of molecules.

$$\text{molarity (M)} = \frac{\text{moles of solute}}{\text{liters of solution}}$$

The liter is named after the Frenchman Claude Litre (1716–1778), who named his daughter Millicent. I suppose her friends called her Millie Litre.

"Mole Day" is celebrated at 6:02 A.M. on October 23 (10/23) at many schools.

Example Molarity of Salts in the Sea

(a) Typical seawater contains 2.7 g of salt (sodium chloride, NaCl) per deciliter ($= \text{dL} = 0.1 \text{ L}$). What is the molarity of NaCl in the ocean? (b) MgCl₂ has a typical concentration of 0.054 M in the ocean. How many grams of MgCl₂ are present in 25 mL of seawater?

SOLUTION (a) The molecular mass of NaCl is 22.99 (Na) + 35.45 (Cl) = 58.44 g/mol. The moles of salt in 2.7 g are

$$\text{moles of NaCl} = \frac{(2.7 \text{ g})}{\left(58.44 \frac{\text{g}}{\text{mol}}\right)} = 0.046 \text{ mol}$$

so the molarity is

$$[\text{NaCl}] = \frac{\text{mol NaCl}}{\text{L of seawater}} = \frac{0.046 \text{ mol}}{0.1 \text{ L}} = 0.46 \text{ M}$$

(b) The molecular mass of MgCl₂ is 24.30 (Mg) + [2 × 35.45] (Cl) = 95.20 g/mol, so the number of grams in 25 mL is

You can find atomic masses in the periodic table inside the front cover of this book. Physical constants such as Avogadro's number appear inside the back cover.

$$\text{grams of MgCl}_2 = 0.054 \frac{\text{mol}}{\text{L}} \times 95.20 \frac{\text{g}}{\text{mol}} \times (25 \times 10^{-3} \text{ L}) = 0.13 \text{ g}$$

 **Test Yourself** Sulfate (SO_4^{2-}) has a typical concentration of 0.038 M in seawater. Find the concentration of SO_4^{2-} in grams per 100 mL. (Answer: 0.37 g/100 mL)

Strong electrolyte: mostly dissociated into ions in solution

Weak electrolyte: partially dissociated into ions in solution

An **electrolyte** dissociates into ions in aqueous solution. Magnesium chloride is a **strong electrolyte**, which means that it is mostly dissociated into ions in most solutions. In seawater, about 89% of the magnesium is present as Mg^{2+} and 11% is found as the *complex ion*, MgCl^+ . The concentration of MgCl_2 molecules in seawater is close to 0. Sometimes the molarity of a strong electrolyte is referred to as **formal concentration** (F) to indicate that the substance is really converted to other species in solution. When we commonly, and inaccurately, say that the “concentration” of MgCl_2 is 0.054 M in seawater, we really mean that its formal concentration is 0.054 F. The “molecular mass” of a strong electrolyte is more properly called the **formula mass** (which we will abbreviate FM), because it is the sum of atomic masses in the formula, even though there may be few molecules with that formula in the solution.

For a **weak electrolyte** such as acetic acid, $\text{CH}_3\text{CO}_2\text{H}$, only a small portion of the molecules are split into ions in solution:

	Formal concentration	Percent dissociated
$\text{CH}_3\text{CO}_2\text{H}$ Acetic acid (undissociated)	0.1 F	1.3%
CH_3CO_2^- Acetate ion (dissociated)	0.01 F	4.1%
H^+	0.001 F	12.4%

A solution prepared by dissolving 0.010 00 mol of acetic acid in 1.000 L has a formal concentration of 0.010 00 F. The actual molarity of $\text{CH}_3\text{CO}_2\text{H}$ is 0.009 59 M because 4.1% is dissociated into CH_3CO_2^- and 95.9% remains as $\text{CH}_3\text{CO}_2\text{H}$. Nevertheless, we customarily say that the solution is 0.010 00 M acetic acid and understand that some of the acid is dissociated.

Molality (m) is a designation of concentration expressing the number of moles of a solute per kilogram of solvent (not total solution). The masses of solute and solvent do not change with temperature, as long as neither one is allowed to evaporate. Therefore molality does not change when temperature changes. By contrast, molarity changes with temperature because the volume of a solution usually increases when it is heated.

Percent Composition

The percentage of a component in a mixture or solution is usually expressed as a **weight percent** (wt%):

$$\text{Definition of weight percent: } \text{weight percent} = \frac{\text{mass of solute}}{\text{mass of total solution or mixture}} \times 100 \quad (1-1)$$

A common form of ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) is 95 wt%; it has 95 g of ethanol per 100 g of total solution. The remainder is water. Another common expression of composition is **volume percent** (vol%):

$$\text{Definition of volume percent: } \text{volume percent} = \frac{\text{volume of solute}}{\text{volume of total solution}} \times 100 \quad (1-2)$$

Although “wt%” or “vol%” should always be written to avoid ambiguity, wt% is usually implied when you see just “%.”

Example Converting Weight Percent to Molarity

Find the molarity of HCl in a reagent labeled “37.0 wt% HCl, density = 1.188 g/mL.” The **density** of a substance is the mass per unit volume.

SOLUTION We need to find the moles of HCl per liter of solution. To find moles of HCl, we need to find the mass of HCl. The mass of HCl in 1 L is 37.0% of the mass of 1 L of solution. The mass of 1 L of solution is $(1.188 \text{ g/mL}) \times (1000 \text{ mL/L}) = 1188 \text{ g/L}$. The mass of HCl in 1 L is

$$\text{HCl} \left(\frac{\text{g}}{\text{L}} \right) = 1188 \frac{\text{g solution}}{\text{L}} \times 0.370 \frac{\text{g HCl}}{\text{g solution}} = 439.6 \frac{\text{g HCl}}{\text{L}}$$

↑

This is what 37.0 wt% HCl means.

The molecular mass of HCl is 36.46 g/mol, so the molarity is

$$\text{molarity} = \frac{\text{mol HCl}}{\text{L solution}} = \frac{439.6 \text{ g HCl/L}}{36.46 \text{ g HCl/mol}} = 12.1 \frac{\text{mol}}{\text{L}} = 12.1 \text{ M}$$

 **Test Yourself** Phosphoric acid (H_3PO_4 , FM = formula mass = 97.99 g/mol) is commonly sold as an 85.5 wt% aqueous solution with a density of 1.69 g solution/mL. Find the molarity of H_3PO_4 . (Answer: Look inside the back cover for the molarity of 85.5 wt% H_3PO_4 .)

Parts per Million and Parts per Billion

Concentrations of trace components of a sample can be expressed as **parts per million** (ppm) or **parts per billion** (ppb), terms that mean grams of substance per million or billion grams of total solution or mixture.

$$\text{Definition of parts per million: } \text{ppm} = \frac{\text{mass of substance}}{\text{mass of sample}} \times 10^6 \quad (1-3)$$

$$\text{Definition of parts per billion: } \text{ppb} = \frac{\text{mass of substance}}{\text{mass of sample}} \times 10^9 \quad (1-4)$$

Masses must be expressed in the same units in the numerator and denominator.

The density of a dilute aqueous solution is close to 1.00 g/mL; so we frequently equate 1 g of water with 1 mL of water, although this equivalence is only approximate. Therefore 1 ppm corresponds to 1 $\mu\text{g/mL}$ (= 1 mg/L) and 1 ppb is 1 ng/mL (= 1 $\mu\text{g/L}$).

$$\text{density} = \frac{\text{mass}}{\text{volume}} = \frac{\text{g}}{\text{mL}}$$

A closely related dimensionless quantity is

$$\text{specific gravity} = \frac{\text{density of a substance}}{\text{density of water at } 4^\circ\text{C}}$$

Because the density of water at 4°C is very close to 1 g/mL, specific gravity is nearly the same as density.

A familiar analogy is percent, which is *parts per hundred*:

$$\text{parts per hundred} = \frac{\text{mass of substance}}{\text{mass of sample}} \times 100$$

Question What would be the definition of parts per trillion?

1 ppm \approx 1 $\mu\text{g/mL}$

1 ppb \approx 1 ng/mL

The symbol \approx is read “is approximately equal to.”

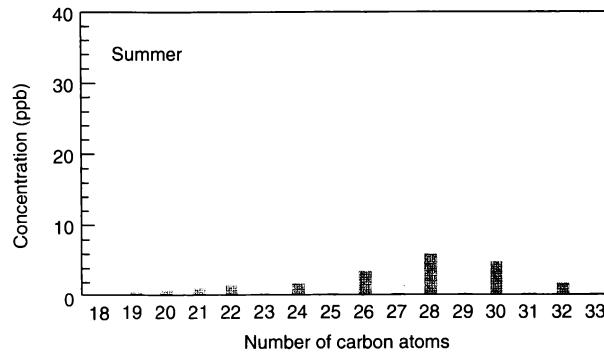
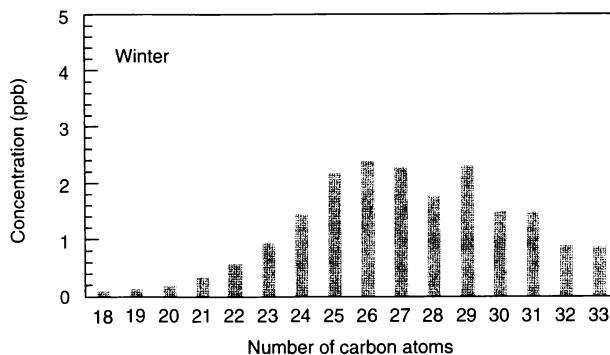


Figure 1-3 Concentrations of alkanes (hydrocarbons with the formula C_nH_{2n+2}) found in rainwater in Hannover, Germany, in the winter and summer in 1989 are measured in parts per billion ($= \mu\text{g hydrocarbon/L of rainwater}$). Summer concentrations are higher, and compounds with an odd number of carbon atoms (colored bars) predominate. Plants produce mainly hydrocarbons with an odd number of carbon atoms. [From K. Levesen, S. Behnert, and H. D. Winkeler, *Fresenius J. Anal. Chem.* **1991**, 340, 665.]

Example Converting Parts per Billion to Molarity

Hydrocarbons are compounds containing only hydrogen and carbon. Plants manufacture hydrocarbons as components of the membranes of cells and vesicles. The biosynthetic pathway leads mainly to compounds with an odd number of carbon atoms. Figure 1-3 shows the concentrations of hydrocarbons washed from the air by rain in the winter and summer. The preponderance of odd-number hydrocarbons in the summer suggests that the source is mainly from plants. The more uniform distribution of odd- and even-number hydrocarbons in the winter suggests a man-made origin. The concentration of $C_{29}H_{60}$ in summer rainwater is 34 ppb. Find the molarity of this compound in nanmoles per liter (nM).

SOLUTION A concentration of 34 ppb means $34 \times 10^{-9} \text{ g} (= 34 \text{ ng})$ of $C_{29}H_{60}$ per gram of rainwater, which we equate to 34 ng/mL. To find moles per liter, we first find grams per liter:

$$34 \times 10^{-9} \frac{\text{g}}{\text{mL}} \times \frac{1000 \text{ mL}}{\text{L}} = 34 \times 10^{-6} \frac{\text{g}}{\text{L}}$$

Because the molecular mass of $C_{29}H_{60}$ is 408.8 g/mol, the molarity is

$$\begin{aligned} \text{molarity of } C_{29}H_{60} \text{ in rainwater} &= \frac{34 \times 10^{-6} \text{ g/L}}{408.8 \text{ g/mol}} = 8.3 \times 10^{-8} \text{ M} \\ &= 83 \times 10^{-9} \text{ M} = 83 \text{ nM} \end{aligned}$$

Test Yourself The molarity of $C_{29}H_{60}$ in winter rainwater is 5.6 nM. Find the concentration in ppb. (Answer: 2.3 ppb)

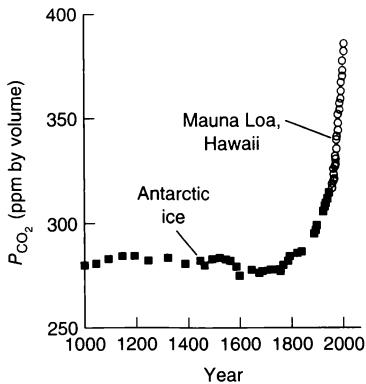


Figure 1-4 Thousand-year record of atmospheric CO_2 from measurements of CO_2 trapped in ice in Antarctica and from direct atmospheric measurements. [Data from D. M. Etheridge, L. P. Steele, R. L. Langenfelds, R. J. Francey, J.-M. Barnola, and V. I. Morgan, in *Trends: A Compendium of Data on Global Change*, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, TN, 1998 and C. D. Keeling and T. P. Whorf, Scripps Institution of Oceanography, and http://scrippsc02.ucsd.edu/data/in_situ_co2/monthly_mlo.csv.]

For gases, ppm usually refers to volume. For example, 300 ppm carbon dioxide in air means 300 μL of CO_2 per liter of air. Write units to avoid confusion. Figure 1-4 shows the increase in atmospheric CO_2 as a result of our burning fossil fuels (coal, oil, and natural gas) and destroying Earth's forests in the last 200 years. Increasing atmospheric CO_2 is acidifying the oceans (Box 11-1) and, possibly, altering Earth's climate.

Ask Yourself

- 1-C. The density of 70.5 wt% aqueous perchloric acid is 1.67 g/mL. Note that grams refers to grams of *solution* (= g HClO₄ + g H₂O).
- (a) How many grams of solution are in 1.00 L?
 - (b) How many grams of HClO₄ are in 1.00 L?
 - (c) How many moles of HClO₄ are in 1.00 L? This is the molarity.



1-4 Preparing Solutions

To prepare a solution with a desired molarity, weigh out the correct mass of pure reagent, dissolve it in solvent in a *volumetric flask* (Figure 1-5), dilute with more solvent to the desired final volume, and mix well by inverting the flask many times. A more complete description of the procedure is given in Section 2-5.

Example* Preparing a Solution with Desired Molarity

Cupric sulfate is commonly sold as the pentahydrate, CuSO₄·5H₂O, which has 5 moles of H₂O for each mole of CuSO₄ in the solid crystal. The formula mass of CuSO₄·5H₂O (= CuSO₄·9H₂O) is 249.69 g/mol. How many grams of CuSO₄·5H₂O should be dissolved in a 250-mL volumetric flask to make a solution containing 8.00 mM Cu²⁺?

SOLUTION An 8.00 mM solution contains 8.00×10^{-3} mol/L. Because 250 mL is 0.250 L, we need

$$8.00 \times 10^{-3} \frac{\text{mol}}{\text{L}} \times 0.250 \text{ L} = 2.00 \times 10^{-3} \text{ mol CuSO}_4 \cdot 5\text{H}_2\text{O}$$

The required mass of reagent is

$$(2.00 \times 10^{-3} \text{ mol}) \left(249.69 \frac{\text{g}}{\text{mol}} \right) = 0.499 \text{ g}$$

The procedure is to weigh 0.499 g of solid CuSO₄·5H₂O into a 250-mL volumetric flask, add about 200 mL of distilled water, and swirl to dissolve the reagent. Then dilute with distilled water up to the 250-mL mark and invert the stoppered flask many times to ensure complete mixing. The solution contains 8.00 mM Cu²⁺.

Test Yourself The anion EDTA⁴⁻ strongly binds metal ions with a charge ≥2. How many grams of the reagent Na₂H₂(EDTA)·2H₂O (FM 372.24 g/mol) should be dissolved in 0.500 L to give a 20.0 mM EDTA solution? What will be the molarity of Na⁺ in this solution? (Answer: 3.72 g, 40.0 mM)

Dilute solutions can be prepared from concentrated solutions. Typically, a desired volume or mass of the concentrated solution is transferred to a volumetric flask and diluted to the intended volume with solvent. The number of moles of reagent in V liters containing M moles per liter is the product $M \cdot V = (\text{mol/L})(\text{L}) = \text{mol}$. When a solution is diluted from a high concentration to a low concentration, the number of moles

Figure 1-5 A *volumetric flask* contains a specified volume (at 20°C) when the liquid level is adjusted to the middle of the mark in the thin neck of the flask. [Photograph courtesy A. H. Thomas Co., Philadelphia, PA.]

of solute is unchanged. Therefore we equate the number of moles in the concentrated (conc) and dilute (dil) solutions:

$$\text{Dilution formula: } M_{\text{conc}} \cdot V_{\text{conc}} = M_{\text{dil}} \cdot V_{\text{dil}} \quad (1-5)$$

Moles taken from
concentrated solution Moles placed in
dilute solution

Example Preparing 0.1 M HCl

The symbol \sim is read “approximately.”

The molarity of “concentrated” HCl purchased for laboratory use is ~ 12.1 M. How many milliliters of this reagent should be diluted to 1.00 L to make 0.100 M HCl?

SOLUTION The required volume of concentrated solution is found with Equation 1-5:

$$M_{\text{conc}} \cdot V_{\text{conc}} = M_{\text{dil}} \cdot V_{\text{dil}}$$

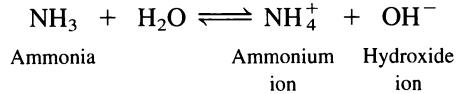
$$(12.1 \text{ M}) \cdot (x \text{ mL}) = (0.100 \text{ M}) \cdot (1000 \text{ mL}) \Rightarrow x = 8.26 \text{ mL}$$

It is all right to express both volumes in mL or both in L. The important point is to use the same units for volume on both sides of the equation so that the units cancel. To make 0.100 M HCl, place 8.26 mL of concentrated HCl in a 1-L volumetric flask and add ~ 900 mL of water. After swirling to mix, dilute to the 1-L mark with water and invert the flask many times to ensure complete mixing.

 **Test Yourself** Concentrated nitric acid has a molarity of ~ 15.8 M. How many milliliters should be used to prepare 1.00 L of 1.00 M HNO₃? (**Answer:** See inside the back cover of the book. Your answer could be slightly different because of round-off errors.)

Example A More Complicated Dilution Calculation

A solution of ammonia in water is called “ammonium hydroxide” because of the equilibrium



The density of concentrated ammonium hydroxide, which contains 28.0 wt% NH₃, is 0.899 g/mL. What volume of this reagent should be diluted to 500 mL to make 0.250 M NH₃?

SOLUTION To use Equation 1-5, we need to know the molarity of the concentrated reagent. The density tells us that the reagent contains 0.899 grams of solution per milliliter of solution. The weight percent tells us that the reagent contains 0.280 grams of NH₃ per gram of solution. To find the molarity of NH₃ in the concentrated reagent, we need to know the number of moles of NH₃ in 1 liter:

$$\text{grams of NH}_3 \text{ per liter} = 899 \frac{\text{g solution}}{\text{L}} \times 0.280 \frac{\text{g NH}_3}{\text{g solution}} = 252 \frac{\text{g NH}_3}{\text{L}}$$

$$\text{molarity of NH}_3 = \frac{252 \frac{\text{g NH}_3}{\text{L}}}{17.03 \frac{\text{g NH}_3}{\text{mol NH}_3}} = 14.8 \frac{\text{mol NH}_3}{\text{L}} = 14.8 \text{ M}$$

Now we use Equation 1-5 to find the volume of 14.8 M NH₃ required to prepare 500 mL of 0.250 M NH₃:

$$\begin{aligned} M_{\text{conc}} \cdot V_{\text{conc}} &= M_{\text{dil}} \cdot V_{\text{dil}} \\ 14.8 \frac{\text{mol}}{\text{L}} \times V_{\text{conc}} &= 0.250 \frac{\text{mol}}{\text{L}} \times 0.500 \text{ L} \\ \Rightarrow V_{\text{conc}} &= 8.45 \times 10^{-3} \text{ L} = 8.45 \text{ mL} \end{aligned}$$

The correct procedure is to place 8.45 mL of concentrated reagent in a 500-mL volumetric flask, add about 400 mL of water, and swirl to mix. Then dilute to exactly 500 mL with water and invert the stoppered flask many times to mix well.

 **Test Yourself** What volume of 28.0 wt% NH₃ should be diluted to 1.00 L to make 1.00 M NH₃? (Answer: See inside back cover of the book.)

Example Preparing a Parts per Million Concentration

Drinking water usually contains 1.6 ppm fluoride (F⁻) to help prevent tooth decay. Consider a reservoir with a diameter of 450 m and a depth of 10 m. (a) How many liters of 0.10 M NaF should be added to produce 1.6 ppm F⁻? (b) How many grams of solid NaF could be used instead?

SOLUTION (a) If we assume that the density of water in the reservoir is close to 1.00 g/mL, 1.6 ppm F⁻ corresponds to $1.6 \times 10^{-6} \text{ g F}^-/\text{mL}$ or

$$1.6 \times 10^{-6} \frac{\text{g F}^-}{\text{mL}} \times 1000 \frac{\text{mL}}{\text{L}} = 1.6 \times 10^{-3} \frac{\text{g F}^-}{\text{L}}$$

The atomic mass of fluorine is 19.00, so the desired molarity of fluoride in the reservoir is

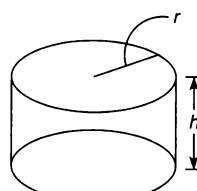
$$\text{desired } [\text{F}^-] \text{ in reservoir} = \frac{1.6 \times 10^{-3} \frac{\text{g F}^-}{\text{L}}}{19.00 \frac{\text{g F}^-}{\text{mol}}} = 8.42 \times 10^{-5} \text{ M}$$

The volume of the reservoir is $\pi r^2 h$, where r is the radius and h is the height.

$$\text{volume of reservoir} = \pi \times (225 \text{ m})^2 \times 10 \text{ m} = 1.59 \times 10^6 \text{ m}^3$$

To use the dilution formula, we need to express volume in liters. Table 1-4 told us that there are 1 000 L in 1 cubic meter. Therefore the volume of the reservoir in liters is

$$\text{volume of reservoir (L)} = 1.59 \times 10^6 \frac{\text{m}^3}{\text{m}^3} \times 1000 \frac{\text{L}}{\text{m}^3} = 1.59 \times 10^9 \text{ L}$$



$$\begin{aligned} \text{volume of cylinder} &= \text{end area} \times \text{height} \\ &= \pi r^2 h \end{aligned}$$

Finally, we are in a position to use the dilution formula 1-5:

$$M_{\text{conc}} \cdot V_{\text{conc}} = M_{\text{dil}} \cdot V_{\text{dil}}$$

$$0.10 \frac{\text{mol}}{\text{L}} \times V_{\text{conc}} = \left(8.42 \times 10^{-5} \frac{\text{mol}}{\text{L}} \right) \times (1.59 \times 10^9 \text{ L})$$
$$\Rightarrow V_{\text{conc}} = 1.3 \times 10^6 \text{ L}$$

We require 1.3 million liters of 0.10 M F^- . Note that our calculation assumed that the final volume of the reservoir is $1.59 \times 10^9 \text{ L}$. Even though we are adding more than 10^6 L of reagent, this amount is small relative to 10^9 L . Therefore the approximation that the reservoir volume remains $1.59 \times 10^9 \text{ L}$ is pretty good.

(b) The number of moles of F^- in the reservoir is $(1.59 \times 10^9 \text{ L}) \times (8.42 \times 10^{-5} \text{ mol/L}) = 1.34 \times 10^5 \text{ mol F}^-$. Because 1 mole of NaF provides 1 mole of F^- , we need $(1.34 \times 10^5 \text{ mol NaF}) \times (41.99 \text{ g NaF/mol NaF}) = 5.6 \times 10^6 \text{ grams of NaF}$.

 **Test Yourself** If the diameter of the reservoir is doubled to 900 m, how many metric tons of NaF are required? A metric ton is 1 000 kg. (**Answer:** 22 metric tons)

Ask Yourself

- 1-D. A 48.0 wt% solution of HBr in water has a density of 1.50 g/mL.
(a) How many grams of solution are in 1.00 L?
(b) How many grams of HBr are in 1.00 L?
(c) What is the molarity of HBr?
(d) How much solution is required to prepare 0.250 L of 0.160 M HBr?

1-5 The Equilibrium Constant

At equilibrium, the rates of the forward reaction



and the reverse reaction



are equal.

The equilibrium constant is more correctly expressed as a ratio of *activities* rather than of concentrations. See Section 12-2.

Equation 1-7, also called the *law of mass action*, was formulated by the Norwegians C. M. Guldenberg and P. Waage and published in 1864. Their derivation was based on the idea that the forward and reverse rates of a reaction at equilibrium must be equal.

Equilibrium describes the state that a system will reach “if you wait long enough.” Most reactions of interest in analytical chemistry reach equilibrium in times ranging from fractions of a second to many minutes.

If the reactants A and B are converted to products C and D with the stoichiometry



we write the **equilibrium constant**, *K*, in the form

$$\text{Equilibrium constant:} \quad K = \frac{[C]^c[D]^d}{[A]^a[B]^b} \quad (1-7)$$

where the small superscript letters denote stoichiometric coefficients and each capital letter stands for a chemical species. The symbol [A] stands for the concentration of A relative to its standard state (defined below). We say that a reaction is favored whenever *K* > 1.

In deriving the equilibrium constant, each quantity in Equation 1-7 is expressed as the *ratio* of the concentration of a species to its concentration in its *standard state*.

For solutes, the standard state is 1 M. For gases, the standard state is 1 bar, which is close to 1 atmosphere (Table 1-4). For solids and liquids, the standard states are the pure solid or liquid. It is understood (but rarely written) that the term [A] in Equation 1-7 really means [A]/(1 M) if A is a solute. If D is a gas, [D] really means (pressure of D in bars)/(1 bar). To emphasize that [D] means pressure of D, we usually write P_D in place of [D]. If C were a pure liquid or solid, the ratio [C]/(concentration of C in its standard state) would be unity (1) because the standard state is the pure liquid or solid. If [C] is a solvent, the concentration is so close to that of pure liquid C that the value of [C] is essentially 1. Each term of Equation 1-7 is dimensionless because each is a ratio in which the units cancel; therefore *all equilibrium constants are dimensionless*.

$$1 \text{ bar} = 10^5 \text{ Pa} \approx 0.987 \text{ atm}$$

The take-home lesson is this: To evaluate an equilibrium constant,

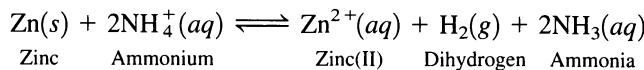
1. Concentrations of solutes should be expressed as moles per liter.
2. Concentrations of gases should be expressed in bars.
3. Concentrations of pure solids, pure liquids, and solvents are omitted because they are unity.

These conventions are arbitrary, but you must use them so that your results are consistent with tabulated values of equilibrium constants and standard reduction potentials.

Equilibrium constants are dimensionless; but, when specifying concentrations, you must use units of molarity (M) for solutes and bars for gases.

Example Writing an Equilibrium Constant

Write the equilibrium constant for the reaction



(In chemical equations, *s* stands for solid, *aq* stands for aqueous, *g* stands for gas, and *l* stands for liquid.)

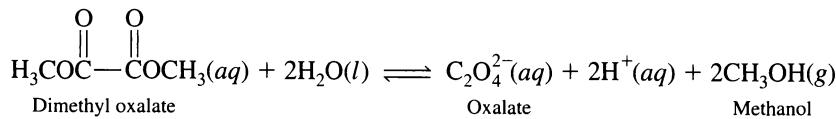
SOLUTION Omit the concentration of pure solid and express the concentration of gas as a pressure in bars:

$$K = \frac{[\text{Zn}^{2+}]P_{\text{H}_2}[\text{NH}_3]^2}{[\text{NH}_4^+]^2}$$

P_{H_2} stands for the pressure of $\text{H}_2(g)$ in bars.



Test Yourself Write the equilibrium constant for the reaction

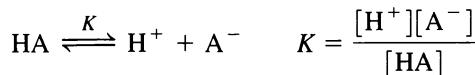


$$\left(\text{Answer: } K = \frac{[\text{C}_2\text{O}_4^{2-}][\text{H}^+]^2 P_{\text{CH}_3\text{OH}}^2}{[\text{dimethyl oxalate}]} \right)$$

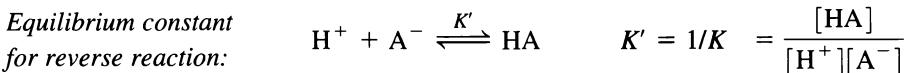
Manipulating Equilibrium Constants

Throughout this text, you should assume that all species in chemical equations are in aqueous solution, unless otherwise specified.

Consider the reaction of the acid HA that dissociates into H⁺ and A⁻:

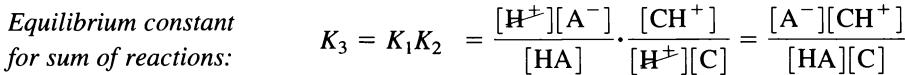
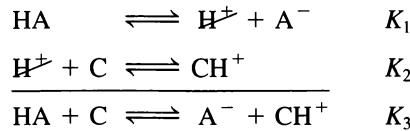


If the reverse reaction is written, the new K' is the reciprocal of the original K:



If a reaction is reversed, then K' = 1/K. If two reactions are added, then K₃ = K₁K₂.

If reactions are added, the new K is the product of the original K's. The equilibrium of H⁺ between the species HA and CH⁺ can be derived by adding two equations:

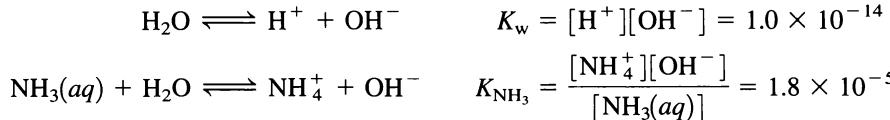


If n reactions are added, the overall equilibrium constant is the product of all n individual equilibrium constants.

Example Combining Equilibrium Constants

From the equilibria

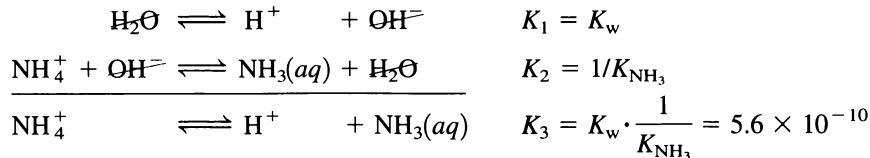
H₂O is omitted from K because it is a pure liquid. Its concentration remains nearly constant.



find the equilibrium constant for the reaction



SOLUTION The third reaction is obtained by reversing the second reaction and adding it to the first reaction:

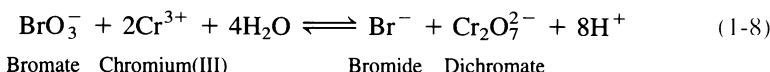


 **Test Yourself** From the reactions $\text{NH}_3(aq) + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-$ ($K_{\text{NH}_3} = 1.8 \times 10^{-5}$) and $\text{CH}_3\text{NH}_2(aq) + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{NH}_3^+ + \text{OH}^-$ ($K_{\text{CH}_3\text{NH}_2} = 4.5 \times 10^{-4}$), find the equilibrium constant for the reaction $\text{CH}_3\text{NH}_2(aq) + \text{NH}_4^+ \rightleftharpoons \text{CH}_3\text{NH}_3^+ + \text{NH}_3(aq)$. (Answer: K = 25)

Le Châtelier's Principle

Le Châtelier's principle states that if a system at equilibrium is disturbed, the direction in which the system proceeds back to equilibrium is such that the disturbance is partly offset.

Let's see what happens when we change the concentration of one species in the reaction:



for which the equilibrium constant is

$$K = \frac{[\text{Br}^-][\text{Cr}_2\text{O}_7^{2-}][\text{H}^+]^8}{[\text{BrO}_3^-][\text{Cr}^{3+}]^2} = 1 \times 10^{11} \text{ at } 25^\circ \text{ C}$$

H_2O is omitted from K because it is the solvent. Its concentration remains nearly constant.

In one particular equilibrium state of this system, the following concentrations exist:

$$[\text{H}^+] = 5.0 \text{ M} \quad [\text{Cr}_2\text{O}_7^{2-}] = 0.10 \text{ M} \quad [\text{Cr}^{3+}] = 0.0030 \text{ M}$$

$$[\text{Br}^-] = 1.0 \text{ M} \quad [\text{BrO}_3^-] = 0.043 \text{ M}$$

Suppose that equilibrium is disturbed by increasing the concentration of dichromate from 0.10 to 0.20 M. In what direction will the reaction proceed to reach equilibrium?

According to the principle of Le Châtelier, the reaction should go in the reverse direction to partly offset the increase in dichromate, which is a product in Reaction 1-8. We can verify this algebraically by setting up the *reaction quotient*, Q , which has the same form as the equilibrium constant. The only difference is that Q is evaluated with whatever concentrations happen to exist, even though the solution is not at equilibrium. When the system reaches equilibrium, $Q = K$. For Reaction 1-8,

$$Q = \frac{(1.0)(0.20)(5.0)^8}{(0.043)(0.0030)^2} = 2 \times 10^{11} > K$$

Q has the same form as K , but the concentrations are generally not the equilibrium concentrations.

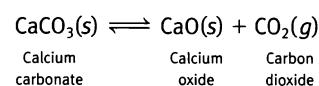
Because $Q > K$, the reaction must go in reverse to decrease the numerator and increase the denominator, until $Q = K$.

In general,

1. If a reaction is at equilibrium and products that appear in the reaction quotient are added (or reactants that appear in the reaction quotient are removed), the reaction goes in the reverse direction (to the left).
 2. If a reaction is at equilibrium and reactants that appear in the reaction quotient are added (or products that appear in the reaction quotient are removed), the reaction goes in the forward direction (to the right).

In equilibrium problems, we predict what must happen for a system to reach equilibrium, but not how long it will take. Some reactions are over in an instant;

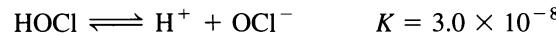
A species must appear in the reaction quotient to affect the equilibrium. If solid CaO is present in the reaction below, adding more CaO does not consume $\text{CO}_2(g)$.



others do not reach equilibrium in a million years. Dynamite remains unchanged indefinitely, until a spark sets off the spontaneous, explosive decomposition. The size of an equilibrium constant tells us nothing about the rate of reaction. A large equilibrium constant does not imply that a reaction is fast.

Ask Yourself

1-E. (a) Show how the following equations can be rearranged and added to give the reaction $\text{HOBr} \rightleftharpoons \text{H}^+ + \text{OBr}^-$:



(b) Find the value of K for the reaction $\text{HOBr} \rightleftharpoons \text{H}^+ + \text{OBr}^-$.

(c) If the reaction $\text{HOBr} \rightleftharpoons \text{H}^+ + \text{OBr}^-$ is at equilibrium and a substance is added that consumes H^+ , will the reaction proceed in the forward or reverse direction to reestablish equilibrium?

Key Equations

Molarity (M)

$$[\text{A}] = \frac{\text{moles of solute A}}{\text{liters of solution}}$$

Weight percent

$$\text{wt\%} = \frac{\text{mass of solute}}{\text{mass of solution or mixture}} \times 100$$

Volume percent

$$\text{vol\%} = \frac{\text{volume of solute}}{\text{volume of solution or mixture}} \times 100$$

Density

$$\text{density} = \frac{\text{grams of substance}}{\text{milliliters of substance}}$$

Parts per million

$$\text{ppm} = \frac{\text{mass of substance}}{\text{mass of sample}} \times 10^6$$

Parts per billion

$$\text{ppb} = \frac{\text{mass of substance}}{\text{mass of sample}} \times 10^9$$

Dilution formula

$$\text{M}_{\text{conc}} \cdot V_{\text{conc}} = \text{M}_{\text{dil}} \cdot V_{\text{dil}}$$

M_{conc} = concentration (molarity) of concentrated solution

M_{dil} = concentration of dilute solution

V_{conc} = volume of concentrated solution

V_{dil} = volume of dilute solution

Equilibrium constant

$$a\text{A} + b\text{B} \xrightleftharpoons{K} c\text{C} + d\text{D} \quad K = \frac{[\text{C}]^c [\text{D}]^d}{[\text{A}]^a [\text{B}]^b}$$

Concentrations of solutes are in M and gases are in bars.

Omit solvents and pure solids and liquids.

Reversed reaction

$$K' = 1/K$$

Add two reactions

$$K_3 = K_1 K_2$$

Le Châtelier's principle

1. Adding product (or removing reactant) drives reaction in reverse.

2. Adding reactant (or removing product) drives reaction forward.

Important Terms

atomic mass concentration	Le Châtelier's principle	order of magnitude	strong electrolyte
density	liter	parts per billion	volume percent
equilibrium constant	molality	parts per million	weak electrolyte
formal concentration	molarity	SI units	weight percent
formula mass	mole	solute	
	molecular mass	solvent	

Problems

1-1. (a) List the SI units of length, mass, time, electric current, temperature, and amount of substance. Write the abbreviation for each.

(b) Write the units and symbols for frequency, force, pressure, energy, and power.

1-2. Write the name and number represented by each abbreviation. For example, for kW you should write kW = kilowatt = 10^3 watts.

- (a) mW (c) kΩ (e) TJ (g) fg
(b) pm (d) μC (f) ns (h) dPa

1-3. Express the following quantities with abbreviations for units and prefixes from Tables 1-1 through 1-3:

- (a) 10^{-13} joules (d) 10^{-10} meters
(b) $4.317\ 28 \times 10^{-8}$ coulombs (e) 2.1×10^{13} watts
(c) $2.997\ 9 \times 10^{14}$ hertz (f) 48.3×10^{-20} moles

1-4. Table 1-4 states that 1 horsepower = 745.700 watts. Consider a 100.0-horsepower engine. Express its power output in (a) watts; (b) joules per second; (c) calories per second; (d) calories per hour.

1-5. (a) Refer to Table 1-4 and calculate how many meters are in 1 inch. How many inches are in 1 m?

(b) A mile is 5 280 feet and a foot is 12 inches. The speed of sound in the atmosphere at sea level is 345 m/s. Express the speed of sound in miles per second and miles per hour.

(c) There is a delay between lightning and thunder in a storm, because light reaches us almost instantaneously, but sound is slower. How many meters, kilometers, and miles away is lightning if the sound reaches you 3.00 s after the light?

1-6. Scientists at the U.S. National Institute of Standards and Technology measured a force of 174 yN exerted by a weak electric field on a vibrating “crystal” of $60\ ^9\text{Be}^+$ ions cooled to 0.5 mK in an electromagnetic trap. (M. J. Biercuk et al., arXiv.org:1004.0780v3 [quant-ph] 24 Apr 2010.)

(a) Express the force in newtons in scientific notation.

(b) Express the temperature in kelvins in scientific notation.

(c) The gravitational force on a mass m at Earth’s surface is $F = GMm/r^2$, where G is the gravitational constant (inside back cover), M is the mass of the Earth (5.98×10^{24} kg), and r is the radius of the Earth (6.38×10^6 m). Find the mass of 60 Be^+ ions in kg. Find the gravitational force on 60 Be^+ ions in N and yN.

1-7. Define the following measures of concentration:

- (a) molarity (e) volume percent
(b) molality (f) parts per million
(c) density (g) parts per billion
(d) weight percent (h) formal concentration

1-8. What is the formal concentration (expressed as mol/L = M) of NaCl when 32.0 g are dissolved in water and diluted to 0.500 L?

1-9. For 0.250 L of aqueous solution with a density of 1.00 g/mL containing 13.7 μg of pesticide, express the concentration of pesticide in ppm and ppb.

1-10. The concentration of the sugar glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) in human blood ranges from about 80 mg/dL before meals to 120 mg/dL after eating. Find the molarity of glucose in blood before and after eating.

1-11. Hot perchloric acid is a powerful (and potentially explosive) reagent used to decompose organic materials and dissolve them for chemical analysis.

(a) How many grams of perchloric acid, HClO_4 , are contained in 100.0 g of 70.5 wt% aqueous perchloric acid?

(b) How many grams of water are in 100.0 g of solution?

(c) How many moles of HClO_4 are in 100.0 g of solution?

1-12. How many grams of boric acid [$\text{B}(\text{OH})_3$, FM 61.83] should be used to make 2.00 L of 0.050 0 M solution?

1-13. Water is fluoridated to prevent tooth decay.

(a) How many liters of 1.0 M H_2SiF_6 should be added to a reservoir with a diameter of 100 m and a depth of 20 m to give 1.2 ppm F^- ? (1 mol H_2SiF_6 provides 6 mol F.)

(b) How many grams of solid H_2SiF_6 should be added to the same reservoir to give 1.2 ppm F^- ?

1-14. How many grams of 50 wt% NaOH (FM 40.00) should be diluted to 1.00 L to make 0.10 M NaOH?

1-15. A bottle of concentrated aqueous sulfuric acid, labeled 98.0 wt% H₂SO₄, has a concentration of 18.0 M.

(a) How many milliliters of reagent should be diluted to 1.00 L to give 1.00 M H₂SO₄?

(b) Calculate the density of 98.0 wt% H₂SO₄.

1-16. How many grams of methanol (CH₃OH, FM 32.04) are contained in 0.100 L of 1.71 M aqueous methanol?

1-17. A dilute aqueous solution containing 1 ppm of solute has a density of 1.00 g/mL. Express the concentration of solute in g/L, µg/L, µg/mL, and mg/L.

1-18. The concentration of C₂₀H₄₂ (FM 282.55) in winter rainwater in Figure 1-3 is 0.2 ppb. Assuming that the density of rainwater is close to 1.00 g/mL, find the molar concentration of C₂₀H₄₂.

1-19. I have always enjoyed eating tuna fish. Unfortunately, a study of the mercury content of canned tuna in 2010 found that chunk white tuna contains 0.6 ppm Hg and chunk light tuna contains 0.14 ppm. (S. L. Gerstenberger, A. Martinson, and J. L. Kramer, *Environ. Toxicol. Chem.* **2010**, 29, 237.) The U.S. Environmental Protection Agency recommends no more than 0.1 µg Hg/kg body weight per day. I weigh 68 kg. How often may I eat a can containing 6 ounces (1 lb = 16 oz) of chunk white tuna so that I do not average more than 0.1 µg Hg/kg body weight per day? If I switch to chunk light tuna, how often may I eat one can?

1-20. A 95.0 wt% solution of ethanol (CH₃CH₂OH, FM 46.07) in water has a density of 0.804 g/mL.

(a) Find the mass of 1.00 L of this solution and the grams of ethanol per liter.

(b) What is the molar concentration of ethanol in this solution?

1-21. (a) How many grams of nickel are contained in 10.0 g of a 10.2 wt% solution of nickel sulfate hexahydrate, NiSO₄ · 6H₂O (FM 262.85)?

(b) The concentration of this solution is 0.412 M. Find its density.

1-22. A 500.0-mL solution was prepared by dissolving 25.00 mL of methanol (CH₃OH, density = 0.7914 g/mL) in chloroform. Find the molarity of the methanol.

1-23. Describe how to prepare exactly 100 mL of 1.00 M HCl from 12.1 M HCl reagent.

1-24. Cesium chloride is used to prepare dense solutions required for isolating cellular components with a centrifuge. A 40.0 wt% solution of CsCl (FM 168.36) has a density of 1.43 g/mL.

(a) Find the molarity of CsCl.

(b) How many milliliters of the concentrated solution should be diluted to 500 mL to make 0.100 M CsCl?

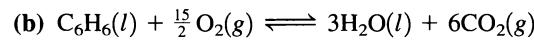
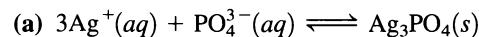
1-25. Protein and carbohydrates provide 4.0 Cal/g, whereas fat gives 9.0 Cal/g. (Remember that 1 Calorie, with a capital C, is really 1 kcal.) The weight percent of these components in some foods are

Food	Protein (wt%)	Carbohydrate (wt%)	Fat (wt%)
Shredded Wheat	9.9	79.9	—
Doughnut	4.6	51.4	18.6
Hamburger (cooked)	24.2	—	20.3
Apple	—	12.0	—

Calculate the number of calories per gram and calories per ounce in each of these foods. (Use Table 1-4 to convert grams into ounces, remembering that there are 16 ounces in 1 pound.)

1-26. Even though we need to express concentrations of solutes in mol/L and the concentrations of gases in bars, why do we say that equilibrium constants are dimensionless?

1-27. Write the expression for the equilibrium constant for each of the following reactions. Write the pressure of a gaseous molecule, X, as P_X.



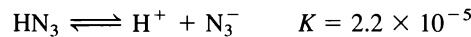
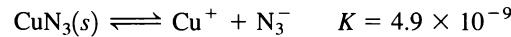
1-28. For the reaction 2A(g) + B(aq) + 3C(l) ⇌ D(s) + 3E(g), the concentrations at equilibrium are P_A = 2.8 × 10³ Pa, [B] = 1.2 × 10⁻² M, [C] = 12.8 M, [D] = 16.5 M, and P_E = 3.6 × 10⁴ Torr. (760 Torr = 1 atm)

(a) Gas pressure should be expressed in bars when writing the equilibrium constant. Express the pressures of A and E in bars.

(b) Find the numerical value of the equilibrium constant that would appear in a table of equilibrium constants.

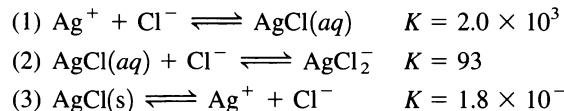
1-29. Suppose that the reaction Br₂(l) + I₂(s) + 4Cl⁻(aq) ⇌ 2Br⁻(aq) + 2ICl₂⁻(aq) has come to equilibrium. If more I₂(s) is added, will the concentration of ICl₂⁻ in the aqueous phase increase, decrease, or remain unchanged?

1-30. From the reactions



find the value of K for the reaction Cu⁺ + HN₃ ⇌ CuN₃(s) + H⁺.

1-31. Consider the following equilibria in aqueous solution:



- (a) Find K for the reaction $\text{AgCl}(s) \rightleftharpoons \text{AgCl}(aq)$. The species $\text{AgCl}(aq)$ is an *ion pair* consisting of Ag^+ and Cl^- associated with each other in solution.
- (b) Find $[\text{AgCl}(aq)]$ in equilibrium with excess $\text{AgCl}(s)$.
- (c) Find K for $\text{AgCl}_2^- \rightleftharpoons \text{AgCl}(s) + \text{Cl}^-$.

How Would You Do It?

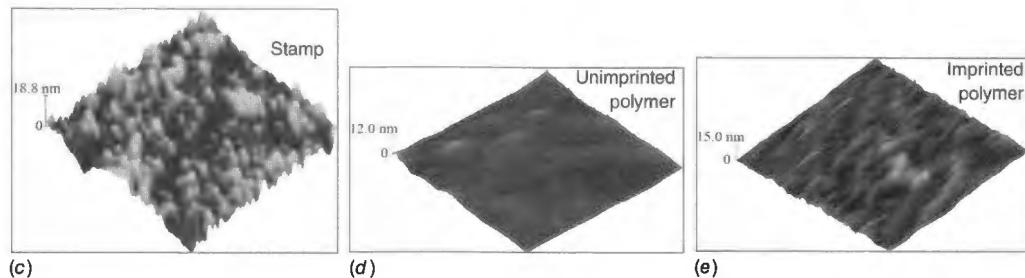
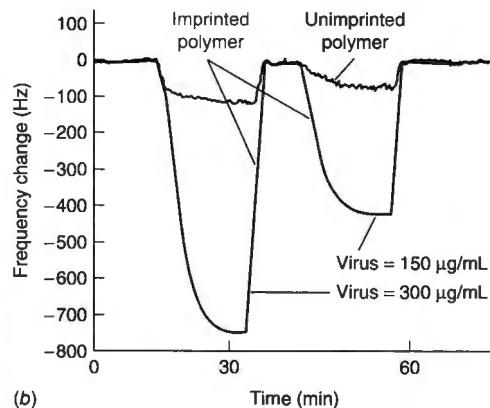
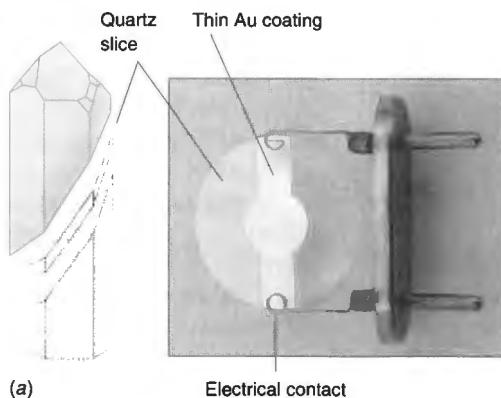
1-32.  The ocean is a *heterogeneous* fluid with different concentrations of zinc at different depths, as shown in Figure 1-2. Suppose that you want to know how much zinc is contained

in an imaginary cylinder of ocean water that is 1 m in diameter and 2 000 m deep. How would you construct a representative sample to measure the average concentration of zinc in the cylinder?

1-33. Chemical characteristics of the Naugatuck River in Connecticut were monitored by students from Sacred Heart University (J. Clark and E. Alkhatab, *Am. Environ. Lab.*, February 1999, p. 421.) The mean flow of the river is 560 cubic feet per second. The concentration of nitrate anion (NO_3^-) in the river was reported to range from 2.05 to 2.50 milligrams of nitrate nitrogen per liter during dry weather and from 0.81 to 4.01 mg nitrate nitrogen per liter during wet weather at the outlet of the river. (The unit “mg nitrate nitrogen” refers to the mass of nitrogen in the nitrate anion.) Estimate how many metric tons of nitrate anion per year flow from the river (1 metric ton = 1 000 kg). State your assumptions.

Catching a Cold with a Quartz Crystal Microbalance

(a) Quartz crystal oscillator with gold electrodes on front and back faces. (b) Response of the electrode to rhinovirus when the electrodes are coated with a polymer imprinted by the virus. (c) Topography of virus on a glass surface visualized with an atomic force microscope. (d) Topography of smooth, unimprinted polymer. (e) Topography of polymer after it has been imprinted by “stamp.” [From M. Jenik, R. Schirhagl, C. Schirk, O. Hayden, P. Lieberzeit, D. Blaas, G. Paul, and F. L. Dickert, *Anal. Chem.* 2009, 81, 5320.]



Cryoelectron microscopy image of rhinovirus 14, showing icosahedral protein shell. [Dr. Timothy Baker, N. Olson, T. J. Smith/Visuals Unlimited.]



A microbalance measures small masses such as micrograms and nanograms. Quartz crystals like the one that keeps time in your watch provide a means to measure small masses. A flat crystal is set into oscillation by a 10-MHz radio-frequency field applied to gold electrodes on the crystal faces (panel a). When a substance is *adsorbed* (bound) on the electrode surface, the vibrational frequency of the crystal decreases in proportion to the adsorbed mass (panel b). The change in vibrational frequency tells us how much mass is bound.

Human rhinovirus, which causes the common cold, consists of a 30-nm-diameter protein shell (the *capsid*) enclosing a ribonucleic acid (RNA) genome. A monolayer of virus particles is attached to a glass surface (panel c) to make a “stamp” that will be used to imprint the form of the virus into a soft polymer in the next step. The stamp is pressed into a smooth, partially polymerized organic layer on the gold electrode (panel d), and the polymer is allowed to harden. When the stamp is removed and virus is washed away, the polymer is left with indentations matching the shape of the virus (panel e). Polymer functional groups are positioned to recognize the virus by interactions such as hydrogen bonding.

When a solution containing rhinovirus is passed over the sensor, virus binds reversibly to the imprinted polymer and lowers the vibrational frequency of the crystal. The sensor is selective for different varieties of the virus with different surface chemistry.

Tools of the Trade

Analytical chemists use a range of equipment, from simple glassware to complex instruments that measure spectroscopic or electrical properties of analytes. The object to be analyzed might be as large as a vein of ore in a mountainside or as small as a vesicle inside a living cell. This course should expose you to some of the instrumental techniques of modern analytical chemistry. Along the way, you must gain some understanding and proficiency in “wet” laboratory operations with simple glassware. *The most sophisticated instruments are useless if you cannot prepare accurate standards for calibration or accurate, representative samples of an unknown for analysis.* This chapter describes basic laboratory apparatus and manipulations associated with chemical measurements.

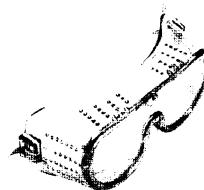


Figure 2-1 Goggles or safety glasses with side shields are required in every laboratory.

2-1 Safety, Waste Disposal, and Green Chemistry

The primary safety rule is to do nothing that you (or your instructor) consider to be dangerous. If you believe that an operation is hazardous, discuss it with your instructor and do not proceed until sensible procedures and precautions are in place. If you still consider an activity to be too dangerous, don’t do it.

Before beginning work, you should be familiar with safety precautions appropriate to your laboratory.¹ Wear goggles (Figure 2-1) or safety glasses with side shields at all times in the lab to protect yourself from flying chemicals and glass. Even if you are very careful, one of your neighbors may be more accident prone. Wear a flame-resistant lab coat, long pants, and shoes that cover your feet to protect yourself from spills and flames. Rubber gloves can protect you when pouring concentrated acids, but organic solvents can penetrate rubber. Food and chemicals should not mix: Don’t bring food or drink into the lab.

Treat chemical spills on your skin *immediately* by flooding the affected area with water and then seek medical attention. Clean up spills on the bench, floor, or reagent bottles immediately to prevent accidental contact by the next person who comes along.

Solvents and concentrated acids that produce harmful fumes should be handled in a fume hood that sweeps vapors away from you and out through a vent on the roof. The hood is not meant to transfer toxic vapors from the chemistry building to the cafeteria. Never generate large quantities of toxic fumes in the hood. If you use a toxic gas in a fume hood, bubble excess gas through a chemical trap or burn it in a flame to prevent its escape from the hood.

Label every vessel to show what it contains. Without labels, you will forget what is in some containers. Unlabeled waste is extremely expensive to discard, because

Why we wear lab coats: In 2008, 23-year-old University of California Los Angeles research assistant Sheharbano Sangji was withdrawing t-butyllithium solution with a syringe from a bottle. She was not wearing a lab coat. The plunger came out of the syringe and the pyrophoric liquid burst into flames, igniting her sweater and gloves. Burns on 40% of her body proved fatal. A flame-resistant lab coat might have protected her.

Limitations of gloves: In 1997, popular Dartmouth College chemistry professor Karen Wetterhahn, age 48, died from a drop of dimethylmercury absorbed through the latex rubber gloves she was wearing. Many organic compounds readily penetrate rubber. Wetterhahn was an expert in the biochemistry of metals and the first female professor of chemistry at Dartmouth. She had two children and played a major role in bringing more women into science and engineering.

you must analyze the contents before you can legally dispose of it. Chemically incompatible wastes should never be mixed.

If we want our grandchildren to inherit a habitable planet, we need to minimize waste production and dispose of chemical waste in a responsible manner.² When it is economically feasible, recycling of chemicals is preferable to waste disposal. Carcinogenic dichromate ($\text{Cr}_2\text{O}_7^{2-}$) waste provides an example of an accepted disposal strategy. Cr(VI) from dichromate should be reduced to less toxic Cr(III) with sodium hydrogen sulfite (NaHSO_3) and precipitated with hydroxide as $\text{Cr}(\text{OH})_3$. The liquid is evaporated and the solid is discarded in an approved landfill that is lined to prevent escape of the chemicals. Wastes such as silver and gold that can be economically recycled should be chemically treated to recover metal.

Green chemistry is a set of principles intended to change our behavior to help sustain a habitable planet.³ Examples of unsustainable behavior are consumption of a limited resource and careless disposal of waste. Green chemistry seeks to design chemical products and processes to reduce the use of resources and energy and the generation of hazardous waste. It is better to design a process to prevent waste than to dispose of waste. For example, NH_3 can be measured with an ion-selective electrode instead of using the spectrophotometric Nessler procedure, which generates HgI_2 waste. Use of “microscale” classroom experiments is encouraged to reduce the cost of reagents and the generation of waste.

2-2 Your Lab Notebook

The lab notebook must

1. State what was done
2. State what was observed
3. Be understandable to someone else

Without a doubt, somebody reading this book today is going to make an important discovery in the future and will seek a patent. The lab notebook is your legal record of your discovery. Therefore each notebook page should be signed and dated. Anything of potential importance should also be signed and dated by a second person.

Do not rely on a computer for long-term storage of information. Even if a file survives, software or hardware required to read the file will become obsolete.

The critical functions of your lab notebook are to state *what you did* and *what you observed*, and it should be *understandable by a stranger* who is trained in your discipline (chemistry in your case). The greatest error is writing ambiguous notes. After a few years, you may not be able to interpret your own notebook when memories of the experiment have faded. Writing in *complete sentences* is an excellent way to reduce this problem. Box 2-1 gives an example.

The measure of scientific “truth” is the ability to reproduce an experiment. A good lab notebook will allow you or anyone else to duplicate an experiment in the exact manner in which it was conducted the first time.

Beginning students find it useful (or required!) to write a complete description of an experiment, with sections describing the purpose, methods, results, and conclusions. Arranging your notebook to accept numerical data prior to coming to the lab is an excellent way to prepare for an experiment.

It is good practice to write a balanced chemical equation for every reaction that you use. This preparation helps you understand what you are doing and may point out what you do not understand.

Record in your notebook the names of computer files where programs and data are stored. *Printed copies* of important data collected on a computer should be pasted into your notebook. The lifetime of a printed page is 10 to 100 times greater than that of a computer file.



Ask Yourself

2-A. What are the three essential attributes of a lab notebook?

Box 2-1 Dan's Lab Notebook Entry

Your lab notebook should (1) state what was done, (2) state what was observed, and (3) be understandable to someone else who is trained in your discipline. The passage below was extracted in 2002 from my notebook of 1974 when, as a “postdoc” at Albert Einstein College of Medicine, I began to isolate the iron storage

protein ferritin. The complete procedure, in which protein was isolated and its purity assessed, occupied 3 weeks and 17 notebook pages. Phrases in brackets were added to help you understand the passage. I do not doubt that you can improve this description.

14 Sept 1974

Isolation of Human Spleen Ferritin

Based on R. R. Crichton *et al.*, *Biochem. J.* **131**, 51 (1973).

Procedure: Mince and homogenize spleen in ~4 vol H₂O
Heat to 70° for 5 min and cool on ice
Centrifuge at 3300 × g for 20 min
Filter through filter paper
Precipitate with 50% (NH₄)₂SO₄ (= 313 g solid/L solution)
Centrifuge at 3300 × g for 20 min
Dissolve in H₂O and dialyze vs. 0.1 M Tris, pH 8
Chromatograph on Sepharose 6B

Today's procedure started with a frozen 41 g human half spleen thawed overnight at 4°. The spleen was healthy and taken from an autopsy about a month ago. The spleen was blended 2 min on the high setting of the Waring blender in a total volume of ~250 mL. Try a smaller volume next time. The mixture was heated to 70–73° in a preheated water bath. It took ~5 min to attain 70° and the sample was then left at that temperature with intermittent stirring for 5 min. It was then cooled in an ice bath to ~10° before centrifugation in the cold at 3300 × g for 20 min (GSA head—4500 rpm). The red supernatant was filtered through Whatman #1 filter paper to give 218 mL solution, pH 6.4. The pH was raised to 7.5 with 10 M KOH and maintained between 7–8 during the addition of 68.2 g (50% saturation) (NH₄)₂SO₄. The solution [with precipitated protein] was left at RT [room temperature] overnight with 60 mg NaN₃ [a preservative]. Final pH = 7.6.

Later, there are tables of numerical data, graphs of results, and original, *well-labeled* instrument output

pasted permanently into the notebook.

2-3 The Analytical Balance

An **electronic balance** uses electromagnetic force compensation to balance the load on the pan. Figure 2-2 shows a typical analytical balance with a capacity of 100–200 g and a readability of 0.01–0.1 mg. *Readability* is the smallest increment of mass that can be indicated. A *microbalance* weighs milligram quantities with a readability of 1 µg.

To weigh a chemical, first place a clean receiving vessel or *weighing paper* (which has a smooth surface to which powders do not easily stick) on the balance pan. The mass of the empty vessel is called the **tare**. On most balances, you can press a button to reset the tare to 0. Add the chemical to the vessel and read its mass. If there is no automatic tare operation, subtract the tare mass from that of the filled vessel. To protect the balance from corrosion, *chemicals should never be placed*



Figure 2-2 Analytical electronic balance. Good-quality balances calibrate themselves with internal weights to correct for variations in the force of gravity, which can be as great as 0.3% from place to place. [Fisher Scientific, Pittsburgh, PA.]

Balances are delicate and expensive. Be gentle when you place objects on the pan and when you adjust the knobs. A balance should be calibrated by measuring a set of standard weights at least once a year.

directly on the weighing pan. Clean up spills on the balance and do not allow chemicals to get into the mechanism below the pan.

An alternative procedure, called *weighing by difference*, is necessary for **hygroscopic** reagents, which rapidly absorb moisture from the air. First weigh a capped bottle containing dry reagent. Then quickly pour some reagent from the weighing bottle into a receiver. Recap the weighing bottle and weigh it again. The difference is the mass of reagent delivered from the weighing bottle. With an electronic balance, you can set the initial mass of the weighing bottle to zero with the tare button. Then deliver reagent from the bottle and reweigh the bottle. The negative reading on the balance is the mass of reagent delivered from the bottle.

Use a paper towel or tissue to handle the vessel that you are weighing, because fingerprints will change its mass. Samples should be at *ambient temperature* (the temperature of the surroundings) when weighed to prevent errors due to convective air currents. The doors of the balance in Figure 2-2 must be closed during weighing so that air currents do not disturb the pan. A top-loading balance without sliding doors has a fence around the pan to deflect air currents. Sensitive balances should be placed on a heavy table, such as a marble slab, to minimize the effect of vibrations on the reading. Use the bubble meter and adjustable feet of a balance to keep it level. For temperature stability, it is best to leave a balance in standby mode (not off) when not in use.

How a Mechanical Balance Works

Figure 2-3 shows the principle of operation of a single-pan **mechanical balance**. The balance beam is suspended on a sharp *knife edge*. The mass of the pan hanging from the balance point (another knife edge) at the left is balanced by a counterweight at the right. You place the object to be weighed on the pan and adjust knobs to remove standard weights from the bar above the pan. The balance beam is restored close to its original position when the weights removed are nearly equal to the mass on the pan. The slight difference from the original position is shown on an optical scale, whose reading is added to that of the knobs.

A mechanical balance should be in its arrested position when you load or unload the pan and in the half-arrested position when you are dialing weights. This practice prevents abrupt forces that would wear down the knife edges and decrease sensitivity.

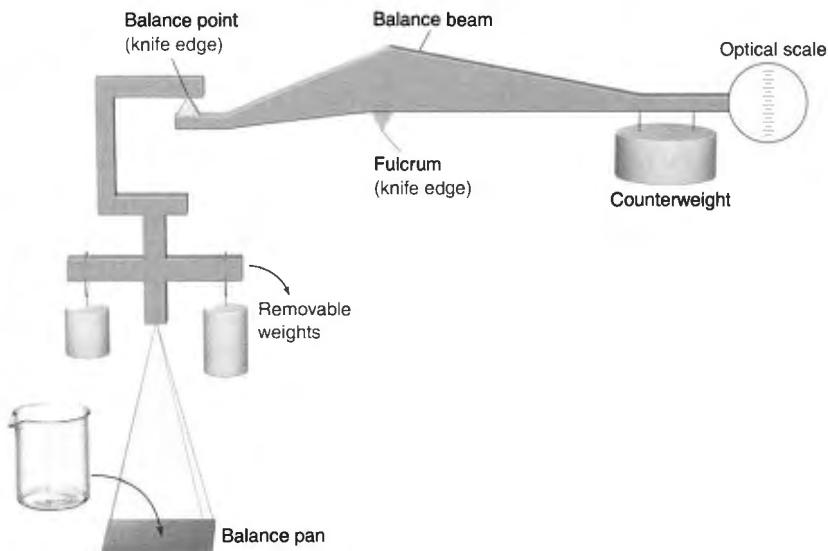


Figure 2-3 Single-pan mechanical balance. After placing an object on the pan, we detach removable weights until the balance beam is restored as close as possible to its original position. The remaining small difference is read on the optical scale.

How an Electronic Balance Works

The operating principle of an electronic balance, such as that in Figure 2-2, is shown in Figure 2-4. An object placed on the balance pan pushes down on a load receptor attached to parallel guides. The force of the sample pushes the left side of the force-transmitting lever down and moves the right side of the lever up. The null position sensor on the far right detects any small movement of the lever arm away from its equilibrium (null) position. When the null sensor detects displacement of the lever arm, a servo amplifier sends electric current through the force compensation wire coil in the field of a permanent magnet. The enlargement at the lower left shows part of the coil and magnet. Electric current in the coil interacts with the magnetic field to produce a downward force. The servo amplifier provides current that exactly compensates for the upward force on the lever arm to maintain a null position. Current flowing through the coil creates a voltage across the precision resistor, which is converted to a digital signal and ultimately to a readout in grams.

The force of gravity varies from location to location and at different elevations above Earth's surface. To compensate for the unknown local gravitational force, an internal calibration mass at the upper left in Figure 2-4 is required. In the automatic calibration step, the balance measures how much current is required to balance the known mass. The same factor is then applied to the weighing of unknown masses.

Buoyancy

When you swim, your weight in the water is nearly zero, which is why you can float. **Buoyancy** is the upward force exerted on an object in a liquid or gaseous fluid. An object weighed in air appears lighter than its actual mass by an amount equal to the

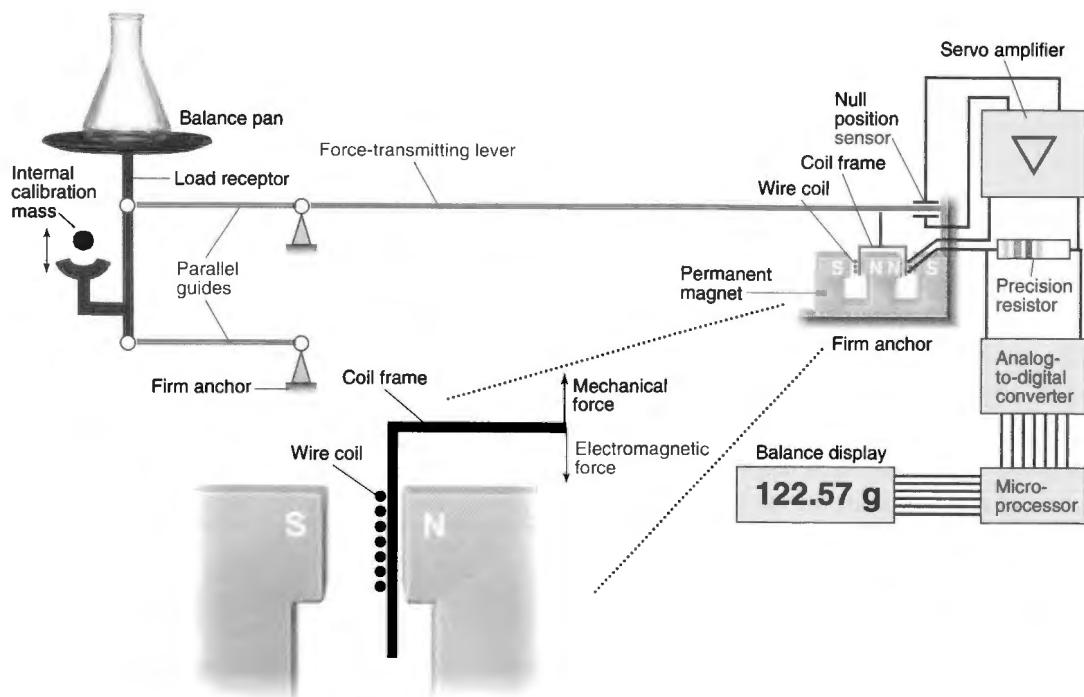


Figure 2-4 Mechanical layout of an electronic balance. The lever ratio is such that the electromagnetic force must be only $\sim 10\%$ of the load on the pan. [Adapted from C. Berg, *The Fundamentals of Weighing Technology* (Goettingen: Sartorius AG, 1996).]

mass of air displaced. True mass is the mass measured in vacuum. The standard weights in a balance also are affected by buoyancy, so they weigh less in air than they would in vacuum. A buoyancy error occurs whenever the density of the object being weighed is not equal to the density of the standard weights.

If mass m' is read from a balance, the true mass m is

Buoyancy equation:

$$m = \frac{m' \left(1 - \frac{d_a}{d_w} \right)}{\left(1 - \frac{d_a}{d} \right)} \quad (2-1)$$

where d_a is the density of air (0.001 2 g/mL near 1 bar and 25°C); d_w is the density of balance weights (8.0 g/mL); and d is the density of the object being weighed. Equation 2-1 applies to an electronic balance and to a mechanical balance.

Example Buoyancy Correction

Find the true mass of water (density = 1.00 g/mL) if the apparent mass is 100.00 g.

SOLUTION Equation 2-1 gives the true mass:

$$m = \frac{100.00 \text{ g} \left(1 - \frac{0.001 2 \text{ g/mL}}{8.0 \text{ g/mL}} \right)}{\left(1 - \frac{0.001 2 \text{ g/mL}}{1.00 \text{ g/mL}} \right)} = 100.11 \text{ g}$$

 **Test Yourself** Find the true mass of 28.0 wt% ammonia (density = 0.90 g/mL) when the apparent mass is 20.000 g. (Answer: 20.024 g)

The buoyancy error for water is 0.11%, which is significant for many purposes. For solid NaCl with a density of 2.16 g/mL, the error is 0.04%.

Ask Yourself

- 2-B. (a) Buoyancy corrections are most critical when you calibrate glassware such as a volumetric flask to see how much volume it actually holds. Suppose you fill a 25-mL volumetric flask with distilled water and find that the mass of water in the flask measured in air is 24.913 g. What is the true mass of the water?
(b) You made the measurement when the lab temperature was 21°C, at which the density of water is 0.998 00 g/mL. What is the true volume of water contained in the volumetric flask?

2-4 Burets

A **buret**⁴ is a precisely manufactured glass tube with graduations enabling you to measure the volume of liquid delivered through the *stopcock* (the valve) at the bottom (Figure 2-5a). Numbers on the buret increase from top to bottom (with 0 mL near the top). A volume measurement is made by reading the level before and after draining liquid from the buret and subtracting the first reading from the second reading. The

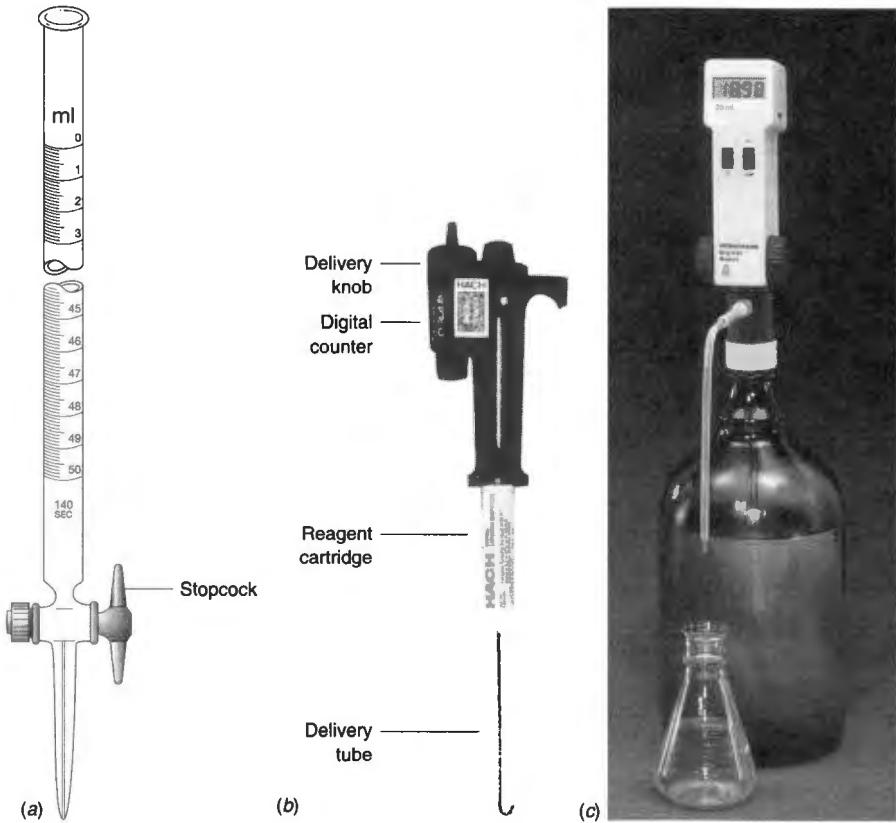


Figure 2-5 (a) Glass buret with Teflon stopcock. Cover your buret with a loose-fitting cap to keep dust out and reduce evaporation. [Fisher Scientific, Pittsburgh, PA.] (b) Digital titrator with its plastic cartridge containing reagent carries out the same function as a buret for analyses in the field. [Hach Co., Loveland, CO.] (c) Battery-operated electronic buret with digital readout delivers 0.01-mL increments from a reagent bottle. [Cole-Parmer Co., Niles, IL.]

graduations of Class A burets (the most accurate grade) are certified to meet the tolerances in Table 2-1. For example, if the reading of a 50-mL buret is 32.50 mL, the true volume can be anywhere in the range 32.45 to 32.55 mL and still be within the manufacturer's stated tolerance of ± 0.05 mL.

When reading the liquid level in a buret, your eye should be at the same height as the top of the liquid. If your eye is too high, the liquid seems to be higher than it actually is. If your eye is too low, the liquid appears too low. The error that occurs when your eye is not at the same height as the liquid is called **parallax error**.

The **meniscus** is the curved upper surface of liquid in the glass buret in Figure 2-6. Water has a concave meniscus because water is attracted to glass and climbs slightly up the glass. It is helpful to use black tape on a white card as a background for locating the meniscus. Align the top of the tape with the bottom of the meniscus and read the position on the buret. Highly colored solutions may appear to have a double meniscus,

Table 2-1 Tolerances of Class A burets

Buret volume (mL)	Smallest graduation (mL)	Tolerance (mL)
5	0.01	± 0.01
10	0.05 or 0.02	± 0.02
25	0.1	± 0.03
50	0.1	± 0.05
100	0.2	± 0.10

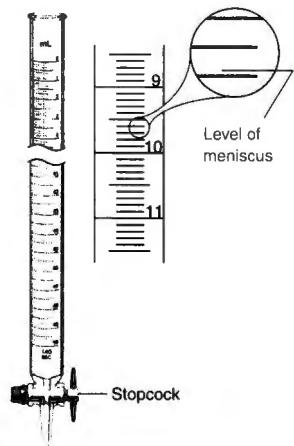


Figure 2-6 Buret with the meniscus at 9.68 mL. Estimate the reading of any scale to the nearest tenth of a division. Because this buret has 0.1-mL divisions, we estimate the reading to 0.01 mL.

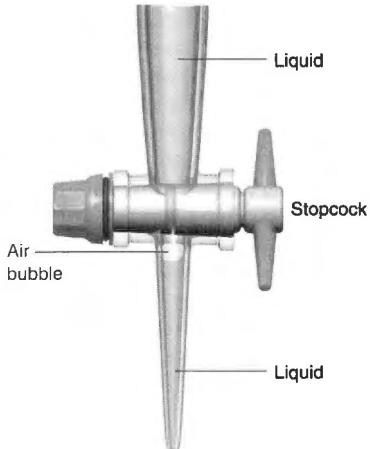


Figure 2-7 Air bubble beneath the stopcock should be expelled before you use a buret.

Operating a buret:

- Read bottom of concave meniscus
- Estimate reading to 1/10 of a division
- Avoid parallax
- Account for graduation thickness in readings
- Drain liquid slowly
- Wash buret with new solution
- Deliver fraction of a drop near end point
- Eliminate air bubble before use

either of which you can use. Volume is determined by subtracting one reading from another, so the important point is to read the position of the meniscus reproducibly. Always estimate the reading to the nearest tenth of a scale division.

The thickness of a graduation line on a 50-mL buret corresponds to about 0.02 mL. To use the buret most accurately, consider the *top* of a graduation line to be 0. When the meniscus is at the bottom of the same graduation line, the reading is 0.02 mL greater.

A drop of liquid from a 50-mL buret is about 0.05 mL. Near the end point of a titration, try to deliver less than one drop at a time so that you can locate the end point more precisely than ± 0.05 mL. To deliver a fraction of a drop, carefully open the stopcock until part of a drop is hanging from the buret tip. Then touch the inside wall of the receiving flask to the buret tip to transfer the droplet to the flask. Carefully tip the flask so that the main body of liquid washes over the newly added droplet. Then swirl the flask to mix the contents. Near the end of a titration, tip and rotate the flask often to ensure that droplets on the wall containing unreacted analyte contact the bulk solution.

Liquid should drain evenly down the wall of a buret. The tendency of liquid to stick to the glass is reduced by draining the buret slowly (<20 mL/min). If many droplets stick to the wall, clean the buret with detergent and a buret brush. If this cleaning is insufficient, soak the buret in peroxydisulfate–sulfuric acid cleaning solution prepared by your instructor.⁵ Cleaning solution eats clothing and people, as well as grease in the buret. Volumetric glassware should not be soaked in alkaline solutions, which attack glass. (A 5 wt% NaOH solution at 95°C dissolves Pyrex glass at a rate of 9 $\mu\text{m}/\text{h}$.)

A common buret error is caused by failure to expel the air bubble often found beneath the stopcock (Figure 2-7). A bubble present at the start of the titration may be filled with liquid during the titration. Therefore some volume that drained out of the graduated part of the buret did not reach the titration vessel. Usually the bubble can be dislodged by draining the buret for a second or two with the stopcock wide open. A tenacious bubble can be expelled by carefully shaking the buret while draining it into a sink.

Before you fill a buret with fresh solution, it is a wonderful idea to rinse the buret several times with small portions of the new solution, discarding each wash. It is not necessary to fill the entire buret with wash solution. Simply tilt the buret so that its whole surface contacts the wash liquid. This same technique should be used with any vessel (such as a spectrophotometer cuvet or a pipet) that is reused without drying.

The *digital titrator* in Figure 2-5b is useful for conducting titrations in the field where samples are collected. The counter tells how much reagent from the cartridge is dispensed by rotation of the delivery knob. Its accuracy of 1% is 10 times poorer than that of a glass buret, but many measurements do not require higher accuracy. The battery-operated *electronic buret* in Figure 2-5c fits on a reagent bottle and delivers up to 99.99 mL in 0.01-mL increments displayed on a digital readout.

Microscale Titrations (A “Green” Idea)

“Microscale” experiments decrease costs, consumption of reagents, and generation of waste. A student buret can be constructed from a 2-mL pipet graduated in 0.01-mL intervals.⁶ Volume can be read to 0.001 mL and titrations can be carried out with a precision of 1%.

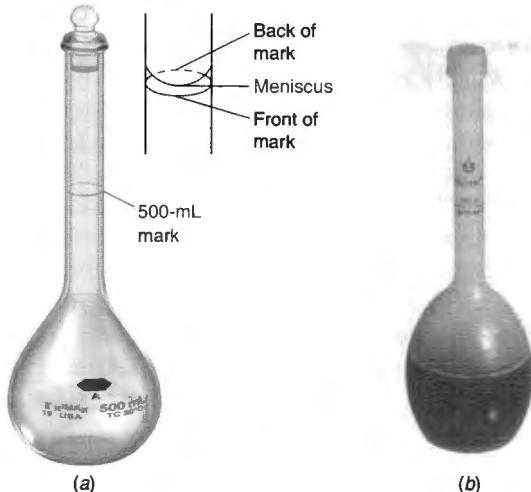


Figure 2-8 (a) Class A glass volumetric flask showing proper position of the meniscus—at the center of the ellipse formed by the front and back of the calibration mark when viewed from above or below. Volumetric flasks and transfer pipets are calibrated to this position. [A. H. Thomas Co., Philadelphia, PA.] (b) Class B polypropylene plastic volumetric flask for trace analysis. [Fisher Scientific, Pittsburgh, PA.] Class A flasks meet tolerances of Table 2-2. Class B tolerances are twice as big as Class A tolerances.

2-5 Volumetric Flasks

A **volumetric flask** (Figure 2-8, Table 2-2) is calibrated to contain a particular volume of solution at 20°C when the bottom of the meniscus is adjusted to the center of the mark on the neck of the flask. Most flasks bear the label “TC 20°C,” which means *to contain* at 20°C. (Other types of glassware may be calibrated *to deliver*, “TD,” their indicated volume.) The temperature of the container is relevant because liquid and glass expand when heated.

We use a volumetric flask to prepare a solution of known volume. Dissolve a known mass of reagent in the flask in *less* than the final volume of liquid. Add more liquid and mix the solution again. Make the final volume adjustment with as much well-mixed liquid in the flask as possible. (When two different liquids are mixed, there is generally a small volume change. The total volume is *not* the sum of the two volumes that were mixed. By swirling the liquid in a nearly full volumetric flask before the liquid reaches the thin neck, you minimize the change in volume when the last liquid is added.) For best control, add the final drops of liquid with a pipet, *not a squirt bottle*. After adjusting the liquid to the correct level, hold the cap firmly in place and invert the flask several times to complete mixing. Before the liquid is homogeneous, we observe streaks (called *schlieren*) arising from regions that refract light differently. After the schlieren are gone, invert the flask a few more times to ensure complete mixing.

Table 2-2 Tolerances of Class A volumetric flasks

Flask capacity (mL)	Tolerance (mL)	Flask capacity (mL)	Tolerance (mL)
1	±0.02	100	±0.08
2	±0.02	200	±0.10
5	±0.02	250	±0.12
10	±0.02	500	±0.20
25	±0.03	1 000	±0.30
50	±0.05	2 000	±0.50

Adsorption: to bind a substance on the surface

Absorption: to bind a substance internally

Glass is notorious for *adsorbing* traces of chemicals—especially cations. **Adsorption** means to stick to the surface. (In contrast, **absorption** means to take inside, as a sponge takes up water.) For critical work, **acid wash** the glassware to replace low concentrations of cations on the glass surface with H⁺. To do this, soak already thoroughly cleaned glassware in 3–6 M HCl or HNO₃ (in a fume hood) for >1 h, followed by several rinses with distilled water and a final soak in distilled water. The HCl can be reused many times, as long as it is only used for soaking clean glassware.

As an example, high-purity nitric acid was delivered from a glass pipet that had been washed normally without acid and another that had been acid washed. The level of the transition elements Ti, Cr, Mn, Fe, Co, Ni, Cu, and Zn in acid delivered from the acid-washed pipet was below the detection level of 0.01 ppb (0.01 ng/g). The concentration of each transition element in acid delivered from the pipet that had not been acid washed was in the range 0.5 to 9 ppb.⁷

Ask Yourself

2-C. How would you use a volumetric flask to prepare 250.0 mL of 0.150 0 M K₂SO₄?

2-6 | Pipets and Syringes

Do not blow the last drop out of a transfer pipet.

Pipets deliver known volumes of liquid. The *transfer pipet* in Figure 2-9 is calibrated to deliver one fixed volume. The last drop of liquid does not drain out of the pipet; *it should not be blown out*. The *measuring pipet* is calibrated to deliver a variable volume, which is the difference between the initial and final volumes. A measuring pipet could be used to deliver 5.6 mL by starting delivery at the 1-mL mark and terminating at the 6.6-mL mark.

A transfer pipet is more accurate than a measuring pipet. Tolerances for Class A (the most accurate grade) transfer pipets in Table 2-3 are the allowed error in the volume that is actually delivered.

Using a Transfer Pipet

Using a rubber bulb, *not your mouth*, suck liquid up past the calibration mark. It is a good idea to discard one or two pipet volumes of liquid to remove traces of previous reagents from the pipet. After taking up a third volume past the calibration mark, quickly replace the bulb with your index finger at the end of the pipet. The liquid should still be above the mark after this maneuver. Pressing the pipet against the bottom of the vessel while removing the rubber bulb helps prevent liquid from draining while you put your finger in place. Wipe the excess liquid off the outside of the pipet

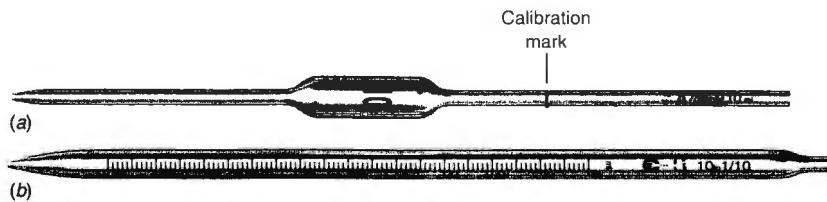


Figure 2-9 (a) Transfer pipet: Do not blow out the last drop. (b) Measuring (Mohr) pipet. [A. H. Thomas Co., Philadelphia, PA.]

with a clean tissue. Touch the tip of the pipet to the side of a beaker and drain the liquid until the bottom of the meniscus just reaches the center of the mark, as in Figure 2-8. Touching the beaker wall draws liquid from the pipet without leaving part of a drop hanging from the pipet when the level reaches the calibration mark.

Transfer the pipet to the desired receiving vessel and drain it *while holding the tip against the wall of the vessel*. After the pipet stops draining, hold it against the wall for a few more seconds to complete draining. *Do not blow out the last drop*. The pipet should be nearly vertical at the end of delivery. When you finish with a pipet, it should be rinsed with distilled water or soaked in a pipet container until it is cleaned. Solutions should never be allowed to dry inside a pipet because dry residue is difficult to remove.

Micropipets

A micropipet (Figure 2-10) is used to deliver volumes of 1 to 1 000 μL ($1 \mu\text{L} = 10^{-6} \text{ L}$) with accuracies given in Table 2-4. The liquid is contained in the disposable plastic tip. Micropipets may have a metal barrel on the inside that can be corroded by pipetting volatile acids such as concentrated HCl. Corrosion slowly diminishes the accuracy of the pipet.

To use a micropipet, place a fresh tip tightly on the barrel. Tips are contained in a package or dispenser so that you do not handle (and contaminate) the points with your fingers. Set the desired volume with the knob at the top of the pipet. Depress the plunger to the first stop, which corresponds to the selected volume. Hold the pipet *vertically*, dip it 3–5 mm into the reagent solution, and *slowly* release the plunger to suck up liquid. Withdraw the tip from the liquid by sliding it along the wall of the vessel to remove liquid from the outside of the tip. To dispense liquid, touch the micropipet tip to the wall of the receiver and gently depress the plunger to the first stop. After a few seconds to allow liquid to drain down the wall of the pipet tip, depress the plunger farther to squirt out the last liquid. It is a good idea to clean and wet a fresh tip by taking up and discarding two or three squirts of reagent first. The

Accuracy: difference between delivered volume and desired volume

Precision: reproducibility of replicate deliveries



Table 2-4 Accuracy (%) of micropipets^{a,b}

Pipet volume (μL)	Accuracy (%) at 10% of pipet volume	Accuracy (%) at 100% of pipet volume
<i>Adjustable volume</i>		
2	± 8	± 1.2
10	± 2.5	± 0.8
25	± 4.5	± 0.8
100	± 1.8	± 0.6
300	± 1.2	± 0.4
1 000	± 1.6	± 0.3
<i>Fixed volume</i>		
10		± 0.8
25		± 0.8
100		± 0.5
500		± 0.4
1 000		± 0.3

a. Data from Hamilton Co., Reno, NV.

b. Precision is typically 2–3 times smaller (better) than the accuracy.

Figure 2-10 (a) Micropipet with disposable plastic tip. (b) Volume selection dial set to 150 μL . [Rainin Instrument Co., Emeryville, CA.]

Figure 2-11 Hamilton syringe with a volume of 1 μL and graduations of 0.01 μL on the glass barrel.
[Hamilton Co., Reno, NV.]

When you use a squirt bottle, never touch the tip to anything.



tip can be discarded or rinsed well with a squirt bottle and reused. When you use a squirt bottle, never touch the tip of the squirt bottle to anything, to avoid contaminating the squirt bottle.

The volume of liquid taken into the tip depends on the angle at which the pipet is held and how far beneath the surface of reagent the tip is held during uptake. As internal parts wear out, both precision and accuracy can decline by an *order of magnitude* (a factor of 10). Micropipets require periodic cleaning, seal replacement, and lubrication. You can check performance by weighing the amount of water delivered from a micropipet. Monthly calibration to identify pipets in need of repair is recommended.

A microliter syringe, such as that in Figure 2-11, dispenses volumes in the range 1 to 500 μL with accuracy and precision near 1%. When using a syringe, take up and discard several volumes of liquid to wash the glass and remove air bubbles from the barrel. The steel needle is attacked by strong acid and will contaminate strongly acidic solutions with iron.

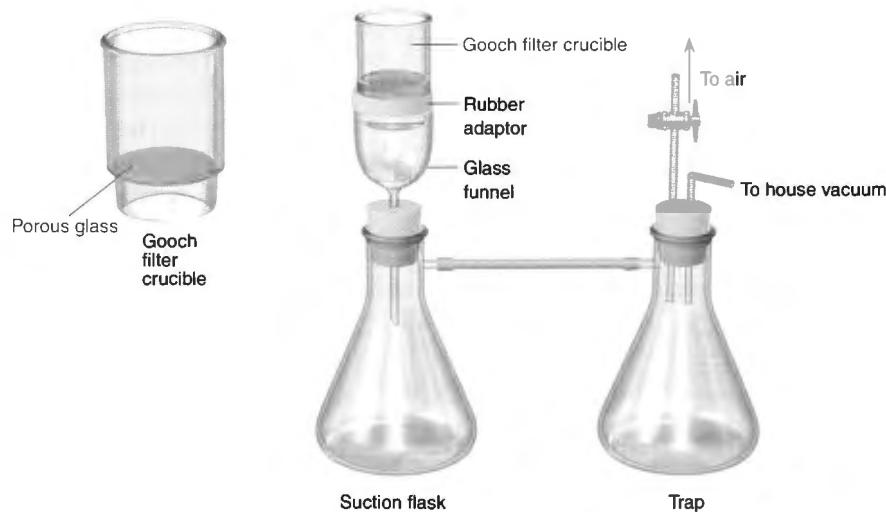
Ask Yourself

2-D. Which is more accurate, a transfer pipet or a measuring pipet? What is the uncertainty in microliters when you deliver either 10 μL or 100 μL from a 100- μL adjustable micropipet?

2-7 Filtration

In **gravimetric analysis**, the mass of product from a reaction is measured to determine how much unknown was present before the reaction. Precipitates from gravimetric analyses are collected by filtration, washed, and then dried. Most precipitates are collected in a *fritted-glass funnel* with suction to speed filtration (Figure 2-12).

Figure 2-12 Filtration with a Gooch filter crucible that has a porous glass (*fritted*) disk through which liquid can pass. Suction is provided by a vacuum line at the lab bench or by an *aspirator* that uses flowing water from a tap to create a vacuum. The trap prevents backup of filtrate into the vacuum system or backup of water from the aspirator into the suction flask.



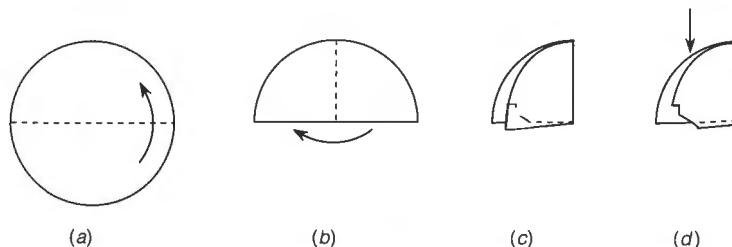


Figure 2-13 Folding filter paper for a conical funnel. (a) Fold the paper in half and (b) in half again. (c) Tear off a corner to better seat the paper in the funnel. (d) Open the side that was not torn and fit the paper in the funnel.

The porous glass plate in the funnel allows liquid to pass but retains solids. Filters with coarse, medium, or fine pores are available to collect precipitates with large, medium, or small particle size. The finer the filter, the slower the filtration. The empty crucible is first dried at 110°C and weighed. After collecting solid and drying again, the crucible and its contents are weighed a second time to determine the mass of solid.

Liquid from which a substance precipitates or crystallizes is called the **mother liquor**. Liquid that passes through the filter is called **filtrate**.

In some gravimetric procedures, **ignition** (heating at high temperature over a burner or in a furnace) is used to convert a precipitate to a known, constant composition. For example, Fe^{3+} precipitates as hydrated $\text{Fe}(\text{OH})_3$ with variable composition. Ignition converts it to Fe_2O_3 prior to weighing. When a gravimetric precipitate is to be ignited, it is collected in **ashless filter paper**, which leaves little residue when burned.

To use filter paper with a conical glass funnel, fold the paper into quarters, tear off one corner (to allow a firm fit into the funnel), and place the paper in the funnel (Figure 2-13). The filter paper should fit snugly and be seated with some distilled water. When liquid is poured in, an unbroken stream of liquid should fill the stem of the funnel. The weight of liquid in the stem helps speed filtration.

For filtration, pour the slurry of precipitate in the mother liquor down a glass rod to prevent splattering (Figure 2-14). (A *slurry* is a suspension of solid in liquid.) Dislodge any particles adhering to the beaker or rod with a **rubber policeman**, which is a flattened piece of rubber at the end of a glass rod. Use a jet of appropriate wash liquid from a squirt bottle to transfer particles from the rubber and glassware to the filter. If the precipitate is going to be ignited, particles remaining in the beaker should be wiped onto a small piece of moist filter paper, which is then added to the filter to be ignited.

2-8 Drying

Reagents, precipitates, and glassware are usually dried in an oven at 110°C. (Some chemicals require other temperatures.) Label everything that you put in the oven. Use a beaker and watchglass (Figure 2-15) to minimize contamination by dust during drying. It is good practice to cover all vessels on the benchtop to prevent dust contamination.

We measure the mass of a gravimetric precipitate by weighing a dry, empty filter crucible before the procedure and weighing the same crucible containing dry product after the procedure. To weigh the empty crucible, first bring it to “constant mass” by drying in the oven for 1 h or longer and then cooling for 30 min in a *desiccator*.

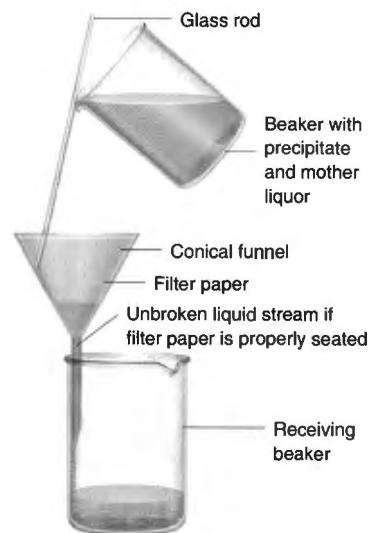


Figure 2-14 Filtering a precipitate.



Figure 2-15 Use a watchglass as a dust cover while drying reagents or crucibles in the oven.

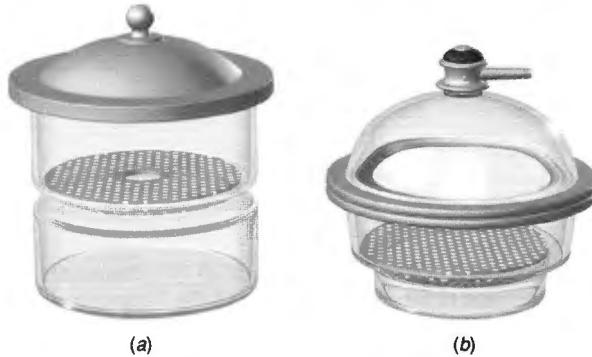


Figure 2-16 (a) Ordinary desiccator. (b) Vacuum desiccator, which can be evacuated through the side arm and then sealed by rotating the joint containing the side arm. Drying is more efficient at low pressure. Drying agents (*desiccants*) are placed at the bottom of each desiccator below the porous porcelain plate.

Weigh the crucible and then heat it again for about 30 min. Cool it and reweigh it. When successive weighings agree to ± 0.3 mg, the filter has reached “constant mass.” If the crucible is warm when it is weighed, it creates convection currents that give a false weight. A microwave oven can be used instead of an electric oven for drying reagents, precipitates, and crucibles. Try an initial heating time of 4 min, with subsequent 2-min heatings.

A **desiccator** (Figure 2-16) is a closed chamber containing a drying agent called a **desiccant**. The lid is greased to make an airtight seal. Desiccant is placed in the bottom beneath the perforated disk. Common desiccants in approximate order of decreasing efficiency are magnesium perchlorate ($Mg(ClO_4)_2$) > barium oxide (BaO) \approx alumina (Al_2O_3) \approx phosphorus pentoxide (P_4O_{10}) \gg calcium chloride ($CaCl_2$) \approx calcium sulfate ($CaSO_4$, called Drierite) \approx silica gel (SiO_2). After placing a hot object in the desiccator, leave the lid cracked open for a minute until the object has cooled slightly. This practice prevents the lid from popping open when the air inside warms up. To open a desiccator, slide the lid sideways rather than trying to pull it straight up.

2-9 Calibration of Volumetric Glassware

Calibration is the process of relating the actual quantity (such as mass, volume, or electric current) to the quantity indicated on the scale of an instrument. Volumetric glassware can be calibrated to measure the volume that is actually contained in or delivered by a particular piece of equipment. Calibration is done by measuring the mass of water contained or delivered and using Table 2-5 to convert mass to volume:

$$\text{true volume} = (\text{mass of water}) \times (\text{correction factor in Table 2-5}) \quad (2-2)$$

To calibrate a 25-mL transfer pipet, first weigh an empty weighing bottle like the one in Figure 2-15. Then fill the pipet to the mark with distilled water, drain it into the weighing bottle, and put on the lid to prevent evaporation. Weigh the bottle again to find the mass of water delivered from the pipet. Use Equation 2-2 to convert mass to volume. Table 2-5 already includes a buoyancy correction.

Table 2-5 Correction factors for volumetric calibration

Temperature (°C)	Correction factor (mL/g) ^a
15	1.002 0
16	1.002 1
17	1.002 3
18	1.002 5
19	1.002 7
20	1.002 9
21	1.003 1
22	1.003 3
23	1.003 5
24	1.003 8
25	1.004 0
26	1.004 3
27	1.004 6
28	1.004 8
29	1.005 1
30	1.005 4

a. Factors are based on the density of water and are corrected for buoyancy with Equation 2-1.

Example Calibration of a Pipet

An empty weighing bottle had a mass of 10.283 g. After water was added from a 25-mL pipet, the mass was 35.225 g. The temperature was 23°C. Find the volume of water delivered by the pipet.

SOLUTION The mass of water is $35.225 - 10.283 = 24.942$ g. From Equation 2-2 and Table 2-5, the volume of water is $(24.942 \text{ g})(1.0035 \text{ mL/g}) = 25.029 \text{ mL}$.

 **Test Yourself** If the temperature had been 29°C, and the mass of water was 24.942 g, what volume was delivered? (Answer: 25.069 mL)

Ask Yourself

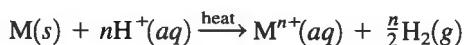
2-E. A 10-mL pipet delivered 10.000 0 g of water at 15°C to a weighing bottle. What is the true volume of the pipet?

2-10 Methods of Sample Preparation

In the analytical process chart in Box 0-1, a homogeneous laboratory sample must be prepared from a representative bulk sample. You can homogenize solids by grinding them to fine powder with a **mortar and pestle** (Figure 2-17) or by dissolving the entire sample.

Dissolving Inorganic Materials with Strong Acids

The acids HCl, HBr, HF, H_3PO_4 , and dilute H_2SO_4 dissolve most metals (M) by the reaction



Many other inorganic substances also can be dissolved. Some anions react with H^+ to form **volatile** products (species that evaporate easily), which are lost from hot solutions in open vessels. Examples include carbonate ($\text{CO}_3^{2-} + 2\text{H}^+ \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{CO}_2(g) \uparrow + \text{H}_2\text{O}$) and sulfide ($\text{S}^{2-} + 2\text{H}^+ \rightarrow \text{H}_2\text{S}(g) \uparrow$). Hot hydro-fluoric acid dissolves silicates found in most rocks. HF also attacks glass, so it is used in Teflon, polyethylene, silver, or platinum vessels. Teflon is inert to attack by most chemicals and can be used up to 260°C.

Substances that do not dissolve in the acids above may dissolve as a result of oxidation by HNO_3 or concentrated H_2SO_4 . Nitric acid attacks most metals, but not Au and Pt, which dissolve in the 3:1 (vol:vol) mixture of HCl:HNO₃ called *aqua regia*.

Acid dissolution is conveniently carried out with a Teflon-lined **bomb** (a sealed vessel, Figure 2-18) in a microwave oven, which heats the contents to 200°C in a minute. The bomb cannot be made of metal, which absorbs microwaves and could be attacked by the acid. The bomb should be cooled prior to opening to prevent loss of volatile products.



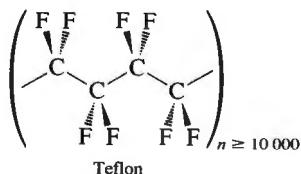
Figure 2-17 Agate mortar and pestle. [Thomas Scientific, Swedesboro, NJ.]

The mortar is the base and the pestle is the grinding tool. Agate is very hard and expensive. Less-expensive porcelain mortars are widely used, but they are somewhat porous and easily scratched. These properties can lead to contamination of the sample by porcelain particles or by traces of previous samples embedded in the porcelain.

HCl	hydrochloric acid
HBr	hydrobromic acid
HF	hydrofluoric acid
H_3PO_4	phosphoric acid
H_2SO_4	sulfuric acid
HNO_3	nitric acid

HF is extremely harmful to touch or breathe. Flood the affected area with water, coat the skin with calcium gluconate (or another calcium salt), and seek medical help.

Teflon is a *polymer* (a chain of repeating units) with the structure



Carbon atoms are in the plane of the page. A solid wedge is a bond coming out of the page toward you and a dashed wedge is a bond going behind the page.

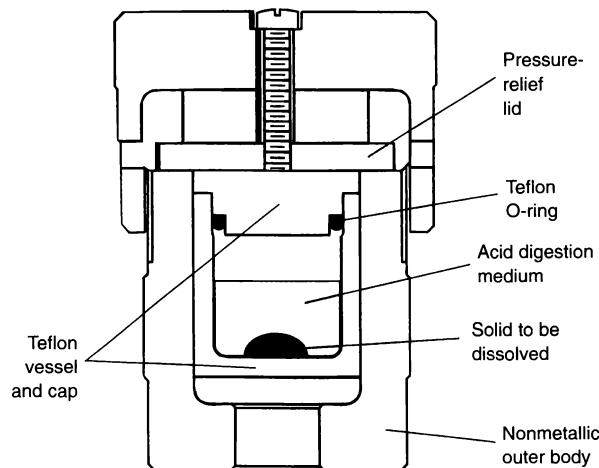


Figure 2-18 Microwave digestion bomb lined with Teflon. [Parr Instrument Co., Moline, IL.] A typical 23-mL vessel can be used to digest as much as 1 g of inorganic material (or 0.1 g of organic material, which releases a great deal of gaseous CO_2) in as much as 15 mL of concentrated acid. The outer container maintains its strength up to 150°C, but rarely rises above 50°C. If the internal pressure exceeds 80 bar, the cap deforms and releases the excess pressure.

Fusion

Inorganic substances that do not dissolve in acid can usually be dissolved by a hot, molten inorganic **flux**, examples of which are lithium tetraborate ($\text{Li}_2\text{B}_4\text{O}_7$) and sodium hydroxide (NaOH). Mix the finely powdered unknown with a mass of solid flux that is 2 to 20 times the mass of the unknown. **Fuse** (melt) the mixture in a platinum–gold alloy crucible at 300° to 1 200°C in a furnace or over a burner. When the sample is homogeneous, carefully pour the molten flux into a beaker containing 10 wt% aqueous HNO_3 to dissolve the product.

Digestion of Organic Substances

To analyze N, P, halogens (F, Cl, Br, I), and metals in an organic compound, first decompose the compound by combustion (described in Section 7-4) or by *digestion*. In **digestion**, a substance is decomposed and dissolved by a reactive liquid. For this purpose, add sulfuric acid or a mixture of H_2SO_4 and HNO_3 to an organic substance and gently boil (or heat it in a microwave bomb) for 10 to 20 min until all particles have dissolved and the solution has a uniform black appearance. After cooling, destroy the dark color by adding hydrogen peroxide (H_2O_2) or HNO_3 , and heat again. Analyze the decomposed sample after digestion.

Extraction

In **extraction**, analyte is dissolved in a solvent that does not dissolve the entire sample and does not decompose the analyte. In a typical extraction of pesticides from soil, a mixture of soil plus the solvents acetone and hexane is placed in a Teflon-lined bomb and heated by microwaves to 150°C. This temperature is 50° to 100° higher than the boiling points of the individual solvents in an open vessel at atmospheric

pressure. Soluble pesticides dissolve, but most of the soil remains behind. To complete the analysis, analyze the solution by chromatography, which is described in Chapters 21 through 23.



Ask Yourself

2-F. Lead sulfide (PbS) is a black solid that is sparingly soluble in water but dissolves in concentrated HCl . If such a solution is boiled to dryness, white, crystalline lead chloride (PbCl_2) remains. What happened to the sulfide?

Key Equation

Buoyancy
$$m = m' \left(1 - \frac{d_a}{d_w} \right) \left(1 - \frac{d_a}{d} \right)$$

m = true mass; m' = mass measured in air

d_a = density of air (0.001 2 g/mL near 1 bar and 25°C)

d_w = density of balance weights (8.0 g/mL)

d = density of object being weighed

Important Terms

absorption	desiccant	gravimetric analysis	parallax error
acid wash	desiccator	green chemistry	pipet
adsorption	digestion	hygroscopic	rubber policeman
ashless filter paper	electronic balance	ignition	tare
bomb	extraction	mechanical balance	volatile
buoyancy	filtrate	meniscus	volumetric flask
buret	flux	mortar and pestle	
calibration	fuse	mother liquor	

Problems

2-1. What is the purpose of the internal calibration mass at the upper left in the electronic balance in Figure 2-4.

2-2. What do the symbols TD and TC mean on volumetric glassware?

2-3. When would it be preferable to use a plastic volumetric flask instead of a glass flask?

2-4. What is the purpose of the trap in Figure 2-12? What does the watchglass do in Figure 2-15?

2-5. Distinguish absorption from adsorption. When you heat glassware in a drying oven, are you removing absorbed or adsorbed water?

2-6. What is the difference between digestion and extraction?

2-7. What is the true mass of water if the mass measured in air is 5.397 4 g?

2-8. Pentane (C_5H_{12}) is a liquid with a density of 0.626 g/mL. Find the true mass of pentane when the mass weighed in air is 14.82 g.

2-9. Ferric oxide (Fe_2O_3 , density = 5.24 g/mL) obtained from ignition of a gravimetric precipitate weighed 0.296 1 g in the atmosphere. What is the true mass in vacuum?

2-10. Your professor has recruited you to work in her lab to help her win the Nobel Prize. It is critical that your work be as accurate as possible. Rather than using the stated volumes of glassware in the lab, you decide to calibrate each piece. An empty 10-mL volumetric flask weighed 10.263 4 g. When filled to the mark with distilled water at 20°C, it weighed 20.214 4 g. What is the true volume of the flask?

2-11. Water from a 5-mL pipet was drained into a weighing bottle whose empty mass was 9.974 g to give a new mass of 14.974 g at 26°C. Find the volume of the pipet.

2-12. Water was drained from a buret between the 0.12- and 15.78-mL marks. The apparent volume was $15.78 - 0.12 = 15.66$ mL. Measured in air at 25°C, the mass of water delivered was 15.569 g. What is the true volume?

2-13. Glass is a notorious source of metal ion contamination. Three glass bottles were crushed and sieved to collect 1-mm pieces.⁸ To see how much Al³⁺ could be extracted, 200 mL of

a 0.05 M solution of the metal-binding compound EDTA were stirred with 0.50 g of ~1-mm glass particles in a polyethylene flask. The Al content of the solution after 2 months was 5.2 µM. The total Al content of the glass, measured after completely dissolving some glass in 48 wt% HF with microwave heating, was 0.80 wt%. What fraction of the Al was extracted from glass by EDTA?

Reference Procedure: Calibrating a 50-mL Buret

This procedure tells you how to construct a graph like that in Figure 3-2 so that you can convert the measured volume delivered by a buret to the true volume delivered at 20°C.

0. Measure the temperature in the laboratory. Distilled water for this experiment must be at laboratory temperature.

1. Fill the buret with distilled water and force any air bubbles out the tip. See whether the buret drains without leaving drops on its walls. If drops are left, clean the buret with soap and water or soak it with cleaning solution.⁵ Adjust the meniscus to be at or slightly below 0.00 mL, and touch the buret tip to a beaker to remove the suspended drop of water. Allow the buret to stand for 5 min while you weigh a 125-mL flask fitted with a rubber stopper. (Hold the flask with a paper towel to prevent fingerprints from changing its mass.) If the level of the liquid in the buret has changed, tighten the

stopcock and repeat the procedure. Record the level of the liquid.

2. Drain approximately 10 mL of water at a rate of <20 mL/min into the weighed flask, and cap it tightly to prevent evaporation. Allow 30 s for the film of liquid on the walls to descend before you read the buret. Estimate all readings to the nearest 0.01 mL. Weigh the flask again to determine the mass of water delivered.

3. Drain the buret from 10 to 20 mL, and measure the mass of water delivered. Repeat the procedure for 30, 40, and 50 mL. Then do the entire procedure (10, 20, 30, 40, 50 mL) a second time.

4. Use Table 2-5 to convert the mass of water to the volume delivered. Repeat any set of duplicate buret corrections that do not agree to within 0.04 mL. Prepare a calibration graph like Figure 3-2, showing the correction factor at each 10-mL interval.

Example Buret Calibration

When draining the buret at 24°C, you observe the following values:

Final reading	10.01	10.08 mL
Initial reading	0.03	0.04
Difference	9.98	10.04 mL
Mass	9.984	10.056 g
Actual volume delivered	10.02	10.09 mL
Correction	+0.04	+0.05 mL
Average correction	+0.045 mL	

To calculate the actual volume delivered when 9.984 g of water are delivered at 24°C, use the conversion factor 1.003 8 mL/g in Table 2-5. We find that 9.984 g occupies $(9.984 \text{ g})(1.003 8 \text{ mL/g}) = 10.02 \text{ mL}$. The average correction for both sets of data is +0.045 mL.

To obtain the correction for a volume greater than 10 mL, add successive masses of water collected in the flask. Suppose that the following masses were measured:

Volume interval (mL)	Mass delivered (g)
0.03–10.01	9.984
10.01–19.90	9.835
<u>19.90–30.06</u>	<u>10.071</u>
Sum 30.03 mL	29.890 g

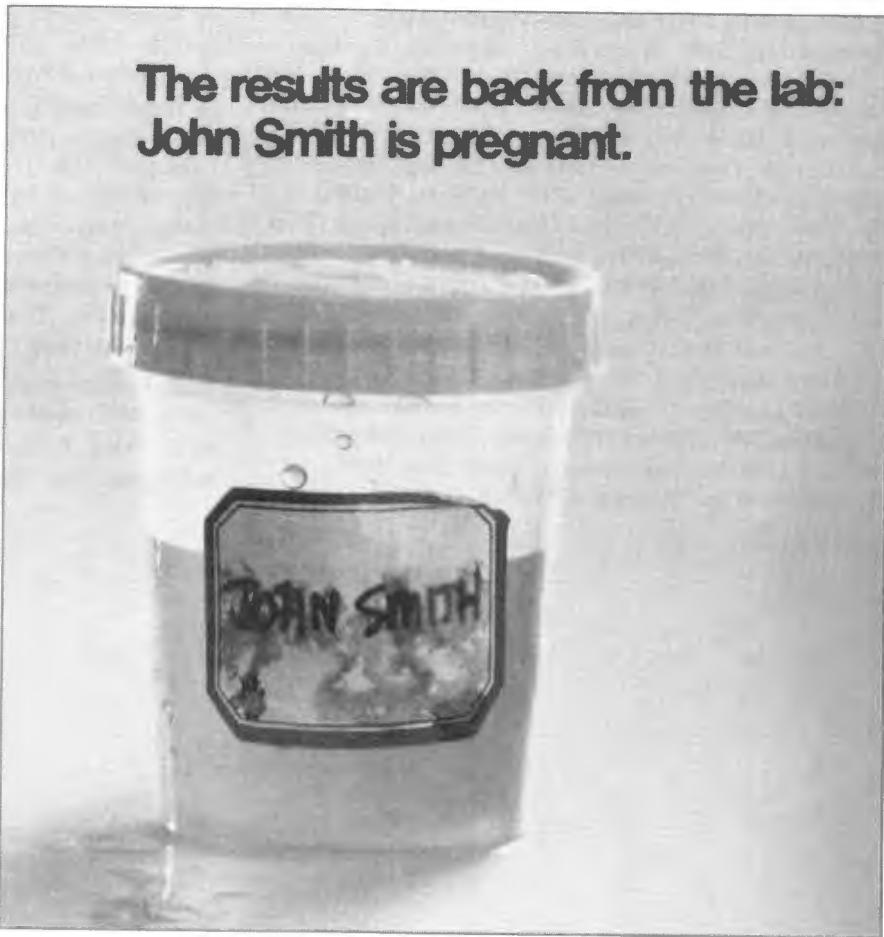
The total volume of water delivered is $(29.890 \text{ g})(1.003 8 \text{ mL/g}) = 30.00 \text{ mL}$. Because the indicated volume is 30.03 mL, the buret correction at 30 mL is -0.03 mL.

What does this mean? Suppose that Figure 3-2 applies to your buret. If you begin a titration at 0.04 mL and end at 29.43 mL, you would deliver 29.39 mL if the buret were perfect. Figure 3-2 tells you that the buret delivers 0.03 mL less than the indicated amount; so only 29.36 mL were actually delivered. To use the calibration curve, either begin all titrations near 0.00 mL or correct both the initial and the final readings. Use the calibration curve whenever you use your buret.

Notes and References

1. R. H. Hill and D. Finster, *Laboratory Safety for Chemistry Students* (Hoboken, NJ: Wiley, 2010).
2. *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards* (Washington: National Academies Press, 2011), http://www.nap.edu/catalog.php?record_id=12654; R. J. Lewis, Sr., *Hazardous Chemicals Desk Reference*, 6th ed. (New York: Wiley, 2008); P. Patnaik, *A Comprehensive Guide to the Hazardous Properties of Chemical Substances*, 3rd ed. (New York: Wiley, 2007); G. Lunn and E. B. Sansone, *Destruction of Hazardous Chemicals in the Laboratory* (New York: Wiley, 1994); and M. A. Armour, *Hazardous Laboratory Chemical Disposal Guide*, 3rd ed. (Boca Raton, FL: CRC Press, 2003).
3. P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice* (New York: Oxford University Press, 1998); M. C. Cann and M. E. Connelly, *Real-World Cases in Green Chemistry* (Washington, DC: American Chemical Society, 2000); M. Lancaster, *Green Chemistry: An Introductory Text* (Cambridge: Royal Society of Chemistry, 2002); C. Baird and M. Cann, *Environmental Chemistry*, 3rd ed. (New York: W. H. Freeman and Company, 2005); J. E. Girard, *Principles of Environmental Chemistry* (Sudbury, MA: Bartlett, 2005); B. Braun, R. Charney, A. Clarens, J. Farrugia, C. Kitchens, C. Lisowski, D. Naistat, and A. O’Neil, *J. Chem. Ed.* **2006**, 83, 1126.
4. Videos illustrating basic laboratory techniques are available from the *Journal of Chemical Education* at www.jce.divched.org/ and also from www.academysavant.com.
5. Prepare cleaning solution by dissolving 36 g of ammonium peroxydisulfate, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, in a *loosely stoppered* 2.2-L (“one gallon”) bottle of 98 wt% sulfuric acid. Add ammonium peroxydisulfate every few weeks to maintain the oxidizing strength. Alternative, far less hazardous cleaners that do not generate toxic waste are available. For example, see the Web site for International Products Corp.: <http://www.ipcol.com/>.
6. M. M. Singh, C. McGowan, Z. Szafran, and R. M. Pike, *J. Chem. Ed.* **1998**, 75, 371; *J. Chem. Ed.* **2000**, 77, 625.
7. R. H. Obenauf and N. Kocherlakota, *Spectroscopy Applications Supplement*, March 2006, p. 12.
8. D. Bohrer, P. Cícero do Nascimento, P. Martins, and R. Binotto, *Anal. Chim. Acta* **2002**, 459, 267.

Experimental Error



[Courtesy 3M Company, St. Paul, MN.]

Some laboratory errors are more obvious than others, but there is error associated with every measurement. There is no way to measure the "true" value of anything. The best we can do in a chemical analysis is to carefully apply a technique that experience tells us is reliable. Repetition of one type of measurement several times tells us the reproducibility (*precision*) of the measurement. Measuring the same quantity by different methods gives us confidence of nearness to the "truth" (*accuracy*), if the results agree with one another.

Math Toolkit

Suppose that you measure the density of a mineral by finding its mass (4.635 ± 0.002 g) and its volume (1.13 ± 0.05 mL). Density is mass per unit volume: $4.635 \text{ g}/1.13 \text{ mL} = 4.101\overline{8}$ g/mL. The uncertainties in mass and volume are ± 0.002 g and ± 0.05 mL, but what is the uncertainty in the computed density? And how many significant figures should be used for the density? This chapter answers these questions and introduces spreadsheets—a powerful tool that will be invaluable to you in and out of this course.

3-1 Significant Figures

The number of **significant figures** is the minimum number of digits needed to write a given value in scientific notation without loss of precision. The number 142.7 has four significant figures because it can be written 1.427×10^2 . If you write $1.427\overline{0} \times 10^2$, you imply that you know the value of the digit after 7, which is not the case for the number 142.7. The number $1.427\overline{0} \times 10^2$ has five significant figures.

The number 6.302×10^{-6} has four significant figures, because all four digits are necessary. You could write the same number as 0.000 006 302, which also has just *four* significant figures. The zeros to the left of the 6 are merely holding decimal places. The number 92 500 is ambiguous. It could mean any of the following:

9.25×10^4	3 significant figures
9.250×10^4	4 significant figures
$9.250\overline{0} \times 10^4$	5 significant figures

You should write one of these three numbers, instead of 92 500, to indicate how many figures are actually known.

Zeros are significant when they are (1) in the middle of a number or (2) at the end of a number on the right-hand side of a decimal point.

The last (farthest to the right) significant figure in a measured quantity always has some associated uncertainty. The minimum uncertainty is ± 1 in the last digit. The scale of a Spectronic 20 spectrophotometer is drawn in Figure 3-1. The needle in the figure appears to be at an absorbance value of 0.234. We say that this number has three significant figures because the numbers 2 and 3 are completely certain and the number 4 is an estimate. The value might be read 0.233 or 0.235 by other people. The percent transmittance is near 58.3. The transmittance scale is smaller than the absorbance scale at this point, so there is more uncertainty in the last digit of transmittance. A reasonable estimate of the uncertainty might be 58.3 ± 0.2 . There are three significant figures in the number 58.3.

Significant figures: minimum number of digits required to express a value in scientific notation without loss of precision

Significant zeros are **bold**:

106 0.010 6 0.106 0.106 0

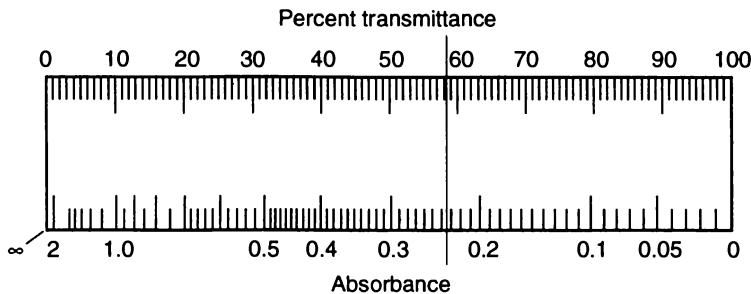


Figure 3-1 Scale of a Bausch and Lomb Spectronic 20 spectrophotometer. Percent transmittance is a linear scale and absorbance is a logarithmic scale.

Interpolation: estimate all readings to the nearest tenth of the distance between scale divisions

When reading the scale of any apparatus, *interpolate* between the markings. Try to estimate to the nearest tenth of the distance between two marks. Thus, on a 50-mL buret, which is graduated to 0.1 mL, read the level to the nearest 0.01 mL. When using a ruler calibrated in millimeters, estimate distances to the nearest tenth of a millimeter.

Ask Yourself

3-A. How many significant figures are there in each number below?

- (a) 1.903 0 (b) 0.039 10 (c) 1.40×10^4
-

3-2 Significant Figures in Arithmetic

We now address the question of how many digits to retain in the answer after you have performed arithmetic operations with your data. Rounding should be done only on the *final answer* (not intermediate results), to avoid accumulating round-off errors.

Addition and Subtraction

If the numbers to be added or subtracted have equal numbers of digits, the answer is given to the *same decimal place* as that in any of the individual numbers:

$$\begin{array}{r} 1.362 \times 10^{-4} \\ + 3.111 \times 10^{-4} \\ \hline 4.473 \times 10^{-4} \end{array}$$

The number of significant figures in the answer may exceed or be less than that in the original data.

$$\begin{array}{r} 5.345 \\ + 6.728 \\ \hline 12.073 \end{array} \quad \begin{array}{r} 7.26 \times 10^{14} \\ - 6.69 \times 10^{14} \\ \hline 0.57 \times 10^{14} \end{array}$$

If the numbers being added do not have the same number of significant figures, we are limited by the least-certain one. For example, in a calculation of the molecular mass of KrF₂, the answer is known only to the third decimal place, because we are limited by our knowledge of the atomic mass of Kr.

$$\begin{array}{r}
 18.998\ 403\ 2 \quad (\text{F}) \\
 + 18.998\ 403\ 2 \quad (\text{F}) \\
 + 83.798 \quad (\text{Kr}) \\
 \hline
 121.794\ \underbrace{806\ 4}_{\text{Not significant}}
 \end{array}$$

The number 121.794 806 4 should be rounded to 121.795 as the final answer.

When rounding off, look at *all* the digits *beyond* the last place desired. In the preceding example, the digits 806 4 lie beyond the last significant decimal place. Because this number is more than halfway to the next higher digit, we round the 4 up to 5 (that is, we round up to 121.795 instead of down to 121.794). If the insignificant figures were less than halfway, we would round down. For example, 121.794 3 is rounded to 121.794.

In the special case where the number is exactly halfway, round to the nearest *even* digit. Thus, 43.55 is rounded to 43.6, if we can have only three significant figures. If we are retaining only three figures, 1.425×10^{-9} becomes 1.42×10^{-9} . The number $1.425\ 01 \times 10^{-9}$ would become 1.43×10^{-9} , because 501 is more than halfway to the next digit. The rationale for rounding to an even digit is to avoid systematically increasing or decreasing results through successive round-off errors. Half the round-offs will be up and half down.

In adding or subtracting numbers expressed in scientific notation, we should express all numbers with the same exponent:

$$\begin{array}{r}
 1.632 \times 10^5 \qquad \qquad 1.632 \times 10^5 \\
 + 4.107 \times 10^3 \Rightarrow + 0.041\ 07 \times 10^5 \\
 + 0.984 \times 10^6 \qquad \qquad \qquad + 9.84 \times 10^5 \\
 \hline
 \qquad \qquad \qquad 11.51 \times 10^5
 \end{array}$$

The sum $11.513\ 07 \times 10^5$ is rounded to 11.51×10^5 because the number 9.84×10^5 limits us to two decimal places when all numbers are expressed as multiples of 10^5 .

Rules for rounding off numbers.

Addition and subtraction: Express all numbers with the same exponent and align all numbers with respect to the decimal point. Round off the answer according to the number of decimal places in the number with the fewest decimal places.

Challenge Show that the answer has four significant figures even if all numbers are expressed as multiples of 10^4 instead of 10^5 .

Multiplication and Division

In multiplication and division, we are normally limited to the number of digits contained in the number with the fewest significant figures:

$$\begin{array}{r}
 3.26 \times 10^{-5} \qquad \qquad 4.317\ 9 \times 10^{12} \qquad \qquad 34.60 \\
 \times 1.78 \qquad \qquad \times 3.6 \qquad \times 10^{-19} \qquad \div 2.462\ 87 \\
 \hline
 5.80 \times 10^{-5} \qquad 1.6 \qquad \times 10^{-6} \qquad \qquad \qquad 14.05
 \end{array}$$

The power of 10 has no influence on the number of figures that should be retained.

Example Significant Figures in Molecular Mass

Find the molecular mass of $C_{14}H_{10}$ with the correct number of significant digits.

SOLUTION Multiply the atomic mass of C by 14 and the atomic mass of H by 10 and add the products together.

$$14 \times 12.010\ 7 = 168.149_8 \leftarrow 6 \text{ significant figures because } 12.010\ 7 \text{ has 6 digits}$$
$$10 \times 1.007\ 94 = \frac{10.079\ 4}{178.229_2} \leftarrow 6 \text{ significant figures because } 1.007\ 94 \text{ has 6 digits}$$

A reasonable answer is 178.229. The molecular mass is limited to three decimal places because the last significant digit in $14 \times 12.010\ 7 = 168.149_8$ is the third decimal place. We often retain an extra (subscripted) digit beyond the last significant figure to avoid introducing round-off error into subsequent calculations.

 **Test Yourself** Find the molecular mass of $C_{14}H_{10}O_8$ with the correct number of significant digits. (Answer: 306.224)

Logarithms and Antilogarithms

If $n = 10^a$, then we say that a is the base 10 **logarithm** of n :

$$\text{Logarithm of } n: \quad n = 10^a \text{ means that } \log n = a \quad (3-1)$$

$$10^{-3} = \frac{1}{10^3} = \frac{1}{1\ 000} = 0.001$$

For example, 2 is the logarithm of 100 because $100 = 10^2$. The logarithm of 0.001 is -3 because $0.001 = 10^{-3}$. To find the logarithm of a number with your calculator, enter the number and press the *log* function.

In Equation 3-1, the number n is said to be the **antilogarithm** of a . That is, the antilogarithm of 2 is 100 because $10^2 = 100$ and the antilogarithm of -3 is 0.001 because $10^{-3} = 0.001$. Your calculator may have a 10^x key or an *antilog* key or an *INV log* key. To find the antilogarithm of a number, enter it in your calculator and press 10^x (or *antilog* or *INV log*).

A logarithm is composed of a **characteristic** and a **mantissa**. The characteristic is the integer part and the mantissa is the decimal part:

$$\begin{array}{ll} \log 339 = \underbrace{2.530}_{\substack{\text{Characteristic} \\ = 2}} & \log 3.39 \times 10^{-5} = \underbrace{-4.470}_{\substack{\text{Mantissa} \\ = 0.470}} \\ & \text{Characteristic} \quad \text{Mantissa} \\ & = -4 \quad = 0.470 \end{array}$$

The number 339 can be written 3.39×10^2 . The number of digits in the mantissa of $\log 339$ should equal the number of significant figures in 339. The logarithm of 339 is properly expressed as 2.530. The characteristic, 2, corresponds to the exponent in 3.39×10^2 .

To see that the third decimal place is the last significant place, consider the following results:

$$10^{2.531} = 340 \ (339.6)$$

$$10^{2.530} = 339 \ (338.8)$$

$$10^{2.529} = 338 \ (338.1)$$

Number of digits in *mantissa* of $\log x$ = number of significant figures in x :

$$\log(\underbrace{5.403 \times 10^{-8}}_{\substack{4 \text{ digits}}}) = -7.267\ 4 \quad \underbrace{4 \text{ digits}}$$

The numbers in parentheses are the results prior to rounding to three figures. Changing the exponent by one digit in the third decimal place changes the answer by one digit in the last (third) place of 339.

In converting a logarithm to its antilogarithm, *the number of significant figures in the antilogarithm should equal the number of digits in the mantissa*. Thus

$$\text{antilog } (-3.42) = \underbrace{10}_{\substack{2 \\ \text{digits}}}^{-3.\underbrace{42}_{2 \text{ digits}}} = \underbrace{3.8}_{2 \text{ digits}} \times \underbrace{10^{-4}}_{2 \text{ digits}}$$

Here are some examples showing the proper use of significant figures:

$$\begin{array}{ll} \log 0.001\,237 = -2.907\,6 & \text{antilog } 4.37 = 2.3 \times 10^4 \\ \log 1\,237 = 3.092\,4 & 10^{4.\underbrace{37}_{3 \text{ digits}}} = 2.3 \times 10^4 \\ \log 3.2 = 0.51 & 10^{-2.\underbrace{600}_{3 \text{ digits}}} = 2.51 \times 10^{-3} \end{array}$$

Number of digits in antilog x ($= 10^x$) =
number of digits in *mantissa* of x :

$$10^{6.\underbrace{142}_{3 \text{ digits}}} = 1.39 \times 10^6$$

Ask Yourself

3-B. How would you express each answer with the correct number of digits?

- (a) $1.021 + 2.69 = 3.711$
- (b) $12.3 - 1.63 = 10.67$
- (c) $4.34 \times 9.2 = 39.928$
- (d) $0.060\,2 \div (2.113 \times 10^4) = 2.849\,03 \times 10^{-6}$
- (e) $\log(4.218 \times 10^{12}) = ?$
- (f) $\text{antilog}(-3.22) = ?$
- (g) $10^{2.384} = ?$

3-3 Types of Error

Every measurement has some uncertainty, which is called *experimental error*. Scientific conclusions can be expressed with a high or low degree of confidence, but never with complete certainty. Experimental error is classified as either *systematic* or *random*.

Systematic Error

A **systematic error**, also called a **determinate error**, is repeatable if you make the measurement over again in the same way. In principle, a systematic error can be discovered and corrected. For example, suppose you think that the pH of a buffer used to standardize a pH meter is 7.00, but it is really 7.08. If the meter is otherwise working properly, all pH readings will be 0.08 pH unit too low. When you read a pH of 5.60, the actual pH of the sample is 5.68. This systematic error could be discovered by using another buffer of known pH to test the meter.

Another systematic error arises from an uncalibrated buret. The manufacturer's tolerance for a Class A 50-mL buret is ± 0.05 mL. When you think you have delivered 29.43 mL, the real volume could be 29.40 mL and still be within tolerance. One way to correct for an error of this type is by constructing an experimental calibration curve (Figure 3-2). To do this, deliver distilled water from the buret into a flask and weigh it. Convert the mass of water into volume by using Table 2-5. The graph tells us to apply a correction factor of -0.03 mL to the measured value of 29.43 mL to reach the correct value of 29.40 mL.

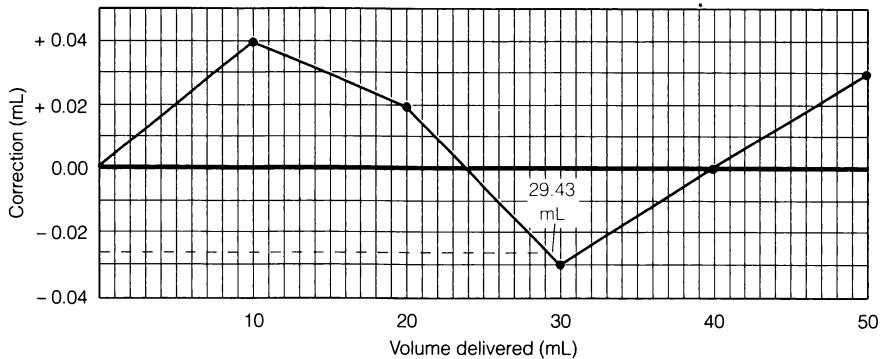
Systematic error might be positive in some regions and negative in others. The error is repeatable and, with care and cleverness, you can detect and correct it.

Systematic error is a consistent error that can be detected and corrected. Standard Reference Materials described in Box 3-1 are designed to reduce systematic errors. Box 3-2 provides a case study.

Ways to detect systematic error:

1. Analyze known sample, such as a Standard Reference Material. You should observe the known answer. (See Box 15-1 for an example.)
2. Analyze "blank" sample containing no analyte. If you observe a nonzero result, your method responds to more than you intend.
3. Use different analytical methods for the same analyte. If results do not agree, there is error in one (or more) of the methods.
4. *Round robin* experiment: Analyze identical samples in different laboratories by different people using the same or different methods. Disagreement beyond the expected random error is systematic error.

Figure 3-2 Calibration curve for a 50-mL buret.



Random Error

Random error cannot be eliminated, but it might be reduced by a better experiment.

Random error, also called **indeterminate error**, arises from limitations on our ability to make physical measurements and on natural fluctuations in the quantity being measured. Random error has an equal chance of being positive or negative. It is always present and cannot be corrected. One random error is that associated with reading a scale. Different people reading the scale in Figure 3-1 report a range of

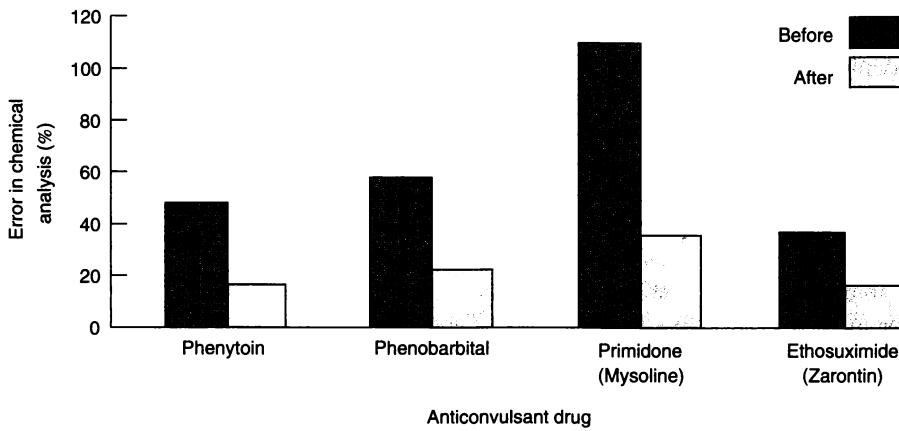
Box 3-1 What Are Standard Reference Materials?

Inaccurate laboratory measurements can mean wrong medical diagnosis and treatment, lost production time, wasted energy and materials, manufacturing rejects, and product liability. To minimize errors, the U.S. National Institute of Standards and Technology and institutes of standards of other nations distribute standard reference materials, such as metals, chemicals, rubber, plastics, engineering materials, radioactive substances, and environmental and clinical standards that can be used to test the accuracy of analytical procedures.

For example, in treating patients with epilepsy, physicians depend on laboratory tests to measure blood serum concentrations of anticonvulsant drugs. Low

drug levels lead to seizures, and high levels are toxic. Tests of identical serum specimens at different laboratories gave an unacceptably wide range of results. Therefore the National Institute of Standards and Technology developed a standard reference serum containing known levels of antiepilepsy drugs. The reference serum allows different laboratories to detect and correct errors in their assay procedures.

Before introduction of this reference material, five laboratories analyzing identical samples reported a range of results with relative errors of 40% to 110% of the expected value. After distribution of the reference material, the error was reduced to 20% to 40%.



Box 3-2 Case Study: Systematic Error in Ozone Measurement*

Ozone (O_3) is a corrosive gas that harms your lungs and all forms of life. It is formed near the surface of the Earth by the action of sunlight on air pollutants largely derived from automobile exhaust. The U.S. Environmental Protection Agency sets an 8-h average O_3 limit of 80 ppb (80 nL/L by volume) in air.

In the past, O_3 monitors often exhibited erratic behavior on hot, humid days. It was conjectured that half of the regions deemed to be out of compliance with the O_3 standard might actually have been under the legal limit. This error could force expensive remediation measures when none were required. Conversely, there were rumors that some unscrupulous operators of O_3 monitors were aware that zeroing their instrument at night when the humidity is higher produced lower O_3 readings the next day, thereby reducing the number of days when a region is deemed out of compliance.

Common instruments measure O_3 by its absorption of ultraviolet radiation. Prior to measuring an air sample, the instrument is zeroed while measuring air from which O_3 has been removed by a chemical scrubber. A study of commercial O_3 monitors found that controlled changes in humidity led to *systematic errors in the apparent O_3 concentration of tens to hundreds of ppb*—up to several times greater than the O_3 being measured. Increasing humidity produced systematic *positive* errors in some types of instruments and systematic *negative* errors in other types. The error could be eliminated by installing water-permeable tubing to equalize the humidity in air being measured and air used to zero the instrument.

* From K. L. Wilson and J. W. Birks, *Environ. Sci. Technol.* 2006, 40, 6361.

values representing their interpolation between the markings. One person reading an instrument several times might report several different readings. Another indeterminate error comes from random electrical noise in an instrument. Positive and negative fluctuations occur with approximately equal frequency and cannot be completely eliminated. Still another source of random error is actual variation in the quantity being measured. If you were to measure the pH of blood in your body, you might get different answers for blood from different parts of the body and the pH at a given location might vary with time. There is some random uncertainty in “the pH of your blood” even if there were no variations in the measuring device.

Precision and Accuracy

Precision is a measure of the reproducibility of a result. **Accuracy** is how close a measured value is to the “true” value.

A measurement might be reproducible, but wrong. For example, if you made a mistake preparing a solution for a titration, the solution would not have the desired concentration. You might then do a series of reproducible titrations but report an incorrect result because the concentration of the titrating solution was not what you intended. In this case, the precision is good but the accuracy is poor. Conversely, it is possible to make poorly reproducible measurements clustered around the correct value. In this case, the precision is poor but the accuracy is good. An ideal procedure is both accurate and precise.

Accuracy is defined as nearness to the “true” value. The word *true* is in quotes because somebody must *measure* the “true” value, and there is error associated with *every* measurement. The “true” value is best obtained by an experienced person using a well-tested procedure. It is desirable to test the result by using different procedures because, even though each method might be precise, systematic error could lead to poor agreement between methods. Good agreement among several methods affords us confidence, but never proof, that the results are “true.”

Precision: reproducibility

Accuracy: nearness to the “truth”

Absolute and Relative Uncertainty

Absolute uncertainty expresses the margin of uncertainty associated with a measurement. If the estimated uncertainty in reading a calibrated buret is ± 0.02 mL, we say that ± 0.02 mL is the absolute uncertainty associated with the reading.

Relative uncertainty compares absolute uncertainty with its associated measurement. The relative uncertainty of a buret reading of 12.35 ± 0.02 mL is a dimensionless quotient:

$$\begin{aligned} \text{Relative uncertainty:} \quad \text{relative uncertainty} &= \frac{\text{absolute uncertainty}}{\text{magnitude of measurement}} \quad (3-2) \\ &= \frac{0.02 \text{ mL}}{12.35 \text{ mL}} = 0.002 \end{aligned}$$

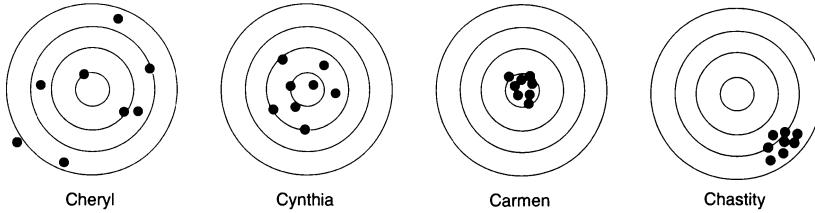
Percent relative uncertainty is simply

$$\begin{aligned} \text{Percent relative uncertainty:} \quad \text{percent relative uncertainty} &= 100 \times \text{relative uncertainty} \quad (3-3) \\ &= 100 \times 0.002 = 0.2\% \end{aligned}$$

If the absolute uncertainty in reading a buret is constant at ± 0.02 mL, the percent relative uncertainty is $\pm 0.2\%$ for a volume of 10 mL and $\pm 0.1\%$ for a volume of 20 mL.

Ask Yourself

3-C. Cheryl, Cynthia, Carmen, and Chastity shot these targets at Girl Scout camp. Match each target with the proper description.



- (a) accurate and precise (c) precise but not accurate
(b) accurate but not precise (d) neither precise nor accurate

3-4 Propagation of Uncertainty

We can usually estimate or measure the random error associated with a measurement, such as the length of an object or the temperature of a solution. The uncertainty might be based on how well we can read an instrument or on our experience with a particular method. If possible, uncertainty is expressed as the *standard deviation* or as a *confidence interval* based on a series of replicate measurements. The

Standard deviation and confidence interval are discussed in Chapter 4.

following discussion applies only to random error. We assume that systematic error has been detected and corrected.

In most experiments, it is necessary to perform arithmetic operations on several numbers, each of which has an associated random error. The most likely uncertainty in the result is not simply the sum of the individual errors, because some of them are likely to be positive and some negative. We expect some cancellation of errors.

Addition and Subtraction

Suppose you wish to perform the following arithmetic, in which experimental uncertainties, designated e_1 , e_2 , and e_3 , are given in parentheses.

$$\begin{array}{r} 1.76 (\pm 0.03) \leftarrow e_1 \\ + 1.89 (\pm 0.02) \leftarrow e_2 \\ - 0.59 (\pm 0.02) \leftarrow e_3 \\ \hline 3.06 (\pm e_4) \end{array} \quad (3-4)$$

The arithmetic answer is 3.06; but what is the uncertainty associated with this result?

For addition and subtraction, the uncertainty in the answer is obtained from the *absolute uncertainties* of the individual terms as follows:

*Uncertainty in addition
and subtraction:*

$$e_4 = \sqrt{e_1^2 + e_2^2 + e_3^2} \quad (3-5)$$

For the sum in expression 3-4, we can write

$$e_4 = \sqrt{(0.03)^2 + (0.02)^2 + (0.02)^2} = 0.04_1$$

The absolute uncertainty e_4 is ± 0.04 , and we can write the answer as 3.06 ± 0.04 . Although there is only one significant figure in the uncertainty, we wrote it initially as 0.04_1 , with the first insignificant figure subscripted. We retain one or more insignificant figures to avoid introducing round-off errors into later calculations through the number 0.04_1 . The insignificant digit was subscripted to remind us where the last significant digit should be at the conclusion of the calculations.

To find the percent relative uncertainty in the sum of expression 3-4, we write

$$\text{percent relative uncertainty} = \frac{0.04_1}{3.06} \times 100 = 1.3\%$$

The uncertainty, 0.04_1 , is 1.3% of the result, 3.06. The subscript 3 in 1.3% is not significant. It is sensible to drop the insignificant figures now and express the final result as

$$\begin{array}{ll} 3.06(\pm 0.04) & \text{(absolute uncertainty)} \\ 3.06(\pm 1\%) & \text{(relative uncertainty)} \end{array}$$

For addition and subtraction, use absolute uncertainty.

For addition and subtraction, use absolute uncertainty. Relative uncertainty can be found at the end of the calculation.

Example Uncertainty in a Buret Reading

The volume delivered by a buret is the difference between the final and initial readings. If the uncertainty in each reading is ± 0.02 mL, what is the uncertainty in the volume delivered?

SOLUTION Suppose that the initial reading is 0.05 (± 0.02) mL and the final reading is 17.88 (± 0.02) mL. The volume delivered is the difference:

$$\begin{array}{r} 17.88 (\pm 0.02) \\ - 0.05 (\pm 0.02) \\ \hline 17.83 (\pm e) \end{array} \quad e = \sqrt{0.02^2 + 0.02^2} = 0.03$$

Regardless of the initial and final readings, if the uncertainty in each one is ± 0.02 mL, the uncertainty in volume delivered is ± 0.03 mL.

 **Test Yourself** Suppose that the uncertainty in measuring pH is ± 0.03 pH units. The pH of two solutions is measured to be 8.23 and 4.01. Find the difference in pH and its uncertainty. (Answer: 4.22 ± 0.04)

Multiplication and Division

For multiplication and division, first convert all uncertainties to percent relative uncertainties. Then calculate the error of the product or quotient as follows:

For multiplication and division, use percent relative uncertainty.

Uncertainty in multiplication and division:

$$\%e_4 = \sqrt{(\%e_1)^2 + (\%e_2)^2 + (\%e_3)^2} \quad (3-6)$$

For example, consider the following operations:

$$\frac{1.76 (\pm 0.03) \times 1.89 (\pm 0.02)}{0.59 (\pm 0.02)} = 5.64 \pm e_4$$

First, convert absolute uncertainties to percent relative uncertainties:

$$\frac{1.76 (\pm 1.7\%) \times 1.89 (\pm 1.1\%)}{0.59 (\pm 3.4\%)} = 5.64 \pm e_4$$

Then, find the percent relative uncertainty of the answer by using Equation 3-6.

$$\%e_4 = \sqrt{(1.7)^2 + (1.1)^2 + (3.4)^2} = 4.0\%$$

The answer is $5.64 (\pm 4.0\%)$.

To convert relative uncertainty to absolute uncertainty, find 4.0% of the answer:

$$4.0\% \times 5.64 = 0.04_0 \times 5.64 = 0.2_3$$

The answer is $5.64 (\pm 0.2_3)$. Finally, drop the insignificant digits:

$$\begin{array}{ll} 5.6 (\pm 0.2) & \text{(absolute uncertainty)} \\ 5.6 (\pm 4\%) & \text{(relative uncertainty)} \end{array}$$

For multiplication and division, use percent relative uncertainty. Absolute uncertainty can be found at the end of the calculation.

The denominator of the original problem, 0.59, limits the answer to two significant digits.

Example Scientific Notation and Propagation of Uncertainty

Express the absolute uncertainty in

(a) $\frac{3.43 (\pm 0.08) \times 10^{-8}}{2.11 (\pm 0.04) \times 10^{-3}}$

(b) $[3.43 (\pm 0.08) \times 10^{-8}] + [2.11 (\pm 0.04) \times 10^{-7}]$

SOLUTION (a) The uncertainty ± 0.08 applies to the number 3.43. Therefore the uncertainty in the numerator is $100 \times 0.08/3.43 = 2.332\%$. (Remember to keep extra digits in your calculator until the end of the problem. Do not round off until the end.) The uncertainty in the denominator is $100 \times 0.04/2.11 = 1.896\%$. The uncertainty in the answer is $\sqrt{2.332\%^2 + 1.896\%^2} = 3.006\%$. The quotient is $(3.43 \times 10^{-8})/(2.11 \times 10^{-3}) = 1.63 \times 10^{-5}$ and the uncertainty is 3.006% of 1.63 = 0.05. The answer is $1.63 (\pm 0.05) \times 10^{-5}$.

(b) For addition and subtraction, we must express each term with the same power of ten. Let's write the second number as a multiple of 10^{-8} instead of 10^{-7} . To do this, multiply 2.11 and 0.04 by 10 and divide 10^{-7} by 10:

$$\begin{array}{rcl} 3.43 (\pm 0.08) \times 10^{-8} & & 3.43 (\pm 0.08) \times 10^{-8} \\ + 2.11 (\pm 0.04) \times 10^{-7} & \Rightarrow & + 21.1 (\pm 0.4) \times 10^{-8} \\ & & \hline 24.5_3 (\pm e) \times 10^{-8} \end{array}$$

$$e = \sqrt{0.08^2 + 0.4^2} = 0.4_1 \quad \Rightarrow \quad \text{Answer: } 24.5 (\pm 0.4) \times 10^{-8}$$

 **Test Yourself** Find the difference $4.22 (\pm 0.04) \times 10^{-3} - 3.8 (\pm 0.6) \times 10^{-4}$ and the product $[4.22 (\pm 0.04) \times 10^{-3}][3.8 (\pm 0.6) \times 10^{-4}]$. (**Answers:** 3.84 (± 0.07) $\times 10^{-3}$, $1.60 (\pm 0.25) \times 10^{-6}$)

In the Test Yourself problem above, you evaluated the product $[4.22 (\pm 0.04) \times 10^{-3}][3.8 (\pm 0.6) \times 10^{-4}] = 1.6036 (\pm 0.2537) \times 10^{-6}$. In deciding how many decimal places to retain in the answer, two reasonable choices are 1.6 (± 0.3) or 1.60 (± 0.25). When an answer lies between 1 and 2, it is good practice to retain an extra decimal place to avoid excessive round-off. The uncertainty 0.3 differs from the uncertainty 0.25 by $(0.3 - 0.25)/0.25 = 20\%$. We lose fidelity in the answer by throwing away the extra decimal place, so I prefer 1.60 (± 0.25) rather than 1.6 (± 0.3). You could write $1.6_0 (\pm 0.2_5)$ to emphasize that the first uncertain digit is 6 and the subscripted 0 is even less certain.

It is fine to keep an extra decimal place in an answer to avoid excessively large round-off.

Mixed Operations

Now consider an operation containing subtraction and division:

$$\frac{[1.76 (\pm 0.03) - 0.59 (\pm 0.02)]}{1.89 (\pm 0.02)} = 0.619_0 \pm ?$$

First work out the difference in the numerator, by using absolute uncertainties:

$$1.76 (\pm 0.03) - 0.59 (\pm 0.02) = 1.17 (\pm 0.03_6)$$

because $\sqrt{(0.03)^2 + (0.02)^2} = 0.03_6$.

Then convert to percent relative uncertainties:

$$\frac{1.17 (\pm 0.03_6)}{1.89 (\pm 0.02)} = \frac{1.17 (\pm 3.1\%)}{1.89 (\pm 1.1\%)} = 0.619_0 (\pm 3.3\%)$$

because $\sqrt{(3.1\%)^2 + (1.1\%)^2} = 3.3\%$

The percent relative uncertainty is 3.3%, so the absolute uncertainty is $0.03_3 \times 0.619_0 = 0.02_0$. The final answer can be written

$$\begin{array}{ll} 0.619 (\pm 0.02_0) & \text{(absolute uncertainty)} \\ 0.619 (\pm 3.3\%) & \text{(relative uncertainty)} \end{array}$$

The result of a calculation ought to be written in a manner consistent with the uncertainty in the result.

Because the uncertainty begins in the 0.01 decimal place, it is reasonable to round the result to the 0.01 decimal place:

$$\begin{array}{ll} 0.62 (\pm 0.02) & \text{(absolute uncertainty)} \\ 0.62 (\pm 3\%) & \text{(relative uncertainty)} \end{array}$$

The Real Rule for Significant Figures

The real rule: The first uncertain figure is the last significant figure. There is no harm keeping an extra, subscripted digit beyond the first uncertain digit to avoid excessive round-off.

$$\frac{0.002\ 364 (\pm 0.000\ 003)}{0.025\ 00 (\pm 0.000\ 05)} = 0.094\ 6 (\pm 0.000\ 2)$$

the uncertainty ($\pm 0.000\ 2$) is in the fourth decimal place. Therefore the answer is reasonably expressed with *three* significant figures, even though the original data have four figures. The first uncertain figure of the answer is the last significant figure. The quotient

$$\frac{0.002\ 664 (\pm 0.000\ 003)}{0.025\ 00 (\pm 0.000\ 05)} = 0.106\ 6 (\pm 0.000\ 2)$$

is expressed with *four* significant figures because the uncertainty is in the fourth decimal place. The quotient

$$\frac{0.821 (\pm 0.002)}{0.803 (\pm 0.002)} = 1.022 (\pm 0.004)$$

is expressed with *four* figures even though the dividend and divisor each have *three* figures. If we rounded the answer to three digits, it would be $1.02 (\pm 0.00)$, which loses all information about the uncertainty. We need to keep enough digits to meaningfully represent the uncertainty.

Example Significant Figures in Laboratory Work

You prepared a 0.250 M NH_3 solution by diluting 8.45 (± 0.04) mL of 28.0 (± 0.5) wt% NH_3 [density = 0.899 (± 0.003) g/mL] up to 500.0 (± 0.2) mL. Find the uncertainty in 0.250 M. Consider the molecular mass of NH_3 , 17.031 g/mol, to have negligible uncertainty.

SOLUTION To find the uncertainty in molarity, you need to find the uncertainty in moles delivered to the 500-mL flask. The concentrated reagent contains 0.899 (± 0.003) g of solution per milliliter. The weight percent tells you that the reagent contains 0.280 (± 0.005) g of NH₃ per gram of solution. In the following calculations, retain extra insignificant digits and round off only at the end.

$$\left. \begin{array}{l} \text{grams of NH}_3 \\ \text{per mL in} \\ \text{concentrated} \\ \text{reagent} \end{array} \right\} = 0.899 (\pm 0.003) \frac{\text{g solution}}{\text{mL}} \times 0.280 (\pm 0.005) \frac{\text{g NH}_3}{\text{g solution}}$$

$$= 0.899 (\pm 0.334\%) \frac{\text{g solution}}{\text{mL}} \times 0.280 (\pm 1.79\%) \frac{\text{g NH}_3}{\text{g solution}}$$

$$= 0.2517 (\pm 1.82\%) \frac{\text{g NH}_3}{\text{mL}}$$

because $\sqrt{(0.334\%)^2 + (1.79\%)^2} = 1.82\%$.

Next, find the moles of ammonia in 8.45 (± 0.04) mL of concentrated reagent. The relative uncertainty in volume is $\pm 0.04/8.45 = \pm 0.473\%$.

$$\text{mol NH}_3 = \frac{0.2517 (\pm 1.82\%) \frac{\text{g NH}_3}{\text{mL}} \times 8.45 (\pm 0.473\%) \text{mL}}{17.031 \frac{\text{g NH}_3}{\text{mol}}}$$

$$= 0.1249 (\pm 1.88\%) \text{ mol}$$

because $\sqrt{(1.82\%)^2 + (0.473\%)^2 + (0\%)^2} = 1.88\%$.

This much ammonia was diluted to 0.5000 (± 0.0002) L. The relative uncertainty in final volume is $\pm 0.0002/0.5000 = \pm 0.04\%$. The diluted molarity is

$$\frac{\text{mol NH}_3}{\text{L}} = \frac{0.1249 (\pm 1.88\%) \text{ mol}}{0.5000 (\pm 0.04\%) \text{ L}}$$

$$= 0.2498 (\pm 1.88\%) \text{ M}$$

because $\sqrt{(1.88\%)^2 + (0.04\%)^2} = 1.88\%$. The absolute uncertainty is 1.88% of 0.2498 M = 0.0188 × 0.2498 M = 0.0047 M. The result 0.0047 tells us that the uncertainty in molarity begins in the third decimal place, so your final, rounded answer is

$$[\text{NH}_3] = 0.250 (\pm 0.005) \text{ M}$$



Test Yourself The uncertainty in [NH₃] above is $\pm 1.9\%$. If the uncertainty in wt% NH₃ were 1.0% instead of 1.8%, what would be the relative uncertainty in [NH₃]? (Answer: $\pm 1.2\%$)

For multiplication and division, convert absolute uncertainty to percent relative uncertainty.

By far, the largest uncertainty in the initial data is in wt%, which has an uncertainty of 0.005/0.280 = 1.79%. The only way to decrease uncertainty in the result (0.250 \pm 0.005 M) is to know the wt% of NH₃ reagent more precisely. Improving the other numbers does not help.

Uncertainty in pH and [H⁺]

When we study acids and bases, we will use pH, which is the negative logarithm of H⁺ concentration. That is,

$$\text{pH} = -\log[\text{H}^+] \quad \text{and} \quad [\text{H}^+] = 10^{-\text{pH}} \quad (3-7)$$

If $a = -\log b$, then $-a = \log b$. Raising 10 to the quantity on each side of the equation gives

$$10^{-a} = 10^{\log b} = b$$

If the pH of a solution is 3.07, $[H^+] = 10^{-3.07} = 8.511 \times 10^{-4}$ M. If the uncertainty in pH is ± 0.03 , what is the uncertainty in $[H^+]$? We answer this question with the formula

$$\text{uncertainty in } [H^+] = 2.303[H^+] (\text{uncertainty in pH}) \quad (3-8)$$

where 2.303 is the natural logarithm of 10. For $\text{pH} = 3.07 \pm 0.03$,

$$\begin{aligned}\text{uncertainty in } [H^+] &= 2.303[H^+] (\text{uncertainty in pH}) \\ &= 2.303[8.511 \times 10^{-4} \text{ M}] (0.03) = 0.588 \times 10^{-4} \text{ M}\end{aligned}$$

It would be reasonable to write $[H^+] = 8.5 (\pm 0.6) \times 10^{-4}$ M. The uncertainty occurs in the first decimal place, so the answer is rounded to the first decimal place.

Ask Yourself

3-D. To help identify an unknown mineral in your geology class, you measured its mass and volume and found them to be 4.635 ± 0.002 g and 1.13 ± 0.05 mL.

- Find the percent relative uncertainty in the mass and volume.
- Write the density (= mass/volume) and its uncertainty with a reasonable number of digits.
- When the rock is left in contact with water, the liquid comes to $\text{pH} = 8.82 \pm 0.02$. Find $[H^+]$ and its uncertainty.

3-5 Introducing Spreadsheets

Spreadsheets are powerful tools for manipulating quantitative information with a computer. They allow us to conduct “what if” experiments in which we investigate effects such as changing acid strength or concentration on the shape of a titration curve. Any spreadsheet is suitable for exercises in this book. Our specific instructions apply to Microsoft Excel, which is widely available. You will need directions for your particular software. Although this book can be used without spreadsheets, you will be amply rewarded far beyond this course if you invest the time to learn to use spreadsheets.

A Spreadsheet for Temperature Conversions

Let’s prepare a spreadsheet to convert temperature from degrees Celsius to kelvins and degrees Fahrenheit by using formulas from Table 1-4:

$$K = ^\circ C + C_0 \quad (3-9a)$$

$$^\circ F = \left(\frac{9}{5}\right)^* ^\circ C + 32 \quad (3-9b)$$

where C_0 is the constant 273.15.

Figure 3-3a shows a blank spreadsheet as it would appear on your computer. Rows are numbered 1, 2, 3, . . . and columns are lettered A, B, C, . . . Each rectangular box is called a *cell*. The fourth cell down in the second column, for example, is designated cell B4.

We adopt a standard format in this book in which constants are collected in column A. Select cell A1 and type “Constant:” as a column heading. Select cell A2 and type “ $C_0 =$ ” to indicate that the constant C_0 will be written in the next cell down. Now select cell A3 and type the number 273.15. Your spreadsheet should now look like Figure 3-3b.

(a)

Rows	Columns			
	A	B	C	D
1				
2				
3				
4		cell B4		
5				
6				
7				
8				
9				
10				

(b)

	A	B	C	D
1	Constant:			
2	C0 =			
3	273.15			
4				
5				
6				
7				
8				
9				
10				

(c)

	A	B	C	D
1	Constant: °C		kelvin	°F
2	C0 =	-200	73.15	-328
3	273.15	-100	173.15	-148
4		0	273.15	32
5		100	373.15	212
6		200	473.15	392
7				
8				
9				
10				

(d)

	A	B	C	D
1	Constant: °C		kelvin	°F
2	C0 =	-200	73.15	-328
3	273.15	-100	173.15	-148
4		0	273.15	32
5		100	373.15	212
6		200	473.15	392
7				
8	Formulas:			
9	C2 = B2+\$A\$3			
10	D2 = (9/5)*B2+32			

Figure 3-3 Constructing a spreadsheet for temperature conversions.

In cell B1, type the label “°C” (or “Celsius” or whatever you like). For illustration, we enter the numbers -200 , -100 , 0 , 100 , and 200 in cells B2 through B6. This is our *input* to the spreadsheet. The *output* will be computed values of kelvins and °F in columns C and D. (If you want to enter very large or very small numbers, you can write, for example, $6.02E23$ for 6.02×10^{23} and $2E-8$ for 2×10^{-8} .)

Label column C “kelvin” in cell C1. In cell C2, we enter our first *formula*—an entry beginning with an equal sign. Select cell C2 and type “=B2+\$A\$3”. This expression tells the computer to calculate the contents of cell C2 by taking the contents of cell B2 and adding the contents of cell A3 (which contains the constant 273.15). We will explain the dollar signs shortly. When this formula is entered, the computer responds by calculating the number 73.15 in cell C2. This number is the kelvin equivalent of -200°C .

Now comes the beauty of a spreadsheet. Instead of typing many similar formulas, select cells C2, C3, C4, C5, and C6 all together. In the Home ribbon, go to Editing and select Fill and then Down (or just press the two keys Ctrl+D). This command tells the computer to do the same thing in cells C3 through C6 that was done in cell C2. The numbers 173.15, 273.15, 373.15, and 473.15 will appear in cells C3 through C6.

When computing the output in cell C3, the computer automatically uses input from cell B3 instead of cell B2. The reason for the dollar signs in \$A\$3 is that we do not want the computer to go down to cell A4 to find input for cell C3. \$A\$3 is called an *absolute reference* to cell A3. No matter what cell uses the constant C_0 , we want it to come from cell A3. The reference to cell B2 is a *relative reference*. Cell

The formula “=B2+\$A\$3” in cell C2 is equivalent to writing $K = ^{\circ}\text{C} + C_0$.

Absolute reference: \$A\$3
Relative reference: B2

C2 will use the contents of cell B2. Cell C6 will use the contents of cell B6. In general, references to constants in column A will be absolute (with dollar signs). References to numbers in the remainder of a spreadsheet will usually be relative (without dollar signs).

In cell D1, enter the label “°F”. In cell D2, type the formula “= (9/5)*B2+32”. This is equivalent to writing ${}^{\circ}\text{F} = (9/5){}^{\circ}\text{C} + 32$. The slash (/) is a division sign and the asterisk (*) is a multiplication sign. Parentheses are used to make the computer do what we intend. Operations inside parentheses are carried out before operations outside the parentheses. The computer responds to this formula by writing -328 in cell D2. This is the Fahrenheit equivalent to -200°C . Select cells D2 through D6. In the Home ribbon, go to Editing and select Fill and then Down to complete the table shown in Figure 3-3c.

Order of Operations

The arithmetic operations in a spreadsheet are addition, subtraction, multiplication, division, and exponentiation (which uses the symbol ^). The order of operations in formulas is ^ first, followed by * and / (evaluated in order from left to right as they appear), finally followed by + and - (also evaluated from left to right). Make liberal use of parentheses to be sure that the computer does what you intend. The contents of parentheses are evaluated first, before carrying out operations outside the parentheses. Here are some examples:

$$\begin{aligned} 9/5*100+32 &= (9/5)*100+32 = (1.8)*100+32 = (1.8*100)+32 = (180)+32 = 212 \\ 9/5*(100+32) &= 9/5*(132) = (1.8)*(132) = 237.6 \\ 9+5*100/32 &= 9+(5*100)/32 = 9+(500)/32 = 9+(500/32) = 9+(15.625) = 24.625 \\ 9/5^2+32 &= 9/(5^2)+32 = (9/25)+32 = (0.36)+32 = 32.36 \\ -2^2 &= 4 \quad \text{but} \quad -(2^2) = -4 \end{aligned}$$

When in doubt about how an expression will be evaluated by the computer, use parentheses to force it to do what you intend.

Documentation and Readability

If your spreadsheet cannot be read by another person without your help, it needs better documentation. (The same is true of your lab notebook!)

If you look at your spreadsheet next month, you will probably not know what formulas were used. Therefore we *document* the spreadsheet to show how it works by adding the text in cells A8, A9, and A10 in Figure 3-3d. In cell A8, write “Formulas:”. In cell A9, write “C2 = B2+\$A\$3” and, in cell A10, write “D2 = (9/5)*B2+32”. Documentation is an excellent practice for every spreadsheet. As you learn to use your spreadsheet, you should use Copy and Paste commands to copy the formulas used in cells C2 and D2 into the text in cells A9 and A10. This practice saves time and reduces transcription errors. Another basic form of documentation that we will add to future spreadsheets is a title in cell A1. A title such as “Temperature Conversions” tells us immediately what spreadsheet we are looking at.

For additional readability, select how many decimal places are displayed in a cell or column. The computer retains more digits for calculations. It does not throw away digits that are not displayed. You can also control whether numbers are displayed in decimal or exponential notation. To alter the format of a cell, in the Home ribbon, select Number and choose the way it will be displayed and the number of decimal places.

Ask Yourself

- 3-E. Reproduce the spreadsheet in Figure 3-3 on your computer. The boiling point of N₂ at 1 atm pressure is –196°C. Use your spreadsheet to find the kelvin and Fahrenheit equivalents of –196°C. Check your answers with your calculator.

3-6 Graphing in Excel

Humans require a visual display to understand the relation between two columns of numbers. This section introduces the basics of creating a graph with Excel.

First, we will generate some data to plot. The spreadsheet in Figure 3-4 computes the density of water as a function of temperature (°C) with the equation

$$\text{density (g/mL)} = a_0 + a_1*T + a_2*T^2 + a_3*T^3 \quad (3-10)$$

where $a_0 = 0.999\ 89$, $a_1 = 5.332\ 2 \times 10^{-5}$, $a_2 = -7.589\ 9 \times 10^{-6}$, and $a_3 = 3.671\ 9 \times 10^{-8}$. After writing a title in cell A1, enter the constants a_0 to a_3 in column A. Column B is labeled “Temp (°C)” and column C is labeled “Density (g/mL)”. Enter values of temperature in column B. In cell C4, type the formula “=A\$5 + \$A\$7*B4 + \$A\$9*B4^2 + \$A\$11*B4^3”, which uses the exponent symbol ^ to compute T^2 and T^3 . When you enter the formula, the number 0.999 97 is computed in cell C4. The remainder of column C is completed with a Fill Down command. The spreadsheet is not finished until it is documented by entering text in cells A13 and A14 to show what formula was used in column C.

Now we want to make a graph of density in column C versus temperature in column B. Density will appear on the y-axis (the **ordinate**) and temperature will be on the x-axis (the **abscissa**). There may be some variation from the following description in different versions of Excel.

To make a graph in Excel 2007 from the spreadsheet in Figure 3-4, go to the Insert ribbon and select Chart. Click on Scatter and select the icon for Scatter with Smooth Lines and Markers. The other most common graph we will make is Scatter with only Markers. Grab the blank chart with your mouse and move it to the right of the data. In Chart Tools, select Design and click on Select Data. Click on Add. For

Equation 3-10 is accurate to five decimal places in the range 4° to 40°C.

A spreadsheet does not know how many figures are significant. You can choose how many decimal places are displayed to be consistent with the number of significant figures associated with a cell or column.

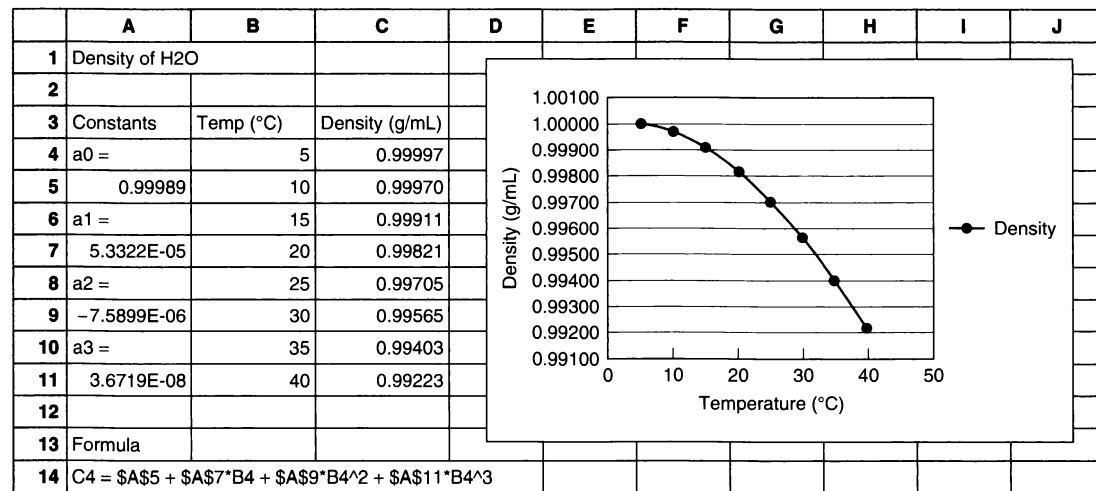


Figure 3-4 Spreadsheet for computing the density of water as a function of temperature.

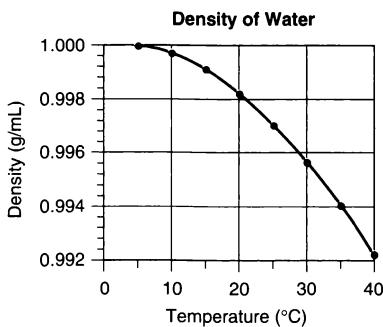


Figure 3-5 Graph from Figure 3-4 after reformatting.

Series name, write “Density” (without quotation marks). For X values, highlight cells B4:B11. For Y values, delete what was in the box and highlight cells C4:C11. Click OK twice. Click inside the plot area and select the Chart Tools Format ribbon. In Plot Area, Format Selection provides options for the border and fill color of the graph. For Fill, select Solid fill and Color white. For Border Color, select solid line and Color black. We now have a white graph surrounded by a black border. Close the Format Plot Area window.

To add an X axis title, select Chart Tools Layout. Click on Axis Titles and Primary Horizontal Axis Title. Click on Title Below Axis. A generic axis title appears on the graph. Highlight it and type “Temperature (°C)” over the title. Get the degree sign from Insert Symbol. To put a title on the Y axis, select Chart Tools Layout again. Click on Axis Titles and Primary Vertical Axis Title. Click on Rotated Title. Then type “Density (g/mL)” for the title. Select the title that appears above the graph and remove it with the delete key. Your graph probably looks like the one in Figure 3-4 now.

Let’s change the graph so that it looks like Figure 3-5. Click on the curve on the graph to highlight all data points. If only one point is highlighted, click elsewhere on the line. Select Chart Tools Format. In Current Selection, choose Format Selection. A Format Data Series window appears. For Marker Options, choose Built-in. Select the Type circle and Size 6. For Marker Fill, select Solid fill and a Color of your choice. Select Marker Line Color, then Solid line, then the same Color as the marker. To change the appearance of the curve on the graph, use Line Color and Line Style. Create a solid black line with a width of 1.5 points.

To change the appearance of the Y axis, click any number on the Y axis and they will all be highlighted. Select Chart Tools and Format and Format Selection. The Format Axis box appears. For Axis Options, Minimum, click on Fixed and set the value to 0.992. For Axis Options, Maximum, click on Fixed and set the value to 1.000. For Major unit, click on Fixed and set the value to 0.002. For Minor unit, click on Fixed and set the value to 0.0004. Set Minor tick mark type to Outside. In the Format Axis window, select Number and set a display of 3 decimal places. Close the Format Axis window to finish with the vertical axis.

In a similar manner, select a number on the X axis and change the appearance so that it looks like Figure 3-5 with a Minimum of 0, Maximum of 40, Major unit of 10, and Minor unit of 5. Place Minor tick marks Outside. To add vertical grid lines, go to Chart Tools and select Layout and Gridlines. Select Primary Vertical Gridlines and Major Gridlines.

Add a title back to the chart. In Chart Tools Layout, select Chart Title and highlight Above Chart. Type “Density of Water”. In the Home ribbon, select a font size of 10 points. Your chart ought to look much like Figure 3-5 now. You can resize the chart from its lower right corner. To draw on an Excel worksheet, select Insert and then Shapes.

To write on the chart, go to the Insert ribbon and select Text Box. Click in the chart and begin typing. Drag the text box where you want it to be. To format the box, click on its border. Go to the Format ribbon and use Shape Fill and Shape Outline. To add arrows or lines, go to the Insert ribbon and select Shapes. To change the data point symbol, click on one point. On the Format Ribbon, click on Format Selection. The box that appears lets you change the appearance of the points and the line.

Ask Yourself

- 3-F. Reproduce the spreadsheet in Figure 3-4 and the graph in Figure 3-5 on your computer.

Key Equations

Definition of logarithm	If $n = 10^a$, then a is the logarithm of n .
Definition of antilogarithm	If $n = 10^a$, then n is the antilogarithm of a .
Relative uncertainty	$\text{relative uncertainty} = \frac{\text{absolute uncertainty}}{\text{magnitude of measurement}}$
Percent relative uncertainty	$\text{percent relative uncertainty} = 100 \times \text{relative uncertainty}$
Uncertainty in addition and subtraction	$e_4 = \sqrt{e_1^2 + e_2^2 + e_3^2}$ (use absolute uncertainties) $e_4 = \text{uncertainty in final answer}$ $e_1, e_2, e_3 = \text{uncertainty in individual terms}$
Uncertainty in multiplication and division	$\%e_4 = \sqrt{\%e_1^2 + \%e_2^2 + \%e_3^2}$ (use percent relative uncertainties)
Uncertainty from pH	Uncertainty in $[\text{H}^+]$ = $2.303[\text{H}^+](\text{uncertainty in pH})$

Important Terms

abscissa	characteristic	mantissa	relative uncertainty
absolute uncertainty	determinate error	ordinate	significant figure
accuracy	indeterminate error	precision	systematic error
antilogarithm	logarithm	random error	

Problems

3-1. Round each number as indicated:

- (a) 1.236 7 to 4 significant figures
(b) 1.238 4 to 4 significant figures
(c) 0.135 2 to 3 significant figures
(d) 2.051 to 2 significant figures
(e) 2.005 0 to 3 significant figures

3-2. Round each number to three significant figures:

- (a) 0.216 74 (b) 0.216 5 (c) 0.216 500 3 (d) 0.216 49

3-3. Indicate how many significant figures there are in

- (a) 0.305 0 (b) 0.003 050 (c) 1.003×10^4

3-4. Write each answer with the correct number of digits:

- (a) $1.0 + 2.1 + 3.4 + 5.8 = 12.300\ 0$
(b) $106.9 - 31.4 = 75.500\ 0$
(c) $107.868 - (2.113 \times 10^2) + (5.623 \times 10^3) = 5\ 519.568$
(d) $(26.14/37.62) \times 4.38 = 3.043\ 413$
(e) $(26.14/37.62 \times 10^8) \times (4.38 \times 10^{-2}) = 3.043\ 413 \times 10^{-10}$
(f) $(26.14/3.38) + 4.2 = 11.933\ 7$
(g) $\log(3.98 \times 10^4) = 4.599\ 9$
(h) $10^{-6.31} = 4.897\ 79 \times 10^{-7}$

3-5. Write each answer with the correct number of digits:

- (a) $3.021 + 8.99 = 12.011$ (e) $\log(2.2 \times 10^{-18}) = ?$
(b) $12.7 - 1.83 = 10.87$ (f) $\text{antilog}(-2.224) = ?$
(c) $6.345 \times 2.2 = 13.959\ 0$ (g) $10^{-4.555} = ?$
(d) $0.030\ 2 \div (2.114\ 3 \times 10^{-3}) = 14.283\ 69$

3-6. Find the formula mass of (a) BaCl_2 and (b) $\text{C}_{31}\text{H}_{32}\text{O}_8\text{N}_2$ with the correct number of significant figures.

3-7. Find the molecular mass of $\text{Mn}_2(\text{CO})_{10}$ with the correct number of significant figures.

3-8. Why do we use quotation marks around the word *true* in the statement that accuracy refers to how close a measured value is to the “true” value?

3-9. (a) Explain the difference between systematic and random errors.

State whether the errors in (b)–(e) are random or systematic.

(b) A 25-mL transfer pipet consistently delivers 25.031 ± 0.009 mL when drained from the mark.

(c) A 10-mL buret consistently delivers 1.98 ± 0.01 mL when drained from exactly 0 to exactly 2 mL and consistently delivers 2.03 ± 0.02 mL when drained from 2 to 4 mL.

(d) A 10-mL buret delivered 1.983 9 g of water when drained from exactly 0.00 to 2.00 mL. The next time I delivered water from the 0.00 to the 2.00 mL mark, the delivered mass was 1.990 0 g.

(e) Four consecutive $20.0\text{-}\mu\text{L}$ injections of a solution into a chromatograph were made (as in Figure 0-6) and the area of a particular peak was 4 383, 4 410, 4 401, and 4 390 units.

(f) A clean funnel that had been in the lab since last semester had a mass of 15.432 9 g. When filled with a solid precipitate and dried thoroughly in the oven at 110°C , the mass was 15.845 6 g. The calculated mass of precipitate was therefore $15.845\ 6 - 15.432\ 9 = 0.412\ 7$ g. Is there systematic or random error (or both) in the mass of precipitate?

3-10. Rewrite the number $3.123\ 56$ (± 0.167 89%) in the forms **(a)** number (\pm absolute uncertainty) and **(b)** number (\pm percent relative uncertainty) with an appropriate number of digits.

3-11. Write each answer with the correct number of digits. Find the absolute uncertainty and percent relative uncertainty for each answer.

(a) $6.2 (\pm 0.2) - 4.1 (\pm 0.1) = ?$

(b) $9.43 (\pm 0.05) \times 0.016 (\pm 0.001) = ?$

(c) $[6.2 (\pm 0.2) - 4.1 (\pm 0.1)] \div 9.43 (\pm 0.05) = ?$

(d) $9.43 (\pm 0.05) \times \{[6.2 (\pm 0.2) \times 10^{-3}] + [4.1 (\pm 0.1) \times 10^{-3}]\} = ?$

3-12. Write each answer with a reasonable number of figures. Find the absolute uncertainty and percent relative uncertainty for each answer.

(a) $[12.41 (\pm 0.09) \div 4.16 (\pm 0.01)] \times 7.068\ 2 (\pm 0.000\ 4) = ?$

(b) $[3.26 (\pm 0.10) \times 8.47 (\pm 0.05)] - 0.18 (\pm 0.06) = ?$

(c) $6.843 (\pm 0.008) \times 10^4 \div [2.09 (\pm 0.04) - 1.63 (\pm 0.01)] = ?$

3-13. Write each answer with the correct number of digits. Find the absolute uncertainty and percent relative uncertainty for each answer.

(a) $9.23 (\pm 0.03) + 4.21 (\pm 0.02) - 3.26 (\pm 0.06) = ?$

(b) $91.3 (\pm 1.0) \times 40.3 (\pm 0.2) / 21.2 (\pm 0.2) = ?$

(c) $[4.97 (\pm 0.05) - 1.86 (\pm 0.01)] / 21.2 (\pm 0.2) = ?$

(d) $2.016\ 4 (\pm 0.000\ 8) + 1.233 (\pm 0.002) + 4.61 (\pm 0.01) = ?$

(e) $2.016\ 4 (\pm 0.000\ 8) \times 10^3 + 1.233 (\pm 0.002) \times 10^2 + 4.61 (\pm 0.01) \times 10^1 = ?$

3-14. Find the absolute and percent relative uncertainty and express each answer with a reasonable number of significant figures.

(a) $3.4 (\pm 0.2) + 2.6 (\pm 0.1) = ?$

(b) $3.4 (\pm 0.2) \div 2.6 (\pm 0.1) = ?$

(c) $[3.4 (\pm 0.2) \times 10^{-8}] \div [2.6 (\pm 0.1) \times 10^3] = ?$

(d) $[3.4 (\pm 0.2) - 2.6 (\pm 0.1)] \times 3.4 (\pm 0.2) = ?$

3-15. (a) The pH of a solution is 5.42 ± 0.05 . Find $[\text{H}^+]$ and its uncertainty.

(b) What is the relative uncertainty in $[\text{H}^+]$?

(c) Show that the relative uncertainty depends on the uncertainty in pH, but not on the value of pH.

3-16. The pH of a liquid is 8.2 ± 0.1 . Find $[\text{H}^+]$ and its absolute and relative uncertainty.

3-17. Uncertainty in molecular mass. The periodic table inside the cover of this book has a note in the legend about

uncertainties in atomic mass. Here is an example of how to find uncertainty in molecular mass. For the compound diborane, B_2H_6 , first multiply the uncertainty in each atomic mass by the number of atoms in the formula:

$$2\text{B}: 2 \times 10.811 \pm 0.007 = 21.622 \pm 0.014$$

$$6\text{H}: 6 \times 1.007\ 94 \pm 0.000\ 07 = \frac{6.047\ 64 \pm 0.000\ 42}{\text{sum}} \\ \text{sum} = 27.669\ 64 \pm ?$$

Then find the uncertainty in the sum of atomic masses by the formula for addition:

$$\text{uncertainty} = \sqrt{e_1^2 + e_2^2} = \sqrt{0.014^2 + 0.000\ 42^2} = 0.014$$

$$\text{molecular mass} = 27.670 \pm 0.014 \text{ (or } 27.67 \pm 0.01)$$

Express the molecular mass (\pm uncertainty) of benzene, C_6H_6 , with the correct number of significant figures.

3-18. As in the previous problem, express the molecular mass of $\text{C}_6\text{H}_{13}\text{B}$ with the correct number of significant figures and find its uncertainty.

3-19. (a) Show that the formula mass of NaCl is 58.443 (± 0.002) g/mol.

(b) You dissolve 2.634 (± 0.002) g of NaCl in a volumetric flask with a volume of 100.00 (± 0.08) mL. Express the molarity of NaCl and its uncertainty with an appropriate number of digits.

3-20. (a) For use in an iodine titration, you prepare a solution from $0.222\ 2$ ($\pm 0.000\ 2$) g of KIO_3 [FM $214.001\ 0$ ($\pm 0.000\ 9$)] in 50.00 (± 0.05) mL. Find the molarity and its uncertainty with an appropriate number of significant figures.

(b) Would your answer be affected significantly if the reagent were only 99.9% pure?

3-21. A 500.0 ± 0.2 -mL solution was prepared by dissolving 25.00 ± 0.03 mL of methanol (CH_3OH , density = $0.791\ 4 \pm 0.000\ 2$ g/mL, molecular mass = $32.041\ 9 \pm 0.000\ 9$ g/mol) in chloroform. Find the molarity \pm uncertainty of the methanol.

3-22. Your instructor has asked you to prepare $2.00\ \text{L}$ of $0.169\ \text{M}$ NaOH from a stock solution of 53.4 (± 0.4) wt% NaOH with a density of 1.52 (± 0.01) g/mL.

(a) How many milliliters of stock solution will you need?

(b) If the uncertainty in delivering the NaOH is ± 0.10 mL, calculate the absolute uncertainty in the molarity ($0.169\ \text{M}$). Assume negligible uncertainty in the formula mass of NaOH and in the final volume, $2.00\ \text{L}$.

3-23. *Formula mass calculator.* Reproduce the spreadsheet shown here. Atomic masses are in column A. Numbers of atoms are in columns B to E. Write a formula in column F to compute formula mass from masses in column A and the number of atoms. I find this spreadsheet

extremely useful. As you need to, add more atomic masses to column A and add columns between E and F for additional atoms.

	A	B	C	D	E	F	G
1	Formula Mass Calculator				Formula		
2		C	H	O	N	mass	
3	C =	1	4	1		32.0419	CH ₃ OH
4	12.0107	5	5	1	1	95.0993	C ₅ H ₅ NO
5	H =						
6	1.00794						
7	O =						
8	15.9994						
9	N =						
10	14.0067						

- 3-24.**  *Graphing.* Figure 1-4 shows that atmospheric CO₂ has been increasing since the dawn of the industrial age. Copy the following data into 2 columns of a spreadsheet and use the spreadsheet to reproduce Figure 1-4. Adjust the axes so that the scales and tick marks are the same as in Figure 1-4.

Year	CO ₂ (ppm)	Year	CO ₂ (ppm)	Year	CO ₂ (ppm)
1603	274	1903	299	1982	341
1646	277	1925	305	1986	347
1691	276	1937	309	1990	354
1747	277	1945	311	1994	359
1776	279	1959	316	1998	367
1795	284	1965	320	2002	373
1823	285	1970	326	2006	382
1843	286	1974	330	2008	385
1889	295	1978	335		

How Would You Do It?

- 3-25.** Here are two methods that you might use to prepare a dilute silver nitrate solution:

Method 1: Weigh out 0.046 3 g AgNO₃ and dissolve it in a 100-mL volumetric flask.

Method 2: Weigh out 0.463 0 g AgNO₃ and dissolve it in a 100-mL volumetric flask. Then pipet 10 mL of this solution into a fresh 100-mL flask and dilute to the mark.

The uncertainty in the balance is ± 3 in the last decimal place. Which method is more accurate?

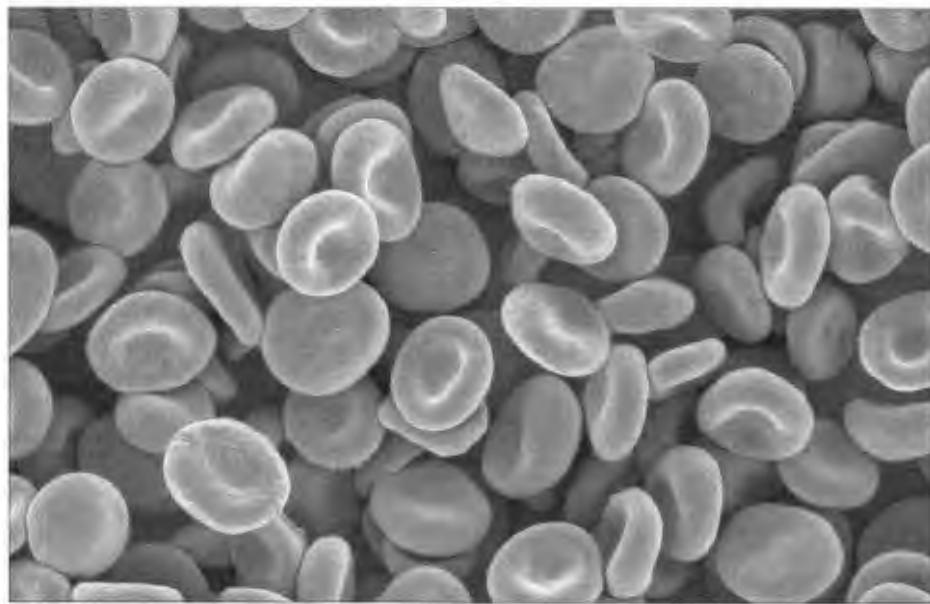
Further Reading

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 E. J. Billio, *Microsoft Excel for Chemists*, 2nd ed. (New York: Wiley, 2001).
 E. J. Billio, *Excel for Scientists and Engineers: Numerical Methods* (New York: Wiley, 2007).

R. de Levie, *How to Use Excel® in Analytical Chemistry and in General Scientific Data Analysis* (Cambridge: Cambridge University Press, 2001).

R. de Levie, *Advanced Excel for Scientific Data Analysis*, 2nd ed. (Oxford: Oxford University Press, 2008).

Is My Red Blood Cell Count High Today?



Red blood cells, also called erythrocytes. [Susumu Nishinaga/Photo Researchers, Inc.]

All measurements contain experimental error, so it is impossible to be completely certain of a result. Nevertheless, we seek to answer questions such as “Is my red blood cell count today higher than usual?” If today’s count is twice as high as usual, it is probably truly higher than normal. But what if the “high” count is not excessively above “normal” counts?

Count on “normal” days	Today’s count
5.1	
5.3	
4.8	$\times 10^6$ cells/ μL
5.4	
5.2	

The number 5.6 is higher than the five normal values, but the random variation in normal values might lead us to expect that 5.6 will be observed on some “normal” days.

The study of statistics allows us to say that, over a long period, today’s value will be observed on 1 out of 20 normal days. It is still up to you to decide what to do with this information.

Statistics

Experimental measurements always have some random error, so no conclusion can be drawn with complete certainty. Statistics gives us tools to accept conclusions that have a high probability of being correct and to reject conclusions that do not. This chapter describes basic statistical tests and introduces the method of least squares for calibration curves.

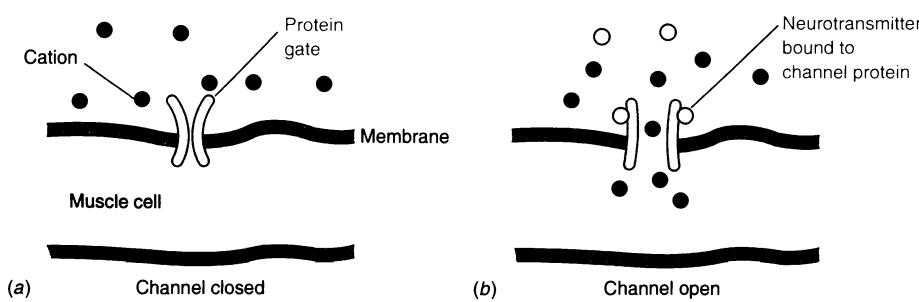
Statistics deals only with random error—not determinate error. We must be ever vigilant and try to detect and eliminate systematic errors. Two ways to detect systematic errors are to analyze certified standards to see whether our method gives the expected results and to use different methods of analysis and see if the results agree.

4-1 The Gaussian Distribution

Nerve cells communicate with muscle cells by releasing neurotransmitter molecules adjacent to the muscle. In Figure 4-1, neurotransmitters bind to membrane proteins of the muscle cell and open up channels that permit cations to diffuse into the cell. Ions entering the cell trigger contraction of the muscle.

Channels are all the same size, so each should allow a similar rate of ion passage across the membrane. Because ions are charged particles, the flow of ions is equivalent to a flow of electricity across the membrane. Of the 922 ion-channel responses in Figure 4-2, 190 are in the narrow range from 2.64 to 2.68 pA (picampères, 10^{-12} amperes), represented by the tallest bar at the center of the chart. The next most probable responses fall just to the right and left of the tallest bar.

Figure 4-2 is typical of many laboratory measurements: The most probable response is at the center, and the probability of observing other responses decreases as the distance from the center increases. The smooth, bell-shaped curve superimposed on the data is called a **Gaussian distribution**. The more measurements made on any physical system, the closer the bar chart comes to the smooth curve.

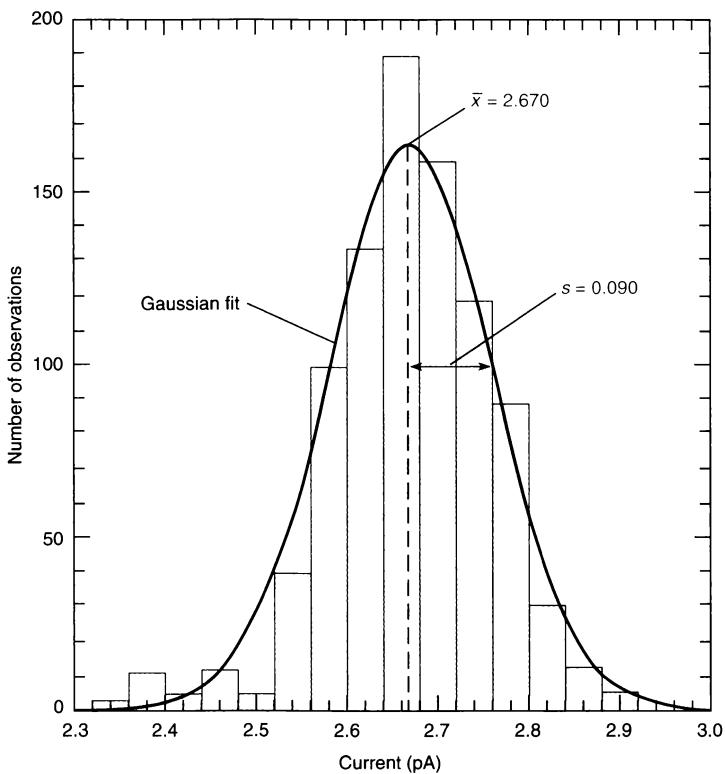


Determinate error, also called *systematic error*, arises from procedural or instrumental factors that make a measurement consistently too large or too small. With care, it can be eliminated.

Random error arises from physical limitations of measurements. It is equally likely to be positive or negative, and it cannot be completely eliminated.

Figure 4-1 (a) In the absence of neurotransmitter, the ion channel is closed and cations cannot enter the muscle cell. (b) In the presence of neurotransmitter, the channel opens, cations enter the cell, and muscle action is initiated. B. Sakmann and E. Neher shared the Nobel Prize in Medicine or Physiology in 1991 for their work on signal transmission at the neuromuscular junction.

Figure 4-2 Observed cation current passing through individual channels of a frog muscle cell. The smooth line is the Gaussian curve that has the same mean and standard deviation as the measured data. The bar chart is also called a *histogram*. [Data from Nobel Lecture of B. Sakmann, *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 830.]



Mean and Standard Deviation

Mean locates center of distribution.
Standard deviation measures width of distribution.

A Gaussian distribution is characterized by a *mean* and a *standard deviation*. The mean is the *center* of the distribution, and the standard deviation measures the *width* of the distribution. Data points tend to be clustered near the mean value.

The arithmetic **mean**, \bar{x} , also called the **average**, is the sum of the measured values divided by the number of measurements.

$$\text{Mean: } \bar{x} = \frac{\sum_i x_i}{n} = \frac{1}{n} (x_1 + x_2 + x_3 + \dots + x_n) \quad (4-1)$$

where each x_i is a measured value. A capital Greek sigma, Σ , is the symbol for a sum. In Figure 4-2, the mean value is indicated by the dashed line at 2.670 pA.

Standard deviation, s , is a measure of the scatter of a data set. *Less scatter gives a smaller standard deviation* or, as we say, a narrower distribution of data.

$$\text{Standard deviation: } s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n - 1}} \quad (4-2)$$

The smaller the standard deviation, the more *precise* (reproducible) the results. Greater precision does not necessarily imply greater *accuracy*, which means nearness to the “truth.”

In Figure 4-2, $s = 0.090$ pA. Figure 4-3 shows that if the standard deviation were doubled, the Gaussian curve for the same number of observations would be shorter and broader.

Relative standard deviation is the standard deviation divided by the average (s/\bar{x}), usually expressed as a percentage. For $s = 0.090 \text{ pA}$ and $\bar{x} = 2.670 \text{ pA}$, the relative standard deviation is $(0.090/2.670) \times 100 = 3.4\%$.

The quantity $n - 1$ in the denominator of Equation 4-2 is called the *degrees of freedom*. Initially we have n independent data points representing n pieces of information. After computing the average, there are only $n - 1$ independent pieces of information left, because we could calculate the n th data point if we know $n - 1$ points and the average.

The symbols \bar{x} and s apply to a finite set of measurements. For an infinite set of data, the true mean (called the *population mean*) is designated μ (Greek mu) and the *population standard deviation* is denoted by σ (lowercase Greek sigma). **Variance** is the square of the standard deviation (s^2).

Example Mean and Standard Deviation

Find the mean, standard deviation, and relative standard deviation for the set of measurements (7, 18, 10, 15).

SOLUTION The mean is

$$\bar{x} = \frac{7 + 18 + 10 + 15}{4} = 12.5$$

To avoid round-off errors, retain one or more subscripted, insignificant digits for the mean and the standard deviation than were present in the original data. The standard deviation is

$$s = \sqrt{\frac{(7 - 12.5)^2 + (18 - 12.5)^2 + (10 - 12.5)^2 + (15 - 12.5)^2}{4 - 1}} = 4.9$$

The mean and the standard deviation should both end at the *same decimal place*. For $\bar{x} = 12.5$, we write $s = 4.9$. The relative standard deviation is $(4.9/12.5) \times 100 = 39\%$.

 **Test Yourself** Use the mean and standard deviation functions on your calculator to show that you can reproduce the results in this example.

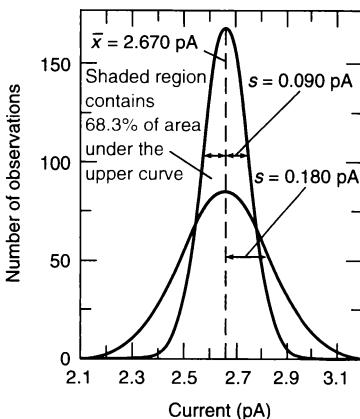


Figure 4-3 Gaussian curves showing the effect of doubling the standard deviation. The number of observations for each curve, which is the area beneath the curve, is the same.

Excel has built-in functions for average and standard deviation. Enter the numbers 7, 18, 10, and 15 in cells A1 through A4 of a spreadsheet. In cell A5, enter the formula “=AVERAGE(A1:A4)”; and in cell A6, enter the formula “=STDEV(A1:A4)”. Results in the spreadsheet in the margin reproduce those in the example above. For a list of built-in functions, go to the Formulas ribbon and select Insert Function. In the window that appears, you can browse categories of functions or all functions.

Other terms that you should know are *median* and *range*. The **median** is the middle number in a series of measurements. When (8, 17, 11, 14, 12) are ordered from lowest to highest to give (8, 11, 12, 14, 17), the middle number (12) is the median. For an even number of measurements, the median is the average of the two middle numbers. For (8, 11, 12, 14), the median is 11.5. Some people prefer to report the median instead of the average, because the median is less influenced by outlying data. The **range** is the difference between the highest and lowest values. The range of (8, 17, 11, 14, 12) is $17 - 8 = 9$.

If your calculator gives 4.3 instead of 4.9, it is using $n = 4$ in the denominator instead of $n - 1 = 4 - 1$. The correct factor is $n - 1$.

	A	B
1	7	
2	18	
3	10	
4	15	
5	12.50	
6	4.93	
7	A5 = AVERAGE(A1:A4)	
8	A6 = STDEV(A1:A4)	

Table 4-1 Percentage of observations in Gaussian distribution

Gaussian Range	Observed in distribution	Figure 4-2
$\mu \pm 1\sigma$	68.3%	71.0%
$\mu \pm 2\sigma$	95.5	95.6
$\mu \pm 3\sigma$	99.7	98.5

Question What fraction of observations in a Gaussian distribution is expected to be below $\mu - 3\sigma$? (Answer: $\frac{1}{2}$ of 0.3% = 0.15%)

Standard Deviation and Probability

In a Gaussian distribution, 68.3% of measurements lie within one standard deviation of the mean (in the interval $\mu \pm \sigma$). That is, 68.3% of the area beneath a Gaussian curve lies in the interval $\mu \pm \sigma$ shown in Figure 4-3. The percentage of measurements lying in the interval $\mu \pm 2\sigma$ is 95.5%, and the percentage in the interval $\mu \pm 3\sigma$ is 99.7%. For real data with a standard deviation s , about 1 in 20 measurements (4.5%) will lie outside the range $\bar{x} \pm 2s$, and only 3 in 1 000 measurements (0.3%) will lie outside the range $\bar{x} \pm 3s$. Table 4-1 shows the correspondence between ideal Gaussian behavior and the observations in Figure 4-2.

The Gaussian distribution is symmetric. If 4.5% of measurements lie outside the range $\mu \pm 2\sigma$, 2.25% of measurements are above $\mu + 2\sigma$ and 2.25% are below $\mu - 2\sigma$.

Ask Yourself

4-A. What are the mean, standard deviation, relative standard deviation, median, and range for the numbers 821, 783, 834, and 855? All but the range should be expressed with an extra digit beyond the last significant digit.

4-2 Comparison of Standard Deviations with the F Test

A most important question in statistics is “Are the mean values of two sets of measurements ‘statistically different’ from each other when experimental uncertainty is considered?” To compare mean values in the next section, we must first learn to decide whether the standard deviations of the two sets are “statistically different.”

Consider the measurement of bicarbonate (HCO_3^-) in the blood of racehorses. Some trainers injected NaHCO_3 into a horse prior to a race to neutralize lactic acid that accumulates during strenuous activity. To enforce a ban on this practice, HCO_3^- in horse blood is measured after a race. When a manufacturer stopped making an instrument that was certified for such measurements, authorities needed to certify a new instrument.

Table 4-2 shows results from two instruments. The averages of 36.14 and 36.20 mM are similar, but the standard deviation (s) from the substitute instrument is almost twice as great as that from the original instrument (0.47 versus 0.28 mM). Is s from the substitute instrument “significantly” greater than s from the original instrument?

Table 4-2 Measurement of HCO_3^- in horse blood^a

	Original instrument	Substitute instrument
Mean (\bar{x} , mM)	36.14	36.20
Standard deviation (s , mM)	0.28	0.47
Number of measurements (n)	10	4

^a Data from M. Jarrett, D. B. Hibbert, R. Osborne, and E. B. Young, *Anal. Bioanal. Chem.* **2010**, 397, 717.

Table 4-3 Critical values of $F = s_1^2/s_2^2$ at 95% confidence level

Degrees of freedom for s_2	Degrees of freedom for s_1													
	2	3	4	5	6	7	8	9	10	12	15	20	30	∞
2	19.0	19.2	19.2	19.3	19.3	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.5	19.5
3	9.55	9.28	9.12	9.01	8.94	8.89	8.84	8.81	8.79	8.74	8.70	8.66	8.62	8.53
4	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.75	5.63
5	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.50	4.36
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.81	3.67
7	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.58	3.51	3.44	3.38	3.23
8	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.08	2.93
9	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.86	2.71
10	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.84	2.77	2.70	2.54
11	3.98	3.59	3.36	3.20	3.10	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.57	2.40
12	3.88	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.47	2.30
15	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.25	2.07
20	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.04	1.84
30	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.84	1.62
∞	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.46	1.00

For n observations, degrees of freedom = $n - 1$. There is a 5% probability of observing F above the tabulated value.

You can compute F for a chosen level of confidence with the Excel function FINV (Probability,Deg_freedom1,Deg_freedom2). The statement “=FINV(0.05,7,6)” reproduces the value $F = 4.21$ in this table.

To answer this question, we use the **F test** with the quotient F , defined as

$$F_{\text{calculated}} = \frac{s_1^2}{s_2^2} \quad (4-3)$$

The square of the standard deviation is called the **variance**.

Put the larger standard deviation in the numerator so that $F \geq 1$. We test the hypothesis that $s_1 > s_2$ by applying the F test in Table 4-3. If $F_{\text{calculated}} > F_{\text{table}}$, then the difference is significant. In using Table 4-3, the *degrees of freedom* for n measurements are $n - 1$. If there are five measurements in one set, there are four degrees of freedom.

Example Is the Standard Deviation from the Substitute Instrument “Significantly” Greater than that of the Original Instrument?

In Table 4-2, the standard deviation from the substitute instrument is $s_1 = 0.47$ ($n_1 = 4$ measurements) and the standard deviation from the original instrument is $s_2 = 0.28$ ($n_2 = 10$).

SOLUTION To answer the question, find F with Equation 4-3:

$$F_{\text{calculated}} = \frac{s_1^2}{s_2^2} = \frac{(0.47)^2}{(0.28)^2} = 2.82$$

In Table 4-3, find F_{table} in the column with 3 degrees of freedom for s_1 (degrees of freedom = $n - 1$) and the row with 9 degrees of freedom for s_2 . Because $F_{\text{calculated}} (= 2.8_2) < F_{\text{table}} (= 3.86)$, we reject the hypothesis that s_1 is significantly larger than s_2 . The difference between s_1 and s_2 is not significant at the 95% confidence level.

 **Test Yourself** If there had been $n = 13$ replications in both data sets, would the difference in standard deviations be significant? (Answer: Yes. $F_{\text{calculated}} = 2.8_2 > F_{\text{table}} = 2.69$)

Hypothesis Testing

Comparing two standard deviations with the F test is an example of what statisticians call a *hypothesis test*. The *null hypothesis* is that the two sets of measurements are drawn at random from populations with the same standard deviation. Because of random variation in all measurements, the observed standard deviations of the two sets are not expected to be equal. Values of F in Table 4-3 are chosen such that there is only a 5% probability that the observed measurements come from populations with the same standard deviation. That is, when $F_{\text{calculated}} < F_{\text{table}}$, there is *more* than a 5% chance that the two sets of measurements come from populations with the same standard deviation. When $F_{\text{calculated}} > F_{\text{table}}$, there is *less* than a 5% chance that the two sets of measurements come from populations with the same standard deviation. We can be 95% confident that measurements come from populations that do not have the same standard deviation if $F_{\text{calculated}} > F_{\text{table}}$. Now you should read Box 4-1 to really appreciate what we mean by the null hypothesis.

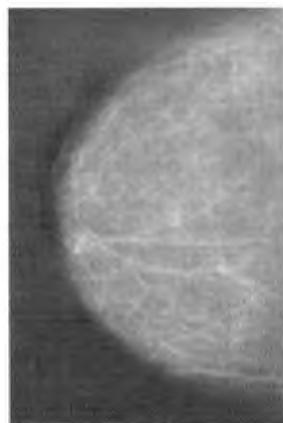
Box 4-1 Choosing the Null Hypothesis in Epidemiology

One fine morning, I found myself seated on a cross-country flight next to Malcolm Pike, an epidemiologist at the University of Southern California. Epidemiologists employ methods of statistics to guide practices in medicine. Pike was studying the relation between menopausal estrogen-progestin hormone therapy and breast cancer in women. His study concluded that there was a 7.6% increase in breast cancer risk per year of estrogen-progestin hormone therapy.¹

How could such therapy have been approved? Pike explained that tests required by the U.S. Food and Drug Administration are designed to test the null hypothesis that “the treatment does no harm.” Instead, he said, the null hypothesis should be “the treatment increases the likelihood of causing breast cancer.”

What did he mean? In the field of statistics, the null hypothesis is assumed to be true. Unless you find strong evidence that it is not true, you continue to believe that it is true. In the U.S. legal system, the null hypothesis is that the accused person is innocent. It is up to the prosecution to produce compelling evidence that the accused person is not innocent; failing that, the jury must acquit the defendant. For drug approval, the null hypothesis is that the treatment does not cause cancer. The burden of the test is to provide compelling evidence that the treatment *does cause* cancer. Pike is saying that if there is evidence that a treatment causes cancer, the null hypothesis should be that the treatment causes cancer. Then it is up to the proponent of the treatment to provide compelling evidence that

the treatment *does not* cause cancer. In Pike’s words, test the hypothesis that “the obvious is likely to be true!”



White regions of mammogram are denser tissue than dark regions. [allOver photography/Alamy.]

Ask Yourself

4-B. Bicarbonate in replicate samples of horse blood was measured four times by each of two methods with the following results:

Method 1: 31.40, 31.24, 31.18, 31.43 mM

Method 2: 30.70, 29.49, 30.01, 30.15 mM

- (a) Find the mean and standard deviation for each analysis.
- (b) Are the standard deviations significantly different at the 95% confidence level?

4-3 Student's *t*

Student's *t* is the statistical tool used to express confidence intervals and to compare mean values from experiments. You could use it on the data at the opening of this chapter to evaluate the probability that your red blood cell count will exceed a certain value on "normal" days.

Confidence Intervals

From a limited number of measurements, it is impossible to find the population mean, μ , or the population standard deviation, σ . What we can determine are \bar{x} and s , the sample mean and the sample standard deviation. The **confidence interval** is a range of values within which there is a specified probability of finding the population mean. We say that the population mean, μ , is likely to lie within a certain distance from the measured mean, \bar{x} . The confidence interval ranges from $-ts/\sqrt{n}$ below \bar{x} to $+ts/\sqrt{n}$ above \bar{x} :

$$\text{Using the confidence interval: } \mu = \bar{x} \pm \frac{ts}{\sqrt{n}} \quad (4-4)$$

where s is the measured standard deviation, n is the number of observations, and t is Student's *t*, taken from Table 4-4. In this table, the *degrees of freedom* are $n - 1$.

Example Calculating Confidence Intervals

In replicate analyses, the carbohydrate content of a glycoprotein (a protein with sugars attached to it) is found to be 12.6, 11.9, 13.0, 12.7, and 12.5 g of carbohydrate per 100 g of protein. Find the 50% and 90% confidence intervals for the carbohydrate content.

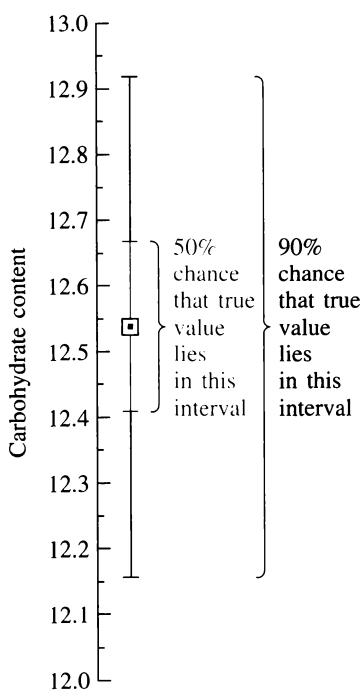
SOLUTION First we calculate $\bar{x} = 12.5_4$ and $s = 0.4_0$ for the $n = 5$ measurements. To find the 50% confidence interval, look up t in Table 4-4 under 50 and across from *four* degrees of freedom (degrees of freedom = $n - 1$). The value of t is 0.741, so the confidence interval is

$$\mu(50\%) = \bar{x} \pm \frac{ts}{\sqrt{n}} = 12.5_4 \pm \frac{(0.741)(0.4_0)}{\sqrt{5}} = 12.5_4 \pm 0.1_3$$

"Student" was the pseudonym of W. S. Gosset, whose employer, the Guinness Breweries of Ireland, restricted publications for proprietary reasons. Because of the importance of his work, Gosset published it under an assumed name in 1908.

Table 4-4 Values of Student's *t*

Degrees of freedom	Confidence level (%)						
	50	90	95	98	99	99.5	99.9
1	1.000	6.314	12.706	31.821	63.656	127.321	636.578
2	0.816	2.920	4.303	6.965	9.925	14.089	31.598
3	0.765	2.353	3.182	4.541	5.841	7.453	12.924
4	0.741	2.132	2.776	3.747	4.604	5.598	8.610
5	0.727	2.015	2.571	3.365	4.032	4.773	6.869
6	0.718	1.943	2.447	3.143	3.707	4.317	5.959
7	0.711	1.895	2.365	2.998	3.500	4.029	5.408
8	0.706	1.860	2.306	2.896	3.355	3.832	5.041
9	0.703	1.833	2.262	2.821	3.250	3.690	4.781
10	0.700	1.812	2.228	2.764	3.169	3.581	4.587
15	0.691	1.753	2.131	2.602	2.947	3.252	4.073
20	0.687	1.725	2.086	2.528	2.845	3.153	3.850
25	0.684	1.708	2.060	2.485	2.787	3.078	3.725
30	0.683	1.697	2.042	2.457	2.750	3.030	3.646
40	0.681	1.684	2.021	2.423	2.704	2.971	3.551
60	0.679	1.671	2.000	2.390	2.660	2.915	3.460
120	0.677	1.658	1.980	2.358	2.617	2.860	3.373
∞	0.674	1.645	1.960	2.326	2.576	2.807	3.291



In calculating confidence intervals, σ may be substituted for s in Equation 4-4 if you have a great deal of experience with a particular method and have therefore determined its "true" population standard deviation. If σ is used instead of s , the value of t to use in Equation 4-4 comes from the bottom row of this table.

The 90% confidence interval is

$$\mu(90\%) = \bar{x} \pm \frac{ts}{\sqrt{n}} = 12.5_4 \pm \frac{(2.132)(0.4_0)}{\sqrt{5}} = 12.5_4 \pm 0.3_8$$

These calculations mean that there is a 50% chance that the true mean, μ , lies in the range $12.5_4 \pm 0.1_3$ (12.4₁ to 12.6₇). There is a 90% chance that μ lies in the range $12.5_4 \pm 0.3_8$ (12.1₆ to 12.9₂).

 **Test Yourself** If \bar{x} and s were unchanged, but there were 10 measurements instead of 5, what would be the 90% confidence interval? (Answer: $12.5_4 \pm 0.2_{3_0}$)

Improving the Reliability of Your Measurements

We wish to be as accurate and precise as possible. Systematic errors reduce the accuracy of a measurement. If a pH meter is not calibrated correctly, it will give inaccurate readings, no matter how precise (reproducible) they are. Making a measurement by two different methods is a good way to detect systematic errors. If results do not agree within the expected uncertainty, systematic error is probably present.

Accuracy: nearness to the "truth"

Precision: reproducibility

Better precision gives smaller confidence intervals. The confidence interval is $\pm ts/\sqrt{n}$. To reduce the size of the confidence interval, we can make more measurements (increase n) or decrease the standard deviation (s). The only way to reduce s is to improve your experimental procedure. In the absence of a procedural change, the way to reduce the confidence interval is to increase the number of measurements. Doubling the number of measurements decreases the factor $1/\sqrt{n}$ by $1/\sqrt{2} = 0.71$.

Comparison of Means with Student's t

Student's t can be used to compare two sets of measurements to decide whether they are "statistically different." We test the *null hypothesis* stating that the means are *not different*. We adopt the following standard: If there is less than 1 chance in 20 that the difference between two sets of measurements arises from random variation in the data, then the difference is significant. This criterion gives us 95% confidence in concluding that two measurements are different. There is a 5% probability that our conclusion is wrong.²

Case A: Standard Deviations Are Not Significantly Different

Let's look again at Table 4-2 and ask whether the two mean values of 36.14 and 36.20 mM are significantly different from each other. We answer this question with the *t test*. If the *F* test tells us that the two standard deviations are not significantly different, then for data sets consisting of n_1 and n_2 measurements (with averages \bar{x}_1 and \bar{x}_2), calculate t from the formula

t test for comparison of means:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{\text{pooled}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \quad (4-5)$$

where

$$s_{\text{pooled}} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}} \quad (4-6)$$

Here s_{pooled} is a *pooled* standard deviation making use of both sets of data. The absolute value of $\bar{x}_1 - \bar{x}_2$ is used in Equation 4-5 so that t is always positive. The value of t from Equation 4-5 is to be compared with the value of t in Table 4-4 for $(n_1 + n_2 - 2)$ degrees of freedom. *If the calculated t is greater than the tabulated t at the 95% confidence level, the two results are considered to be significantly different.*

In Table 4-2, the means are $\bar{x}_1 = 36.14$ and $\bar{x}_2 = 36.20$ mM with $n_1 = 10$ and $n_2 = 4$ measurements. The standard deviations are $s_1 = 0.28$ and $s_2 = 0.47$ mM, which we found with the *F* test in Equation 4-3 not to be significantly different from each other. Therefore we use Equation 4-5 to compare the means. The pooled standard deviation is

$$s_{\text{pooled}} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}} = \sqrt{\frac{0.28^2(10 - 1) + 0.47^2(4 - 1)}{10 + 4 - 2}} = 0.338$$

We retain at least one extra insignificant digit at this point to avoid introducing round-off error into subsequent calculations.

Replicate measurements improve reliability. If $s = 2.0\%$, 3 measurements give a 95% confidence interval of 5.0%:

$$\frac{\pm ts}{\sqrt{n}} = \frac{(4.303)(2.0\%)}{\sqrt{3}} = \pm 5.0\%$$

Making 9 measurements reduces the 95% confidence interval to 1.5%:

$$\frac{\pm ts}{\sqrt{n}} = \frac{(2.306)(2.0\%)}{\sqrt{9}} = \pm 1.5\%$$

(Values of t came from Table 4-4.)

t test when the standard deviations are not significantly different.

If $t_{\text{calculated}} > t_{\text{table}} (95\%)$, the difference is significant.

Table 4-5 Grams of nitrogen-rich gas isolated by Lord Rayleigh^a

From air	From chemical decomposition
2.310 17	2.301 43
2.309 86	2.298 90
2.310 10	2.298 16
2.310 01	2.301 82
2.310 24	2.298 69
2.310 10	2.299 40
2.310 28	2.298 49
—	2.298 89
<i>Average</i>	
2.310 10 ₉	2.299 47 ₂
<i>Standard deviation</i>	
0.000 14 ₃	0.001 37 ₉

a. Data from R. D. Larsen, *J. Chem. Ed.* 1990, 67, 925.

Challenge Rayleigh discovered a systematic error by comparing two different methods of measurement. Which method had the systematic error? Did it overestimate or underestimate the mass of nitrogen in air?

To compare the means, we calculate the value of *t* with Equation 4-5:

$$t_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{\text{pooled}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} = \frac{|36.14 - 36.20|}{0.338} \sqrt{\frac{10 \cdot 4}{10 + 4}} = 0.30_0$$

The calculated value of *t* is 0.30₀. The critical value of *t* in Table 4-4 for (*n*₁ + *n*₂ − 2) = 12 degrees of freedom lies between 2.228 and 2.131 listed for 10 and 15 degrees of freedom in the column for 95% confidence. Because *t*_{calculated} < *t*_{table}, the difference in mean values is not significant. You could expect this conclusion because the difference is less than the standard deviation of either measurement.

Case B: Standard Deviations Are Significantly Different

An example comes from the work of Lord Rayleigh (John W. Strutt), who received the Nobel Prize in 1904 for discovering the inert gas argon—a discovery that came about when he noticed a discrepancy between two sets of measurements of the density of nitrogen. In Rayleigh's time, dry air was known to be ~1/5 oxygen and ~4/5 nitrogen. Rayleigh removed O₂ from air by reaction with red-hot copper [Cu(s) + $\frac{1}{2}$ O₂(g) → CuO(s)] and measured the density of the remaining gas by collecting it in a fixed volume at constant temperature and pressure. He then prepared the same volume of nitrogen by decomposition of nitrous oxide (N₂O), nitric oxide (NO), or ammonium nitrite (NH₄⁺NO₂⁻). Table 4-5 and Figure 4-4 show the mass of gas collected in each experiment. The average mass from air was 0.46% greater than the average mass of the same volume of gas from chemical sources.

If Rayleigh's measurements had not been performed with care, a 0.46% difference might have been attributed to experimental error. Instead, Rayleigh understood that the discrepancy was outside his margin of error, and he postulated that nitrogen from the air was mixed with a heavier gas, which turned out to be argon.

In Figure 4-4, the two sets of data are clustered in different regions. The range of results for chemically generated nitrogen is larger than the range for nitrogen from air. Are the two standard deviations in Table 4-5 statistically different from each other? We answer this question with the *F* test (Equation 4-3):

$$F_{\text{calculated}} = \frac{s_1^2}{s_2^2} = \frac{(0.001 37_9)^2}{(0.000 14_3)^2} = 93.0$$

The critical value of *F* in Table 4-3 for *n* − 1 = 7 degrees of freedom for the numerator (*s*₁) and 6 degrees of freedom for the denominator (*s*₂) is 4.21. Because *F*_{calculated} > *F*_{table}, the difference in standard deviations is significant.

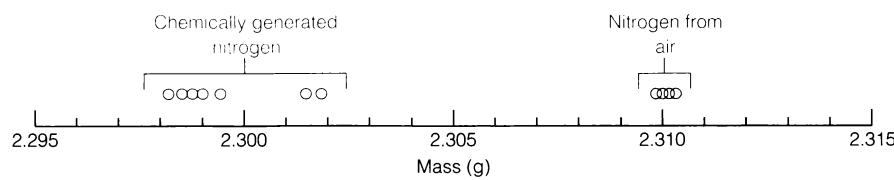


Figure 4-4 Lord Rayleigh's measurements of the mass of nitrogen isolated from air or generated by decomposition of nitrogen compounds. Rayleigh recognized that the difference between the two data sets was too great to be due to experimental error. He deduced that a heavier component, which turned out to be argon, was present in nitrogen isolated from air.

If the standard deviations of two sets of measurements are significantly different, the equations for the t test are

$$t_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{(s_1^2/n_1) + (s_2^2/n_2)}} \quad (4-7)$$

$$\begin{aligned} \text{degrees of freedom} &= \frac{[(s_1^2/n_1) + (s_2^2/n_2)]^2}{\frac{(s_1^2/n_1)^2}{n_1 - 1} + \frac{(s_2^2/n_2)^2}{n_2 - 1}} \end{aligned} \quad (4-8)$$

Round the degrees of freedom from Equation 4-8 to the nearest integer.

Equations 4-7 and 4-8 are a bit of a nuisance on a calculator. You are less likely to make a mistake with a spreadsheet. The Excel function for square root is `SQRT()`.

Example Is Rayleigh's N₂ from Air Denser than N₂ from Chemicals?

The average mass of nitrogen from air in Table 4-5 is $\bar{x}_1 = 2.310\ 10_9$ g, with a standard deviation of $s_1 = 0.000\ 14_3$ (for $n_1 = 7$ measurements). The average mass from chemical sources is $\bar{x}_2 = 2.299\ 47_2$ g, with a standard deviation of $s_2 = 0.001\ 37_9$ (for $n_2 = 8$ measurements). Are the two masses significantly different?

SOLUTION We use Equations 4-7 and 4-8 and compare the calculated value of t to the critical value in Table 4-4 for the appropriate degrees of freedom.

$$t_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{(s_1^2/n_1) + (s_2^2/n_2)}} = \frac{|2.310\ 10_9 - 2.299\ 47_2|}{\sqrt{(0.000\ 14_3^2/7) + (0.001\ 37_9^2/8)}} = 21.7$$

$$\begin{aligned} \text{degrees of freedom} &= \frac{[(s_1^2/n_1) + (s_2^2/n_2)]^2}{\frac{(s_1^2/n_1)^2}{n_1 - 1} + \frac{(s_2^2/n_2)^2}{n_2 - 1}} = \frac{[(0.000\ 14_3^2/7) + (0.001\ 37_9^2/8)]^2}{\frac{(0.000\ 14_3^2/7)^2}{7 - 1} + \frac{(0.001\ 37_9^2/8)^2}{8 - 1}} \\ &= 7.17 \end{aligned}$$

Equation 4-8 gives us 7.17 degrees of freedom, which we round to 7. For 7 degrees of freedom, the critical value of t in Table 4-4 for 95% confidence is 2.365. The observed value $t_{\text{calculated}} = 21.7$ far exceeds t_{table} and is highly significant.

 **Test Yourself** If the difference between the two mean values were half as great as Rayleigh found, but the standard deviations were unchanged, would the difference still be significant? (**Answer:** $t_{\text{calculated}} = 10.8 > t_{\text{table}} = 2.365$ —still highly significant difference)

Ask Yourself

4-C. A reliable assay of ATP (adenosine triphosphate) in a certain type of cell gives a value of $111.0\ \mu\text{mol}/100\ \text{mL}$, with a standard deviation of 2.8 in four replicate measurements. You have developed a new assay, which gave the following values in replicate analyses: 117, 119, 111, 115, 120 $\mu\text{mol}/100\ \text{mL}$.

- (a) Find the mean and standard deviation for your new analysis.
- (b) Are the two standard deviations significantly different?
- (c) Can you be 95% confident that your method produces a result different from the “reliable” value?

4-4 A Spreadsheet for the *t* Test

Excel has built-in procedures for conducting tests with Student's *t*. To compare Rayleigh's two sets of results in Table 4-5, enter his data in columns B and C of the spreadsheet in Figure 4-5. In rows 13 and 14, we compute the averages and standard deviations.

Cell B19 computes F from the standard deviations ($F = C14^2/B14^2$). We need to compare F to F_{table} . You could look in Table 4-3 under 7 degrees of freedom and across from 6 degrees of freedom to find $F_{\text{table}} = 4.21$. Alternatively, Figure 4-5 finds F_{table} in cell B20 with the formula “= FINV(0.05,7,6)”, where 0.05 specifies 95% confidence, 7 is the degrees of freedom for the numerator, and 6 is the degrees of freedom for the denominator. Because $F_{\text{calculated}} > F_{\text{table}}$, we conclude that the experimental standard deviations differ significantly.

On the Data ribbon in Analysis, look for Data Analysis. If Data Analysis does not appear, click the Office button (top left) and select Excel Options at the bottom of the window that opens. Click on Add-Ins at the left and select Analysis ToolPak. Click Go and then OK. Now Data Analysis should appear on your Data ribbon.

Figure 4-5 shows data analysis for both cases in which the standard deviations are not significantly different and are significantly different from each other. On the Data ribbon in Analysis, select Data Analysis. In the window that appears, select *t*-Test: Two-Sample Assuming Equal Variances. Click OK. The next window asks

	A	B	C	D	E	F	G
1	Analysis of Rayleigh's Data				t-Test: Two-Sample Assuming Equal Variances		
2							
3		Mass of gas (g) collected from				Variable 1	Variable 2
4		air	chemical		Mean	2.31010857	2.2994725
5		2.31017	2.30143		Variance	2.0348E-08	1.902E-06
6		2.30986	2.29890		Observations	7	8
7		2.31010	2.29816		Pooled Variance	1.0336E-06	
8		2.31001	2.30182		Hypothesized Mean Difference	0	
9		2.31024	2.29869		df	13	
10		2.31010	2.29940		t Stat	20.2137243	
11		2.31028	2.29849		P(T<=t) one-tail	1.6607E-11	
12			2.29889		t Critical one-tail	1.77093338	
13	Average	2.310109	2.299473		P(T<=t) two-tail	3.3214E-11	
14	Std Dev	0.000143	0.001379		t Critical two-tail	2.16036865	
15							
16	B13 = AVERAGE(B5:B12)				t-Test: Two-Sample Assuming Unequal Variances		
17	B14 = STDEV(B5:B12)						
18						Variable 1	Variable 2
19	$F = s_1^2/s_2^2 =$	93.483			Mean	2.31010857	2.2994725
20	$F_{\text{table}} =$	4.207			Variance	2.0348E-08	1.902E-06
21					Observations	7	8
22	B19: $F = C14^2/B14^2$				Hypothesized Mean Difference	0	
23	B20: $F_{\text{table}} = \text{FINV}(0.05,7,6)$				df	7	
24					t Stat	21.680218	
25					P(T<=t) one-tail	5.6017E-08	
26					t Critical one-tail	1.8945786	
27					P(T<=t) two-tail	1.1203E-07	
28					t Critical two-tail	2.36462425	

Figure 4-5 Spreadsheet for the *t* test.

you where the data are located. Write B5:B12 for Variable 1 and C5:C12 for Variable 2. The blank space in cell B12 is ignored. For the Hypothesized Mean Difference, enter 0; and for Alpha, enter 0.05. With Alpha = 0.05, we are at the 95% confidence level. For Output Range, select cell E1 and click OK.

Excel now prints results in cells E1 to G14 of Figure 4-5. Mean values are in cells F4 and G4. Cells F5 and G5 give *variance*, which is the square of the standard deviation. Cell F7 gives *pooled variance* computed from the square of Equation 4-6. Cell F9 shows degrees of freedom ($df = 13$) and $t_{\text{calculated}} = 20.2$ from Equation 4-5 appears in cell F10.

Excel gives the critical value of $t = 2.160$ in cell F14 of Figure 4-5. Because $t_{\text{calculated}} (= 20.2) > t_{\text{table}} (= 2.160)$, we conclude that the two means are not the same. The difference is significant. Cell F13 states that the probability of observing these two mean values and standard deviations by random chance if the mean values were really the same is 3.32×10^{-11} . The difference is *highly* significant. For any value of $P \leq 0.05$ in cell F13, we reject the *null hypothesis* and conclude that the means are *different*.

For Rayleigh's data in Figure 4-5, $F_{\text{calculated}}$ (cell B19) $> F_{\text{table}}$ (cell B20); therefore the two standard deviations are different. In Data Analysis, select *t-Test: Two-Sample Assuming Unequal Variances* and proceed as above with cell E16 for the output. We find 7 degrees of freedom in cell F23 and $t_{\text{calculated}} = 21.7$ in cell F24, just as we found in the Example in Section 4.3. The critical value of t is 2.36 in cell F28, and the probability that the two means come from the same population is 1.12×10^{-7} in cell F27. We conclude that the two means are significantly different.

We use the two-tail test with results in cells F13 and F14 and in cells F27 and F28. It is beyond the scope of this book to discuss what is meant by one-tail or two-tail.

Ask Yourself

4-D.  Reproduce Figure 4-5 in a spreadsheet.

4-5 Grubbs Test for an Outlier

Freshmen at Phillips University perform an experiment in which they dissolve the zinc from a galvanized nail and measure the mass lost by the nail to tell how much of the nail was zinc. Several students performed the experiment in triplicate and pooled their results:

Mass loss (%): $\underbrace{10.2, 10.8, 11.6}_{\text{Sidney}}$ $\underbrace{9.9, 9.4, 7.8}_{\text{Cheryl}}$ $\underbrace{10.0, 9.2, 11.3}_{\text{Tien}}$ $\underbrace{9.5, 10.6, 11.6}_{\text{Dick}}$

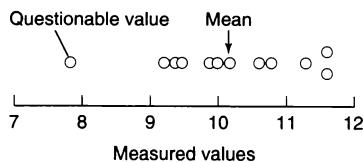
Cheryl's value 7.8 looks out of line from the other data. A datum that is far from the other points is called an *outlier*. Should the group reject 7.8 before averaging the rest of the data or should 7.8 be retained?

We answer this question with the **Grubbs test**. First compute the average (\bar{x}) and the standard deviation (s) of the complete data set (all 12 points in this example):

$$\bar{x} = 10.16 \quad s = 1.11$$

Then compute the Grubbs statistic G , defined as

$$\text{Grubbs test: } G = \frac{|\text{questionable value} - \bar{x}|}{s} \quad (4-9)$$



If $G_{\text{calculated}} > G_{\text{table}}$, reject the questionable point.

Table 4-6 Critical values of G for rejection of outlier^{a,b}

Number of observations	G (95% confidence)
4	1.463
5	1.672
6	1.822
7	1.938
8	2.032
9	2.110
10	2.176
11	2.234
12	2.285
15	2.409
20	2.557

a. From ASTM E 178-02 *Standard Practice for Dealing with Outlying Observations*; F. E. Grubbs and G. Beck, *Technometrics* 1972, 14, 847.

b. $G_{\text{calculated}} = |\text{questionable value} - \text{mean}| / s$. If $G_{\text{calculated}} > G_{\text{table}}$, the value in question can be rejected with 95% confidence. Values in this table are for a one-tail test, as recommended by the American Society for Testing and Materials (ASTM).

where the numerator is the absolute value of the difference between the suspected outlier and the mean value. If G calculated from Equation 4-9 is greater than G in Table 4-6, the questionable point should be discarded.

For the preceding numbers, $G_{\text{calculated}} = |7.8 - 10.16|/1.11 = 2.13$. The value G_{table} is 2.285 for 12 observations in Table 4-6. Because $G_{\text{calculated}}$ is smaller than G_{table} , the questionable point should be retained. There is more than a 5% chance that the value 7.8 is a member of the same population as the other measurements.

Common sense must always prevail. If Cheryl knows that her measurement was low because she spilled some of her unknown, then the probability that the result is wrong is 100% and the datum should be discarded. A datum based on a faulty procedure should be discarded, no matter how well it fits the rest of the data.

Ask Yourself

4-E. Would you reject the value 216 from the set of results 192, 216, 202, 195, and 204?

4-6 Finding the “Best” Straight Line

The *method of least squares* finds the “best” straight line through experimental data points. We will apply this procedure to analytical chemistry calibration curves in the next section.

The equation of a straight line is

$$\text{Equation of straight line: } y = mx + b \quad (4-10)$$

in which m is the **slope** and b is the **y-intercept** (Figure 4-6). If we measure between two points that lie on the line, the slope is $\Delta y/\Delta x$, which is constant for any pair of points on the line. The y-intercept is the point at which the line crosses the y -axis.

Method of Least Squares

The **method of least squares** finds the “best” line by adjusting the line to minimize vertical deviations between the points and the line (Figure 4-7). Reasons to minimize only vertical deviations are (1) experimental uncertainty in y (such as an instrument response) is often greater than uncertainty in x (such as the concentration of a standard) and (2) the calculation for minimizing vertical deviations is relatively simple.

In Figure 4-7, the vertical deviation for the point (x_i, y_i) is $y_i - y$, where y is the *ordinate* of the straight line when $x = x_i$. The ordinate is the value of y that really lies on the line; y_i is the measured value that does not lie exactly on the line.

$$\text{vertical deviation} = d_i = y_i - y = y_i - (mx_i + b) \quad (4-11)$$

Some of the deviations are positive and some are negative. To minimize the magnitude of the deviations irrespective of their signs, we square the deviations to create positive numbers:

$$d_i^2 = (y_i - y)^2 = (y_i - mx_i - b)^2$$

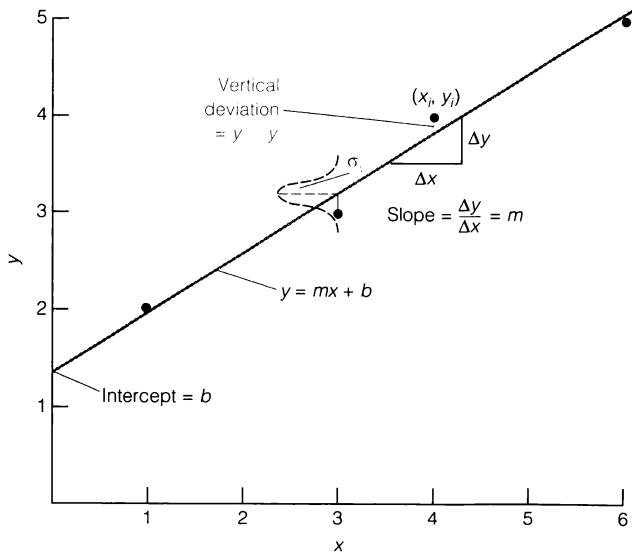


Figure 4-7 Least-squares curve fitting minimizes the sum of the squares of the vertical deviations of the measured points from the line. The Gaussian curve drawn over the point (3, 3) is a schematic indication of the distribution of measured y values about the straight line. The most probable value of y falls on the line, but there is a finite probability of measuring y some distance from the line.

Because we minimize the squares of the deviations, this procedure is called the *method of least squares*.

When we use such a procedure to minimize the sum of squares of the vertical deviations, the slope and the intercept of the “best” straight line fitted to n points are

$$\text{Least-squares slope: } m = \frac{n\sum(x_i y_i) - \sum x_i \sum y_i}{D} \quad (4-12)$$

$$\text{Least-squares intercept: } b = \frac{\sum(x_i^2)\sum y_i - \sum(x_i y_i)\sum x_i}{D} \quad (4-13)$$

where the denominator, D , is given by

$$D = n\sum(x_i^2) - (\sum x_i)^2 \quad (4-14)$$

These equations are not as terrible as they appear. Table 4-7 sets out an example in which the four points ($n = 4$) in Figure 4-7 are treated. The first two columns list x_i and y_i for each point. The third column gives the product $x_i y_i$, and the fourth column lists the square x_i^2 . At the bottom of each column is the sum for that column. That is, beneath the first column is $\sum x_i$ and beneath the third column is $\sum(x_i y_i)$. The last two columns at the right will be used later.

Remember that Σ means summation:
 $\sum x_i = x_1 + x_2 + x_3 + \dots$

Table 4-7 Calculations for least-squares analysis

x_i	y_i	$x_i y_i$	x_i^2	$d_i (= y_i - mx_i - b)$	d_i^2
1	2	2	1	0.038 462	0.001 479
3	3	9	9	-0.192 308	0.036 982
4	4	16	16	0.192 308	0.036 982
6	5	30	36	-0.038 462	0.001 479
$\sum x_i = 14$	$\sum y_i = 14$	$\sum(x_i y_i) = 57$	$\sum(x_i^2) = 62$		$\sum(d_i^2) = 0.076 923$

Quantities required for propagation of uncertainty with Equation 4-19:

$$\bar{x} = (\sum x_i)/n = (1 + 3 + 4 + 6)/4 = 3.50 \quad \bar{y} = (\sum y_i)/n = (2 + 3 + 4 + 5)/4 = 3.50$$

$$\sum(x_i - \bar{x})^2 = (1 - 3.5)^2 + (3 - 3.5)^2 + (4 - 3.5)^2 + (6 - 3.5)^2 = 13$$

With the sums from Table 4-7, we compute the slope and intercept by substituting into Equations 4-14, 4-12, and 4-13:

$$D = n\Sigma(x_i^2) - (\Sigma x_i)^2 = (4 \cdot 62) - 14^2 = 52$$

$$m = \frac{n\Sigma(x_i y_i) - \Sigma x_i \Sigma y_i}{D} = \frac{(4 \cdot 57) - (14 \cdot 14)}{52} = 0.615\ 38$$

$$b = \frac{\Sigma(x_i^2)\Sigma y_i - \Sigma(x_i y_i)\Sigma x_i}{D} = \frac{(62 \cdot 14) - (57 \cdot 14)}{52} = 1.346\ 15$$

The equation of the best straight line through the points in Figure 4-7 is therefore

$$y = 0.615\ 38x + 1.346\ 15$$

Next, we will see how many figures in these numbers are significant.

How Reliable Are Least-Squares Parameters?

The uncertainties in m and b are related to the uncertainty in measuring each value of y . Therefore we first estimate the standard deviation describing the population of y values. This standard deviation, s_y , characterizes the little Gaussian curve inscribed in Figure 4-7. The deviation of each y_i from the center of its Gaussian curve is $d_i = y_i - \bar{y} = y_i - (mx_i + b)$ (Equation 4-11). The standard deviation of these vertical deviations is

$$s_y \approx \sqrt{\frac{\sum(d_i^2)}{n - 2}} \quad (4-15)$$

Analysis of uncertainty for Equations 4-12 and 4-13 leads to the following results:

$$\text{Standard deviation of slope: } s_m = s_y \sqrt{\frac{n}{D}} \quad (4-16)$$

$$\text{Standard deviation of intercept: } s_b = s_y \sqrt{\frac{\sum(x_i^2)}{D}} \quad (4-17)$$

where s_y is given by Equation 4-15 and D is given by Equation 4-14.

At last, we can address significant figures for the slope and the intercept of the line in Figure 4-7. In Table 4-7, we see that $\sum(d_i^2) = 0.076\ 923$. Inserting this value into Equation 4-15 gives

$$s_y = \sqrt{\frac{0.076\ 923}{4 - 2}} = 0.196\ 12$$

Now, we can plug numbers into Equations 4-16 and 4-17 to find

$$s_m = s_y \sqrt{\frac{n}{D}} = (0.196\ 12) \sqrt{\frac{4}{52}} = 0.054\ 394$$

$$s_b = s_y \sqrt{\frac{\sum(x_i^2)}{D}} = (0.196\ 12) \sqrt{\frac{62}{52}} = 0.214\ 15$$

Combining the results for m , s_m , b , and s_b , we write

Slope: $\frac{0.615\ 38}{\pm 0.054\ 39} = 0.62 \pm 0.05$ or $0.61_5 \pm 0.05_4$

Intercept: $\frac{1.346\ 15}{\pm 0.214\ 15} = 1.3 \pm 0.2$ or $1.3_5 \pm 0.2_1$

The first digit of the uncertainty is the last significant figure.

where the uncertainties represent one standard deviation. *The first decimal place of the standard deviation is the last significant figure of the slope or intercept.*

Ask Yourself

- 4-F. Construct a table analogous to Table 4-7 to find the best straight line going through the points (1, 3), (3, 2), and (5, 0). Express your answer in the form $y (\pm s_y) = [m (\pm s_m)]x + [b (\pm s_b)]$, with a reasonable number of significant figures.

4-7 Constructing a Calibration Curve

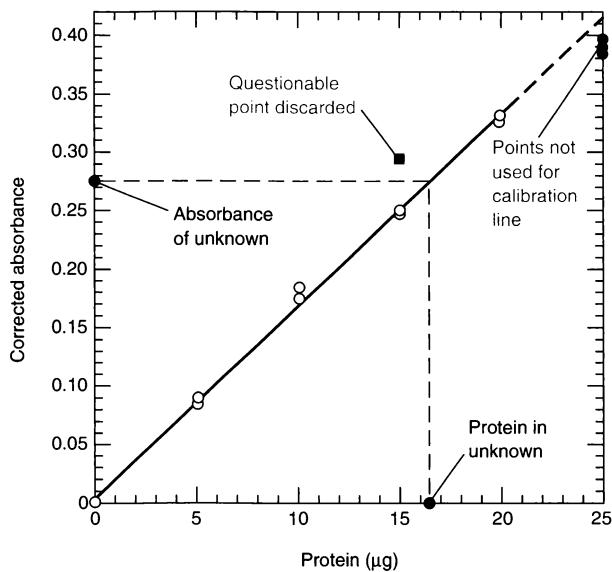
Data from a spectrophotometric analysis are given in Table 4-8. In this procedure, the intensity of a colored product is proportional to the amount of protein in the sample. Color is measured by the absorbance of light recorded on a spectrophotometer. The first row in Table 4-8 shows readings obtained when no protein was present. The nonzero values arise from color in the reagents themselves. A result obtained with zero analyte is called a **blank** (or a *reagent blank*), because it measures effects due to the analytical reagents. The second row shows three readings obtained with 5 μg of protein. Successive rows give results for 10, 15, 20, and 25 μg of protein. A solution containing a known quantity of analyte (or other reagent) is called a **standard solution**.

A **calibration curve** is a graph showing how the experimentally measured property (absorbance) depends on the known concentrations of the standards. To construct the calibration curve in Figure 4-8, we first subtract the average absorbance of

Table 4-8 Spectrophotometer readings for protein analysis by the Lowry method

Sample (μg)	Absorbance of three independent samples			Range	Corrected absorbance (after subtracting average blank)			
0	0.099	0.099	0.100	0.001	-0.000 ₃	-0.000 ₃	0.000 ₇	Data used for calibration curve
5	0.185	0.187	0.188	0.003	0.085 ₇	0.087 ₇	0.088 ₇	
10	0.282	0.272	0.272	0.010	0.182 ₇	0.172 ₇	0.172 ₇	
15	0.392	0.345	0.347	0.047	—	0.245 ₇	0.247 ₇	
20	0.425	0.425	0.430	0.005	0.325 ₇	0.325 ₇	0.330 ₇	
25	0.483	0.488	0.496	0.013	0.383 ₇	0.388 ₇	0.396 ₇	

Figure 4-8 Calibration curve showing the average absorbance values in Table 4-8 versus micrograms of protein analyzed. The average blank value for 0 µg of protein has been subtracted from each point.



the blanks (0.099_3) from those of the standards to obtain *corrected absorbance*. When all points are plotted in Figure 4-8 and a rough straight line is drawn through them, two features stand out:

Inspect your data and use judgment before mindlessly asking a computer to draw a calibration curve!

1. One data point for 15 µg of protein (shown by the square in Figure 4-8) is clearly out of line. When we inspect the range of values for each set of three measurements in Table 4-8, we discover that the range for 15 µg is four times greater than the next greatest range. We discard the absorbance value 0.392 as “bad data.” Perhaps the glassware was contaminated with protein from a previous experiment?
2. All three points at 25 µg lie slightly below the straight line through the remaining data. Many repetitions of this experiment show that these points are consistently below the straight line. Therefore the *linear range* for this determination extends from 0 to 20 µg, but not to 25 µg.

In view of these observations, we discard the 0.392 absorbance value and we do not use the three points at 25 µg for the least-squares straight line. We could use a *non-linear calibration curve* that extends to 25 µg, but we will not do so in this book.

To construct the calibration curve (the straight line) in Figure 4-8, we use the method of least squares with $n = 14$ data points from Table 4-8 (including three blank values) covering the range 0 to 20 µg of protein. The results of applying Equations 4-12 through 4-17 are

Equation of calibration line:

$$y(\pm s_y) = [m(\pm s_m)]x + [b(\pm s_b)] \\ y(\pm 0.005_9) = [0.016\ 3_0 (\pm 0.000\ 2_2)]x \\ + [0.004_7 (\pm 0.002_6)]$$

$$m = 0.016\ 3_0 \quad s_m = 0.000\ 2_2 \\ b = 0.004_7 \quad s_b = 0.002_6 \\ s_y = 0.005_9$$

Finding the Protein in an Unknown

Suppose that the measured absorbance of an unknown sample is 0.373. How many micrograms of protein does it contain, and what uncertainty is associated with the answer?

The first question is easy. The equation of the calibration line is

$$y = mx + b = (0.0163_0)x + 0.004_7$$

where y is corrected absorbance (= observed absorbance – blank absorbance) and x is micrograms of protein. If the absorbance of the unknown is 0.373, its corrected absorbance is $0.373 - 0.099_3 = 0.273_7$. Plugging this value in for y in the preceding equation permits us to solve for x :

$$0.273_7 = (0.0163_0)x + (0.004_7) \quad (4-18a)$$

$$x = \frac{0.273_7 - 0.004_7}{0.0163_0} = 16.50 \text{ } \mu\text{g of protein} \quad (4-18b)$$

But what is the uncertainty in 16.50 μg ?

The uncertainty in x in Equation 4-18 turns out to be

$$\text{uncertainty in } x (= s_x) = \frac{s_y}{|m|} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \sum(x_i - \bar{x})^2}} \quad (4-19)$$

where s_y is the standard deviation of y (Equation 4-15), $|m|$ is the absolute value of the slope, k is the number of replicate measurements of the unknown, n is the number of data points for the calibration line (14 in Table 4-8), \bar{y} is the mean value of y for the points on the calibration line, x_i are individual values of x for the points on the calibration line, and \bar{x} is the mean value of x for the points on the calibration line. For a single measurement of the unknown, $k = 1$ and Equation 4-19 gives $s_x = \pm 0.3_8 \mu\text{g}$. The result of the analysis can therefore be expressed with a reasonable number of significant digits as

$$x = 16.5 (\pm 0.4) \text{ } \mu\text{g of protein}$$

If you measured four replicate unknowns ($k = 4$) and the average corrected absorbance was still 0.273₇, the uncertainty is reduced from $\pm 0.3_8$ to $\pm 0.2_2 \mu\text{g}$.

Example: If 4 replicate samples of an unknown have an average absorbance of 0.373, use the corrected absorbance $y = 0.373 - 0.099_3 = 0.273_7$. In Equation 4-19, $k = 4$ for 4 replicate measurements and $n = 14$ because there are 14 points on the calibration curve (Table 4-8). Other values in Equation 4-19 are

$$x_i = \mu\text{g of protein in standards in Table 4-8} = (0, 0, 0, 5.0, 5.0, 5.0, 10.0, 10.0, 10.0, 15.0, 15.0, 20.0, 20.0, 20.0)$$

$$\bar{x} = \text{average of 14 } x \text{ values} = 9.64_3 \mu\text{g}$$

$$\bar{y} = \text{average of 14 corrected } y \text{ values} = 0.161_8$$

From these values, we calculate $s_x = 0.22 \mu\text{g}$.

Ask Yourself

4-G. Using results from Ask Yourself 4-F, find the value of x (and its uncertainty) corresponding to a mean value of $y = 1.00$ for $k = 5$ replicate measurements.

4-8 A Spreadsheet for Least Squares

Figure 4-9 uses built-in power of Excel for least-squares calculations of straight lines. As an example, enter the x - and y -coordinates from Table 4-7 into cells B4 through B7 and C4 through C7. This range is abbreviated B4:C7. The key portion of the spreadsheet uses the Excel function LINEST to compute the least-squares parameters in cells B10:C12.

Highlight the 3-row \times 2-column region B10:C12 with your mouse. On the Formulas ribbon, go to Insert Function. In the window that appears, go to Statistical

	A	B	C	D	E	F	G	H	I
1	Least-Squares Spreadsheet								
2									
3	Highlight cells B10:C12	x	y						
4	Type "=LINEST(C4:C7, B4:B7,TRUE,TRUE)	1	2						
5		3	3						
6	For PC, press	4	4						
7	CTRL+SHIFT+ENTER	6	5						
8	For Mac, press								
9	APPLE+ENTER	LINEST output:							
10	m	0.6154	1.3462	b					
11	s_m	0.0544	0.2141	s_b					
12	R^2	0.9846	0.1961	s_y					
13									
14	n =	4	B14 = COUNT(B4:B7)						
15	Mean y =	3.5	B15 = AVERAGE(C4:C7)						
16	$\sum(x_i - \text{mean } x)^2 =$	13	B16 = DEVSQ(B4:B7)						
17									
18	Measured y =	2.72	Input						
19	k = Number of replicate measurements of y =	1	Input						
20	Derived x =	2.2325	B20 = (B18-C10)/B10						
21	$s_x =$	0.3735	B21 = (C12/B10)*SQRT((1/B19)+(1/B14)+((B18-B15)^2)/(B10^2*B16))						

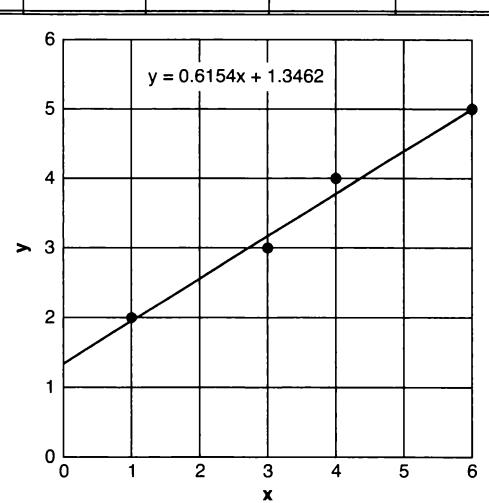


Figure 4-9 Spreadsheet for least-squares calculations.

and double click on LINEST. A new window asks for four inputs to the function. For y values, enter C4:C7. Then enter B4:B7 for x values. The next two entries are both “TRUE”. The first TRUE tells Excel that we want to compute the y-intercept of the least-squares line and not force the intercept to be 0. The second TRUE tells Excel to compute standard deviations of the slope and intercept. The formula you just entered is “=LINEST(C4:C7,B4:B7,TRUE,TRUE)”. Now press CONTROL+SHIFT+ENTER on a PC or APPLE+ENTER on a Mac. Excel dutifully prints out a matrix in cells B10:C12. Write labels around the block to indicate what is in each cell. The slope (m) and intercept (b) are on the top line. The second line contains the standard deviations of slope and intercept, s_m and s_b . Cell C12 contains s_y and cell B12 contains a quantity called R^2 , which measures the goodness of fit of the data to the line. The closer R^2 is to unity, the better the fit.

Cell B14 gives the number of data points with the formula =COUNT(B4:B7). Cell B15 computes the mean value of y. Cell B16 computes the sum $\sum(x_i - \bar{x})^2$ that we need for Equation 4-19. This sum is common enough that Excel has a built-in function called DEVSQ, which you can find in the Formulas ribbon under Insert Function in Statistical Functions. Formulas in Figure 4-9 are documented beside the cell where they are used.

Enter the measured value $y = 2.72$ for replicate measurements of the unknown in cell B18. In cell B19, enter the number of replicate measurements ($k = 1$) of the unknown. In this example, we compute $x = 2.23$ in cell B20 with an uncertainty of ± 0.3 , in cell B21.

We always want a graph to see if the calibration points lie on a straight line. Follow instructions in Section 3-6 to plot the calibration data. To add a straight line, click on one data point and they will all be highlighted. Go to the Chart Tools ribbon and select the Layout Tab. In the Analysis section, select Trendline and choose More Trendline Options. In the Format Trendline Window, select Linear and Display

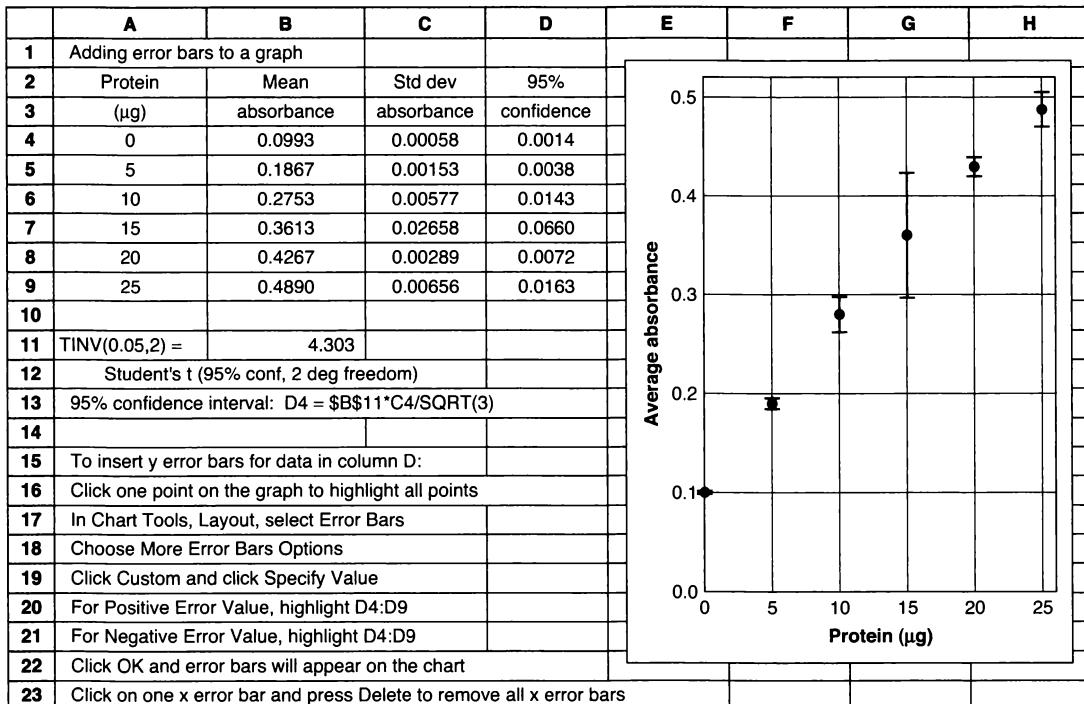


Figure 4-10 Adding 95% confidence error bars to a graph.

Equation on Chart. When you click Close, the least-squares straight line and its equation appear on the graph. The line only extends from the first point to the last point. To extend the line back to $x = 0$, select More Trendline Options again. Forecast Backward by 1 period. Now the trendline extends backward from the first datum at $x = 1$ to the y-axis at $x = 0$. More Trendline Options also allows you to modify the color and style of the trendline.

Adding Error Bars to a Graph

Error bars on a graph help us judge the quality of the data and the fit of a curve to the data. Consider the data in Table 4-8. Let's plot the mean absorbance of columns 2 to 4 versus sample mass in column 1. Then we will add error bars corresponding to the 95% confidence interval for each point. Figure 4-10 lists mass in column A and mean absorbance in column B. The standard deviation of absorbance is given in column C. The 95% confidence interval for absorbance is computed in column D with the formula in the margin. Student's $t = 4.303$ can be found for 95% confidence and $3 - 1 = 2$ degrees of freedom in Table 4-4. Alternatively, compute Student's t with the function TINV(0.05,2) in cell B11. The parameters for TINV are 0.05 for 95% confidence and 2 for degrees of freedom. The 95% confidence interval in cell D4 is computed with “= \$B\$11*C4/SQRT(3)”. You should be able to plot mean absorbance (y) in column B versus protein mass (x) in column A.

To add error bars, click on one of the points to highlight all points on the graph. In Chart Tools, Layout, select Error Bars and choose More Error Bars Options. For Error Amount, choose Custom and Specify Value. For both Positive Error Value and Negative Error Value, enter D4:D9. You just told the spreadsheet to use 95% confidence intervals for error bars. When you click OK, the graph has both x and y error bars. Click on any x error bar and press Delete to remove all x error bars.

$$\text{Confidence interval} = \pm ts/\sqrt{n}$$

t = Student's t for 95% confidence
and $n - 1 = 2$ degrees of freedom

s = standard deviation

n = number of values in average = 3

Ask Yourself

- 4-H.  (a) Reproduce Figure 4-9 to solve linear least-squares problems.
 (b) Plot the data points and least-squares line in Figure 4-9.
 (c) Reproduce the error bar chart in Figure 4-10.
-

Key Equations

Mean	$\bar{x} = \frac{1}{n} \sum_i x_i = \frac{1}{n} (x_1 + x_2 + x_3 + \dots + x_n)$	
	$x_i = \text{individual observation}, n = \text{number of observations}$	
Standard deviation	$s = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{(n - 1)}} \quad (\bar{x} = \text{average})$	
Confidence interval	$\mu = \bar{x} \pm \frac{ts}{\sqrt{n}} \quad (\mu = \text{true mean})$	Student's t comes from Table 4-4 for $n - 1$ degrees of freedom at the selected confidence level.
F test	$F = s_1^2/s_2^2$	If $F_{\text{calculated}} > F_{\text{table}}$ for $n_1 - 1$ and $n_2 - 1$ degrees of freedom, standard deviations are significantly different.
t test for standard deviations that are not significantly different	$t = \frac{ \bar{x}_1 - \bar{x}_2 }{s_{\text{pooled}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$ $s_{\text{pooled}} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}}$	If $t_{\text{calculated}} > t_{\text{table}}$ (for 95% confidence and $n_1 + n_2 - 2$ degrees of freedom), the difference is significant.
		For standard deviations that are significantly different, use Equations 4-7 and 4-8.
Grubbs test	$G = \frac{ \text{questionable value} - \bar{x} }{s}$	If $G_{\text{calculated}} > G_{\text{table}}$, reject questionable point.
Straight line	$y = mx + b \quad m = \text{slope} = \Delta y / \Delta x \quad b = y\text{-intercept}$	
Least-squares equations		You should know how to use Equations 4-12 through 4-17 to derive least-squares slope and intercept and uncertainties.
Calibration curve		You should be able to use Equation 4-19 to find the uncertainty in a result derived from a calibration curve.

Important Terms

average	Gaussian distribution	range	t test
blank	Grubbs test	slope	variance
calibration curve	mean	standard deviation	y -intercept
confidence interval	median	standard solution	
F test	method of least squares	Student's t	

Problems

4-1. What is the relation between the standard deviation and the precision of a procedure? What is the relation between standard deviation and accuracy?

4-2. What fraction of observations in an ideal Gaussian distribution lies within $\mu \pm \sigma$? Within $\mu \pm 2\sigma$? Within $\mu \pm 3\sigma$?

4-3. In Table 4-2, one set of measurements was repeated 10 times and the other was repeated 4 times. If both had been repeated 10 times, would the standard deviations of 0.28 and 0.47 mM be considered “significantly” different from each other?

4-4. The ratio of the number of atoms of the isotopes ^{69}Ga and ^{71}Ga in samples from different sources is listed here:

Sample	$^{69}\text{Ga}/^{71}\text{Ga}$	Sample	$^{69}\text{Ga}/^{71}\text{Ga}$
1	1.526 60	5	1.528 94
2	1.529 74	6	1.528 04
3	1.525 92	7	1.526 85
4	1.527 31	8	1.527 93

(a) Find the mean value of $^{69}\text{Ga}/^{71}\text{Ga}$.

(b) Find the standard deviation and relative standard deviation.

(c) Sample 8 was analyzed seven times, with $\bar{x} = 1.527\ 93$ and $s = 0.000\ 07$. Find the 99% confidence interval for sample 8.

4-5. (a) What is the meaning of a confidence interval?

(b) For a given set of measurements, will the 95% confidence interval be larger or smaller than the 90% confidence interval? Why?

4-6. For the numbers 116.0, 97.9, 114.2, 106.8, and 108.3, find the mean, standard deviation, and 90% confidence interval for the mean.

4-7. The calcium content of a mineral was analyzed five times by each of two methods.

Method	Ca (wt%, five replications)					
1	0.027 1	0.028 2	0.027 9	0.027 1	0.027 5	
2	0.027 1	0.026 8	0.026 3	0.027 4	0.026 9	

(a) Find the mean and standard deviation of each method.

(b) Use the F test to decide whether the standard deviations are “significantly” different.

(c) Use the t test to decide whether the means are different at the 95% confidence level.

4-8. Find the 95% and 99% confidence intervals for the mean mass of nitrogen from chemical sources given in Table 4-5.

4-9. Two methods were used to measure the specific activity (units of enzyme activity per milligram of protein) of an enzyme. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of one micromole of product per minute under specified conditions.

Method	Enzyme activity (five replications)				
1	139	147	160	158	135
2	148	159	156	164	159

Is the mean value of method 1 significantly different from the mean value of method 2 at the 95% confidence level?

4-10. Students measured the concentration of HCl in a solution by various titrations in which different indicators were used to find the end point.

Indicator	Mean HCl concentration (M) (\pm standard deviation)	Number of measurements
1. Bromothymol blue	0.095 65 \pm 0.002 25	28
2. Methyl red	0.086 86 \pm 0.000 98	18
3. Bromocresol green	0.086 41 \pm 0.001 13	29

Data from D. T. Harvey, *J. Chem. Ed.* **1991**, 68, 329.

(a) Show that the standard deviations for indicators 1 and 2 are significantly different. You will not find F_{table} for 27 and 17 degrees of freedom, so use F_{table} for 30 and 20 degrees of freedom. Alternatively, find F_{table} with the Excel function FINV(0.05,27,17).

(b) Is the difference between the mean values for indicators 1 and 2 significant at the 95% confidence level?

(c) Show that standard deviations for indicators 2 and 3 are not significantly different.

(d) Is the difference between the means for indicators 2 and 3 significant?

4-11. The calcium content of a person’s urine was determined on two different days.

Day	[Ca] (mg/L) Average \pm standard deviation	Number of measurements
1	238 \pm 8	4
2	255 \pm 10	5

Are the average values significantly different at the 95% confidence level?

4-12. Lithium isotope ratios are important to medicine, geology, astrophysics, and nuclear chemistry. The $^{6}\text{Li}/^{7}\text{Li}$ ratio in a Standard Reference Material was measured by two methods.

Method 1: 0.082 601, 0.082 621, 0.082 589, 0.082 617, 0.082 598.

Method 2: 0.082 604, 0.082 542, 0.082 599, 0.082 550, 0.082 583, 0.082 561.

Do the two methods give statistically equivalent results?

4-13. Students at Butler University compared the accuracy and precision of delivering 10 mL from a 50-mL buret, a 10-mL volumetric pipet, and a 10-mL volumetric flask. The table shows results for six replicate measurements by each of two students.

Apparatus	Student 1 $\bar{x} \pm s$ (mL)	Student 2 $\bar{x} \pm s$ (mL)
Buret	10.01 ± 0.09	9.98 ± 0.2
Pipet	9.98 ± 0.02	10.004 ± 0.009
Flask	9.80 ± 0.03	9.84 ± 0.02

Data from M. J. Samide, *J. Chem. Ed.* 2004, 81, 1641.

- (a) Do the volumes delivered by student 1 from the buret and pipet differ at the 95% confidence level?
(b) Do the volumes delivered by student 1 from the buret and flask differ at the 95% confidence level?
(c) Do the volumes delivered from the pipet by students 1 and 2 differ at the 95% confidence level?
(d) What can you conclude about the accuracy of the three methods of delivery? Is the observed accuracy within tolerance for Class A glassware in Tables 2-1, 2-2, and 2-3?

4-14. Using the Grubbs test, decide whether the value 0.195 should be rejected from the set of results 0.217, 0.224, 0.195, 0.221, 0.221, 0.223.

4-15. Students at the University of North Dakota measured visible light absorbance of food colorings. Replicate measurements of a solution of the drink Kool-Aid at a wavelength of 502 nm gave the following values: 0.189, 0.169, 0.187, 0.183, 0.186, 0.182, 0.181, 0.184, 0.181, and 0.177. Identify the outlier and decide whether to exclude it from the data set.

4-16. Find the values of m and b in the equation $y = mx + b$ for the straight line going through the points $(x_1, y_1) = (6, 3)$ and $(x_2, y_2) = (8, -1)$. You can do so by writing

$$m = \frac{\Delta y}{\Delta x} = \frac{(y_2 - y_1)}{(x_2 - x_1)} = \frac{(y - y_1)}{(x - x_1)}$$

and rearranging to the form $y = mx + b$. Sketch the curve and satisfy yourself that the value of b is sensible.

4-17. A straight line is drawn through the points $(3.0, -3.87 \times 10^4)$, $(10.0, -12.99 \times 10^4)$, $(20.0, -25.93 \times 10^4)$, $(30.0, -38.89 \times 10^4)$, and $(40.0, -51.96 \times 10^4)$ by using the method of least squares. The results are $m = -1.29872 \times 10^4$, $b = 256.695$, $s_m = 13.190$, $s_b = 323.57$, and $s_y = 392.9$. Express the slope and intercept and their uncertainties with the correct significant figures.

4-18. Consider the least-squares problem illustrated in Figure 4-7. Suppose that a single new measurement produces a y value of 2.58.

- (a) Calculate the corresponding x value and its uncertainty.
(b) Suppose you measure y four times and the average is 2.58. Calculate the uncertainty in x on the basis of four measurements, not one.

4-19. In a common protein analysis, a dye binds to the protein and the color of the dye changes from brown to blue. The intensity of blue color is proportional to the amount of protein present.

Protein (μg): 0.00 9.36 18.72 28.08 37.44
Absorbance: 0.466 0.676 0.883 1.086 1.280

(a) After subtracting the blank absorbance (0.466) from the remaining absorbances, use the method of least squares to determine the equation of the best straight line through these five points ($n = 5$). Use the standard deviation of the slope and intercept to express the equation in the form $y (\pm s_y) = [m (\pm s_m)]x + [b (\pm s_b)]$ with a reasonable number of significant figures.

- (b) Make a graph showing the experimental data and the calculated straight line.
(c) An unknown gave an observed absorbance of 0.973. Calculate the number of micrograms of protein in the unknown, and estimate its uncertainty.

4-20. The equation for a Gaussian curve is

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/2\sigma^2}$$

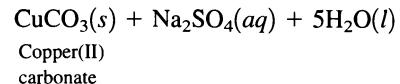
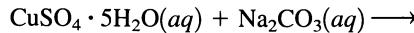
In Excel, the square-root function is SQRT and the exponential function is EXP. To find $e^{-3.4}$, write EXP(-3.4). The Gaussian exponential function is written

$$e^{-(x-\mu)^2/2\sigma^2} = \text{EXP}(-((x-\mu)^2)/(2*\sigma^2))$$

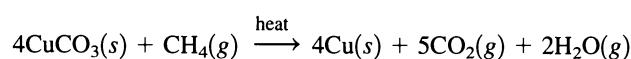
- (a) For $\mu = 10$ and $\sigma = 1$, compute values of y for the range $4 \leq x \leq 16$.
(b) Repeat the calculation for $\sigma = 2$.
(c) Plot the results of (a) and (b) on one graph. For $\sigma = 2$, label the regions that contain 68.3% and 95.5% of all observations.

How Would You Do It?

4-21. Students at Eastern Illinois University³ intended to prepare copper(II) carbonate by adding a solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to a solution of Na_2CO_3 .



After warming the mixture to 60°C, the gelatinous blue precipitate coagulated into an easily filterable pale green solid. The product was filtered, washed, and dried at 70°C. Copper in the product was measured by heating 0.4 g of solid in a stream of methane at high temperature to reduce the solid to pure Cu, which was weighed.



In 1995, 43 students found a mean value of 55.6 wt% Cu with a standard deviation of 2.7 wt%. In 1996, 39 students found 55.9 wt% with a standard deviation of 3.8 wt%. The instructor tried the experiment nine times and measured 55.8 wt% with a standard deviation of 0.5 wt%. Was the product of the synthesis CuCO_3 ? Could it have been a hydrate, $\text{CuCO}_3 \cdot x\text{H}_2\text{O}$?

4-22. Strontium isotopes vary in different rocks, depending on the original content of radioactive elements in those rocks. The isotope ratio $^{87}\text{Sr}/^{86}\text{Sr}$ is used in environmental studies to determine sources of particles and solutes in water and ice. Materials originating from one source should have the same $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. Materials from different sources could have different isotope ratios. Observations for Sr found in micro-

scopic dust particles in ice drilled from Antarctica are shown below. Age is determined from depth in the ice. Uncertainties are expressed as 95% confidence intervals. EH stands for early Holocene and LGM stands for last glacial maximum; both are measures of geologic time.

Location	Age (years before present)	Age	
		Sr (pg/g)	$^{87}\text{Sr}/^{86}\text{Sr}$
Dome C	7 500 [EH]	30.8 ± 0.4	0.7068 ± 0.0006
Dome C	23 000 [LGM]	324 ± 4	0.7082 ± 0.0005
Law Dome	6 500 [EH]	45.6 ± 0.6	0.7097 ± 0.0004
Law Dome	34 000 [LGM]	96 ± 2	0.7093 ± 0.0011

Data from G. R. Burton, V. I. Morgan, C. F. Boutron, and K. J. R. Rosman, *Anal. Chim. Acta* **2002**, 469, 225.

Does it appear that the EH dust at Dome C comes from the same source as EH dust at Law Dome? Does LGM dust at Dome C come from the same source as LGM dust at Law dome? What does the unit pg/g mean? Express pg/g with a term such as “parts per million.”

Notes and References

1. S. A. Lee, R. K. Ross, and M. C. Pike, *Br. J. Cancer* **2005**, 92, 2049.
2. When t calculated with Equation 4-5 is greater than the tabulated t , we conclude that the two means are different with a chosen confidence level. This test does not provide the same confidence that the two means *are equal*. For discussions of

how to show that two means are equal at a certain confidence level, see S. E. Lewis and J. E. Lewis, *J. Chem. Ed.* **2005**, 82, 1408 and G. B. Limentani, M. C. Ringo, F. Ye, M. L. Bergquist, and E. O. McSorley, *Anal. Chem.* **2005**, 77, 221A.

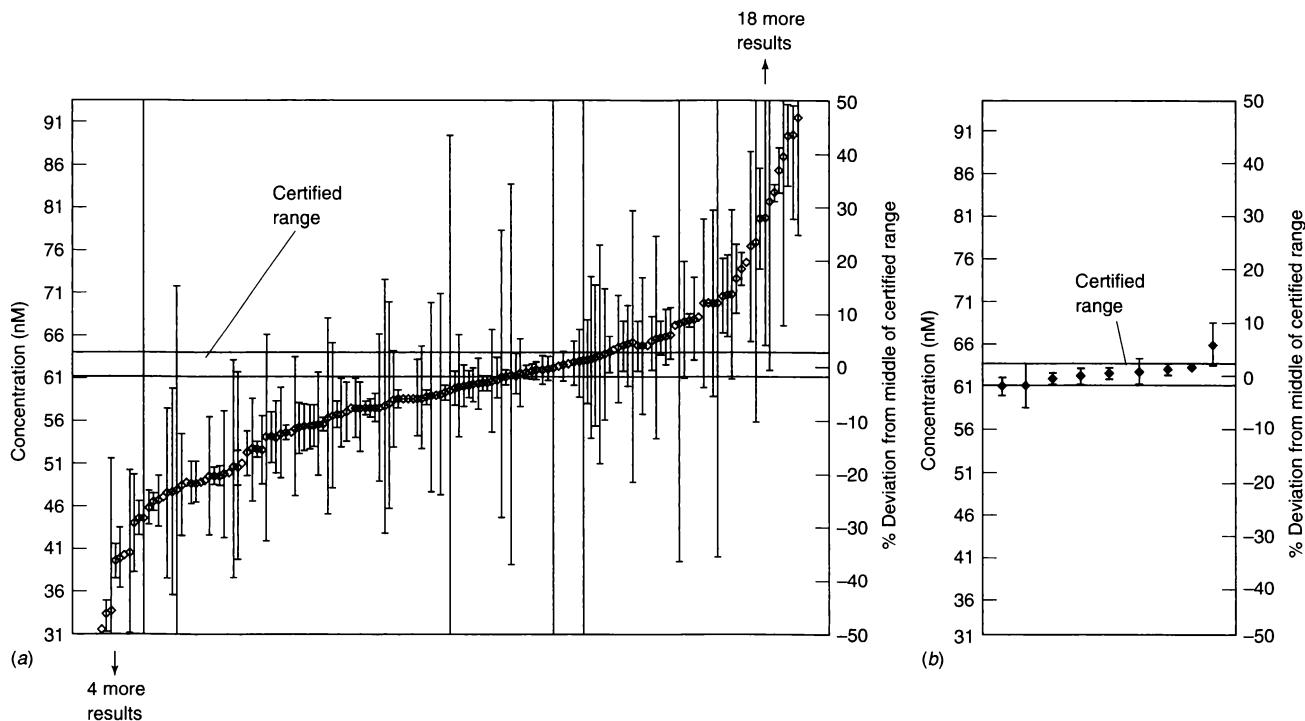
3. D. Sheeran, *J. Chem. Ed.* **1998**, 75, 453. See also H. Gamsjäger and W. Preis, *J. Chem. Ed.* **1999**, 76, 1339.

Further Reading

D. B. Hibbert and J. J. Gooding, *Data Analysis for Chemistry* (Oxford: Oxford University Press, 2006).

NIST/SEMATECH e-Handbook of Statistical Methods, <http://www.itl.nist.gov/div898/handbook/>.

The Need for Quality Assurance



(a) Scattered measurements of Pb in river water by different laboratories, each of which employed a recognized quality management system. (b) Reproducible results from national measurement institutes. [From P. De Bièvre and P. D. P. Taylor, *Fresenius J. Anal. Chem.* **2000**, 368, 567.]

The Institute for Reference Materials and Measurements in Belgium conducts an International Measurement Evaluation Program to allow laboratories to assess the reliability of their analyses. Panel *a* shows results for lead in river water. Of 181 labs, 18 reported results more than 50% above and 4 reported results more than 50% below the certified level of 62.3 ± 1.3 nM. Even though most labs in the study employed recognized quality management procedures, a large fraction of results did not include the certified range. Panel *b* shows that when this same river water was analyzed by nine different national measurement institutes, where the most care is taken, all results were close to the certified range.

This example illustrates that there is no guarantee that results are reliable, even if they are obtained by “accredited” laboratories using accepted procedures. A good way to assess the reliability of a lab working for you is to provide the lab with “blind” samples—similar to your unknowns—for which you know the “right” answer, but the analyst does not. If the lab does not find the known result, there is a problem. Periodic checks with blind samples are required to demonstrate continuing reliability.

Quality Assurance and Calibration Methods

Quality assurance is what we do to get the right answer for our purpose. The answer should have sufficient accuracy and precision to support subsequent decisions. There is no point spending extra money to obtain a more accurate or more precise answer if it is not necessary. This chapter describes basic issues and procedures in quality assurance and introduces two more calibration methods. In Chapter 4, we discussed how to make a calibration curve. In this chapter, we consider the methods of *standard addition* and *internal standards*. These two methods are commonly used for instrumental methods of analysis. Study of Sections 5-3 and 5-4 could logically be deferred until these calibration methods are needed in the lab.

5-1 Basics of Quality Assurance

“Suppose you are cooking for some friends. While making spaghetti sauce, you taste it, season it, taste it some more. Each tasting is a sampling event with a quality control test. You can taste the whole batch because there is only one batch. Now suppose you run a spaghetti sauce plant that makes 1 000 jars a day. You can’t taste each one, so you decide to taste three a day, one each at 11 a.m., 2 p.m., and 5 p.m. If the three jars all taste OK, you conclude all 1 000 are OK. Unfortunately, that may not be true, but the relative risk—that a jar has too much or too little seasoning—is not very important because you agree to refund the money of any customer who is dissatisfied. If the number of refunds is small, say, 100 a year, there is no apparent benefit in tasting 4 jars a day.” You would test 365 additional jars to avoid refunds on 100 jars, a net loss of 265 jars worth of profit.

In analytical chemistry, the product is not spaghetti sauce, but, rather, raw data, treated data, and results. *Raw data* are individual measurements, such as peak areas from a chromatogram or volumes from a buret. *Treated data* are concentrations or amounts found by applying a calibration procedure to the raw data. *Results* are what we ultimately report, such as the mean, standard deviation, and confidence interval, after applying statistics to treated data.

Use Objectives

If you manufacture a drug whose therapeutic dose is just a little less than the lethal dose, you should be more careful than if you make spaghetti sauce. The kind of data that you collect and the way in which you collect them depend on how you plan to use those data. An important goal of quality assurance is making sure that results meet

Quotation from Ed Urbansky, U.S. Environmental Protection Agency, Cincinnati, OH. Section 5-1 is adapted from a description written by Ed Urbansky.

Raw data: individual measurements

Treated data: concentrations derived from raw data by use of calibration method

Results: quantities reported after statistical analysis of treated data

Use objective: states purpose for which results will be used

the customer's needs. A bathroom scale does not have to measure mass to the nearest milligram, but a drug tablet required to contain 2 mg of active ingredient probably cannot contain 2 ± 1 mg. Writing clear, concise **use objectives** for data and results is a critical step in quality assurance and helps prevent misuse of data and results.

Here is an example of a use objective. Drinking water is usually disinfected with chlorine, which kills microorganisms. Unfortunately, chlorine also reacts with organic matter in water to produce "disinfection by-products"—compounds that might harm humans. A disinfection plant was planning to introduce a new chlorination process and wrote the following analytical use objective:

Analytical data and results shall be used to determine whether the modified chlorination process results in at least a 10% reduction of formation of selected disinfection by-products.

The new process was expected to produce less disinfection by-products. The use objective says that uncertainty in the analysis must be small enough that a 10% decrease in selected by-products is clearly distinguishable from experimental error. In other words, is an observed decrease of 10% real?

Specifications

Specifications might include:

- sampling requirements
- accuracy and precision
- rate of false results
- selectivity
- sensitivity
- acceptable blank values
- recovery of fortification
- calibration checks
- quality control samples

Once you have use objectives, you are ready to write **specifications** stating how good the numbers need to be and what precautions are required in the analytical procedure. How shall samples be taken and how many are needed? Are special precautions required to protect samples and ensure that they are not degraded? Within practical restraints, such as cost, time, and limited amounts of material available for analysis, what level of accuracy and precision will satisfy the use objectives? What rate of false positives or false negatives is acceptable? These questions need to be answered in detailed specifications.

Quality assurance begins with sampling. We must collect representative samples, and analyte must be preserved after sample is collected. If our sample is not representative or if analyte is lost after collection, then even the most accurate analysis is meaningless. Samples for trace metal analysis are usually collected in plastic or Teflon containers—not glass—because metal ions found on glass surfaces leach out into the sample over time. Samples for organic analysis are usually collected in glass containers—not plastic—because organic plasticizers leached from plastic containers can contaminate the sample. Samples are often stored in the dark in a refrigerator to minimize degradation of organic analytes.

What do we mean by false positives and false negatives? Suppose you must certify that a contaminant in drinking water is below a legal limit. A *false positive* says that the concentration exceeds the legal limit when, in fact, the concentration is below the limit. A *false negative* says the concentration is below the limit when it is actually above the limit. Even a well-executed procedure produces some false conclusions because of the statistical nature of sampling and measurement. More stringent procedures are required to obtain lower rates of false conclusions. For drinking water, it is more important to have a low rate of false negatives than a low rate of false positives. It would be worse to certify that contaminated water is safe than to certify that safe water is contaminated.

Drug testing of athletes is designed to minimize false positives so that an innocent athlete is not falsely accused of doping. When there is any doubt about a drug test result, it is considered to be negative. In drug testing, the person who collects a sample is not the person who analyzes the sample. To prevent deliberate falsification of a result by an analyst, the identity of the athlete is not known to the analyst.

In choosing a method, we also consider selectivity and sensitivity. **Selectivity** (also called *specificity*) is the ability to distinguish analyte from other species in the sample (avoiding interference). **Sensitivity** is the capability of responding reliably and measurably to changes in analyte concentration. A method must have a *detection limit* (Section 5-2) lower than the concentrations to be measured.

Specifications could include required accuracy and precision, reagent purity, tolerances for apparatus, the use of standard reference materials, and acceptable values for blanks. *Standard reference materials* (see Box 3-1) contain certified levels of analyte in realistic materials that you might be analyzing, such as blood or coal or metal alloys. Your analytical method should produce an answer acceptably close to the certified level; otherwise, there is something wrong with the accuracy of your method. Blanks account for interference by other species in the sample and for traces of analyte found in reagents used for sample preservation, preparation, and analysis. Frequent measurements of blanks detect whether analyte from previous samples is carried into subsequent analyses by adhering to vessels or instruments.

A **method blank** is a sample containing all components except analyte, and it is taken through all steps of the analytical procedure. Subtract the response of the method blank from the response of a real sample prior to calculating the quantity of analyte in the sample. A **reagent blank** is similar to a method blank, but it has not been subjected to all sample preparation procedures. The method blank is a more complete estimate of the blank contribution to the analytical response.

A **field blank** is similar to a method blank, but it has been exposed to the site of sampling. For example, to analyze particulates in air, a certain volume of air could be sucked through a filter, which is then dissolved and analyzed. A field blank would be a filter carried to the collection site in the same package with the collection filters. The filter for the blank would be taken out of its package in the field and placed in the same kind of sealed container used for collection filters. The difference between the blank and the collection filters is that air was not sucked through the blank filter. Volatile organic compounds encountered during transportation or in the field are conceivable contaminants of a field blank.

Another performance requirement often specified is *spike recovery*. Sometimes, response to analyte is affected by something else in the sample. We use the word **matrix** to refer to everything else in the sample other than analyte. A **spike**, also called a *fortification*, is a known quantity of analyte added to a sample to test whether the response to the spike is the same as that expected from a calibration curve. Spiked samples are then analyzed in the same manner as unknowns. For example, if drinking water is found to contain 10.0 µg/L of nitrate upon analysis, a spike of 5.0 µg/L could be added. Ideally, the concentration in the spiked portion found by analysis will be 15.0 µg/L. If a number other than 15.0 µg/L is found, then the matrix could be interfering with the analysis.

Example Spike Recovery

Let C stand for concentration. One definition of spike recovery is

$$\% \text{ recovery} = \frac{C_{\text{spiked sample}} - C_{\text{unspiked sample}}}{C_{\text{added}}} \times 100 \quad (5-1)$$

An unknown was found to contain 10.0 µg of analyte per liter. A spike of 5.0 µg/L was added to a replicate portion of unknown. Analysis of the spiked sample gave a concentration of 14.6 µg/L. Find the percent recovery of the spike.

Sensitivity

$$= \frac{\text{slope of calibration curve}}{\text{change in signal}} = \frac{\text{change in signal}}{\text{change in analyte concentration}}$$

Add a small volume of concentrated standard to avoid changing the volume of the sample significantly. For example, add 50.5 µL of 500 µg/L standard to 5.00 mL (= 5 000 µL) of sample to increase analyte by 5.00 µg/L.

Final concentration

$$\begin{aligned} &= \frac{\text{initial concentration} \times \text{dilution factor}}{\left(500 \frac{\mu\text{g}}{\text{L}}\right) \left(\frac{50.5 \mu\text{L}}{5050.5 \mu\text{L}}\right)} \\ &= 5.00 \frac{\mu\text{g}}{\text{L}} \end{aligned}$$

SOLUTION The percentage of the spike found by analysis is

$$\% \text{ recovery} = \frac{14.6 \mu\text{g/L} - 10.0 \mu\text{g/L}}{5.0 \mu\text{g/L}} \times 100 = 92\%$$

If the acceptable recovery is specified to be in the range 96% to 104%, then 92% is unacceptable. Something in your method or techniques needs improvement.



Test Yourself An unknown containing 93.2 μg of analyte per liter was spiked with an additional 80.0 $\mu\text{g}/\text{L}$. Analysis of the spiked sample gave a concentration of 179.4 $\mu\text{g}/\text{L}$. Find the percent recovery of the spike. (**Answer:** 107.8%)

When dealing with large numbers of samples and replicates, we perform periodic calibration checks to make sure that our instrument continues to work properly and the calibration remains valid. In a **calibration check**, we analyze solutions formulated to contain known concentrations of analyte. A specification might, for example, call for one calibration check for every 10 samples. Solutions for calibration checks should be different from the ones used to prepare the original calibration curve. This practice helps to verify that the initial calibration standards were made properly.

Performance test samples (also called *quality control samples* or *blind samples*) are a quality control measure to help eliminate bias introduced by the analyst who knows the concentration of the calibration check sample. These samples of known composition are provided to the analyst as unknowns. Results are then compared with the known values, usually by a quality assurance manager. For example, the U.S. Department of Agriculture maintains a bank of quality control homogenized food samples for distribution as blind samples to laboratories that measure nutrients in foods.

To gauge accuracy:

- calibration checks
- fortification recoveries
- quality control samples
- blanks

To gauge precision:

- replicate samples
- replicate portions of same sample

Together, raw data and results from calibration checks, spike recoveries, quality control samples, and blanks are used to gauge accuracy. Analytical performance on replicate samples and replicate portions of the same sample measures precision. Fortification also helps ensure that qualitative identification of analyte is correct. If you spike the unknown in Figure 0-5 with extra caffeine and the area of a chromatographic peak not thought to be caffeine increases, then you have misidentified the caffeine peak.

Many labs have their own standard practices, such as recording temperatures in refrigerators, calibrating balances, conducting routine instrument maintenance, or replacing reagents. These are part of the overall quality management plan. The rationale behind standard practices is that some equipment is used by multiple people for different analyses. We save money by having one program to ensure that the most rigorous needs are met.

Assessment

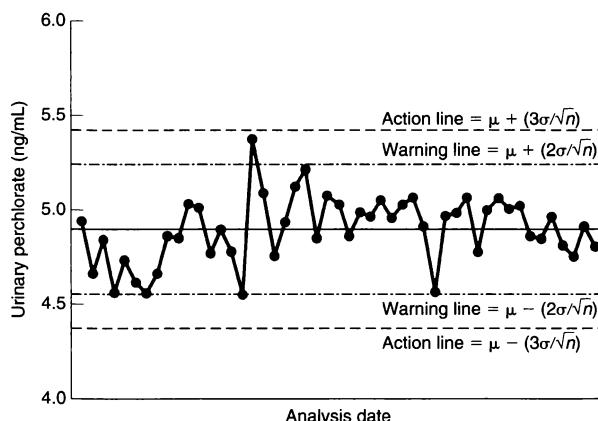
Assessment is the process of (1) collecting data to show that analytical procedures are operating within specified limits and (2) verifying that final results meet use objectives.

Documentation is critical for assessment. Standard *protocols* provide directions for what must be documented and how the documentation is to be done, including how to record information in notebooks. For labs that rely on manuals of standard practices, it is imperative that tasks done to comply with the manuals be monitored and recorded. *Control charts* (Box 5-1) can be used to monitor performance on

Box 5-1 Control Charts

A **control chart** is a visual representation of confidence intervals for a Gaussian distribution. A control chart warns us when a property being monitored strays dangerously far from an intended *target value*.

Consider a laboratory measuring perchlorate (ClO_4^-) in human urine. For quality assurance, $n = 5$ replicate quality control samples made from synthetic urine spiked with perchlorate are measured every day. The control chart shows the mean value of the five samples observed each day over a series of days. The spike contains $\mu = 4.92 \text{ ng/mL}$, and the population standard deviation from many analyses over a long time is $\sigma = 0.40 \text{ ng/mL}$.



Control chart for ClO_4^- in urine. [Data adapted from L. Valentini-Blasini, J. P. Mauldin, D. Maple, and B. C. Blount, *Anal. Chem.* 2005, 77, 2475, in which the standard deviation was smaller.]

For a Gaussian distribution, 95.5% of all observations are within $\pm 2\sigma/\sqrt{n}$ from the mean and 99.7% are within $\pm 3\sigma/\sqrt{n}$. In these expressions, n is the number of replicate measurements ($= 5$) that are averaged each day. The $\pm 2\sigma/\sqrt{n}$ limits are designated *warning lines* and the $\pm 3\sigma/\sqrt{n}$ limits are designated *action lines*. We expect $\sim 4.5\%$ of measurements to be outside the warning lines and $\sim 0.3\%$ to be outside the action lines. It is unlikely that we would observe two consecutive measurements at the warning line (probability = $0.045 \times 0.045 = 0.002$).

The following conditions are considered to be so unlikely that if they occur the process should be shut down for troubleshooting:

- 1 observation outside the action lines
- 2 out of 3 consecutive measurements between the warning and the action lines
- 7 consecutive measurements all above or all below the center line
- 6 consecutive measurements all steadily increasing or all steadily decreasing, wherever they are located
- 14 consecutive points alternating up and down, regardless of where they are located
- an obvious nonrandom pattern

For quality assessment of an analytical process, a control chart might show the mean value of quality control samples or the precision of replicate analyses of unknowns or standards as a function of time.

blanks, calibration checks, and spiked samples to see whether results are stable over time or to compare the work of different employees. Control charts can also monitor sensitivity or selectivity, especially if a laboratory encounters a wide variety of matrixes.

Government agencies such as the U.S. Environmental Protection Agency set requirements for quality assurance for their own labs and for certification of other labs. Published standard methods specify precision, accuracy, numbers of blanks, replicates, and calibration checks. To monitor drinking water, regulations state how often and how many samples are to be taken. Documentation is necessary to demonstrate that all requirements have been met. Table 5-1 summarizes the quality assurance process.

Ask Yourself

5-A. What are the three parts of quality assurance? What questions are asked in each part and what actions are taken in each part?

Table 5-1 Quality assurance process

Question	Actions
<i>Use Objectives</i> Why do you want the data and results and how will you use the results?	<ul style="list-style-type: none"> Write use objectives.
<i>Specifications</i> How good do the numbers have to be?	<ul style="list-style-type: none"> Write specifications. Pick methods to meet specifications. Consider sampling, precision, accuracy, selectivity, sensitivity, detection limit, robustness, and rate of false results. Employ blanks, fortification, calibration checks, quality control samples, and control charts to monitor performance. Write and follow standard operating procedures.
<i>Assessment</i> Were the specifications achieved?	<ul style="list-style-type: none"> Compare data and results with specifications. Document procedures and keep records suitable to meet use objectives. Verify that use objectives were met.

5-2 Validation of an Analytical Procedure

Method validation is the process of showing that a new procedure or an existing procedure applied to a new type of sample meets specifications and is acceptable for its intended purpose. Standard methods published by government and private agencies are validated by multiple labs before they are published. In pharmaceutical chemistry, method validation requirements include studies of *selectivity*, *accuracy*, *precision*, *linearity*, *range*, *robustness*, *limit of detection*, and *limit of quantitation*. Selectivity, accuracy, and precision were mentioned in Section 5-1.

Linearity of a calibration curve is illustrated in Figure 5-1. We prefer a response in which the corrected analytical signal (= signal from sample – signal from blank) is proportional to the quantity of analyte, as it is between 0 and c_1 in Figure 5-1. However, we can obtain valid results in the nonlinear region between c_1 and c_2 by fitting the data to a curve such as a polynomial. **Linear range** is the analyte concentration range over which response is proportional to concentration. **Dynamic range** is the concentration range over which there is a measurable response to analyte, even if the response is not linear. For an analytical method, the word **range** means the concentration interval over which linearity, accuracy, and precision are all acceptable.

Another goal of validation is to show that a method is **robust**, which means that it is not affected by small changes in conditions. For example, a robust chromatographic procedure gives reliable results despite small changes in solvent composition, pH, buffer concentration, temperature, injection volume, and detector wavelength.

Limits of Detection and Quantitation

The **detection limit** (also called the *lower limit of detection*) is the smallest quantity of analyte that is “significantly different” from the blank. The steps below are a procedure that produces a detection limit with ~99% chance of being greater than the blank. That is, only ~1% of samples containing no analyte will give a signal greater than the detection limit (Figure 5-2). We assume that the standard

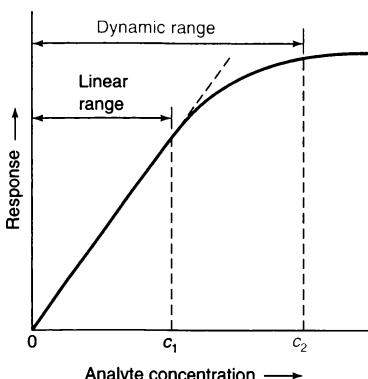


Figure 5-1 Schematic calibration curve with linear and nonlinear regions.

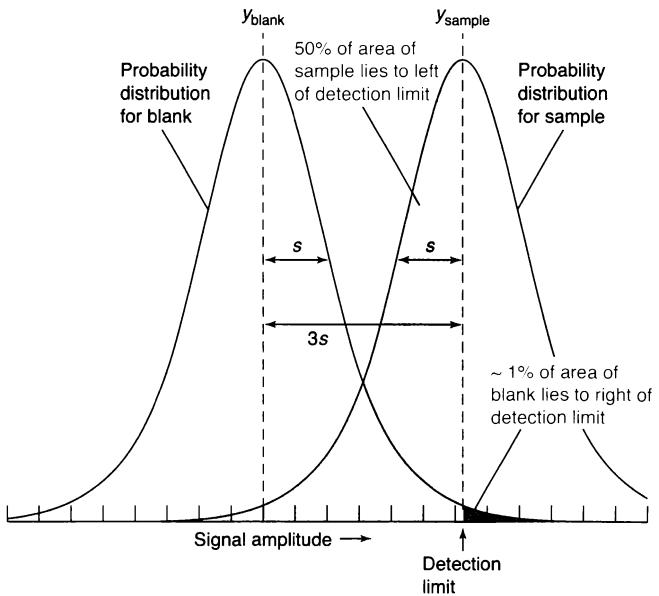


Figure 5-2 Detection limit. Curves show distribution of measurements expected for a blank and a sample whose concentration is at the detection limit. The area of any region is proportional to the number of measurements in that region. Only $\sim 1\%$ of measurements for a blank are expected to exceed the detection limit. However, 50% of measurements for a sample containing analyte at the detection limit will be below the detection limit. There is a 1% chance of concluding that a blank has analyte above the detection limit. If a sample contains analyte at the detection limit, there is a 50% chance of concluding that analyte is *absent* because its signal is below the detection limit. Curves in this figure are Student's *t* distributions, which are broader than the Gaussian distribution.

deviation of the signal from samples near the detection limit is similar to the standard deviation from blanks.

1. After estimating the detection limit from previous experience with the method, prepare a sample whose concentration is ~ 1 to 5 times the detection limit.
2. Measure the signal from n replicate samples ($n \geq 7$).
3. Compute the standard deviation (s) of the n measurements.
4. Measure the signal from n blanks (containing no analyte) and find the mean value, y_{blank} .
5. The minimum detectable signal, y_{dl} , is defined as

$$\text{Signal detection limit: } y_{\text{dl}} = y_{\text{blank}} + 3s \quad (5-2)$$

6. The corrected signal, $y_{\text{sample}} - y_{\text{blank}}$, is proportional to sample concentration:

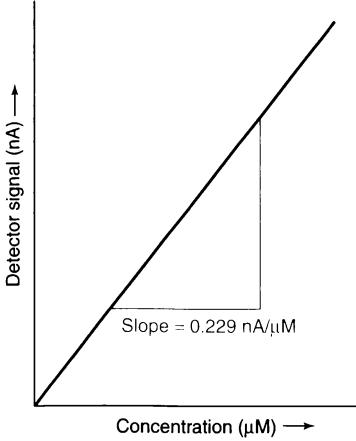
$$\text{Calibration line: } y_{\text{sample}} - y_{\text{blank}} = m \times \text{sample concentration} \quad (5-3)$$

where y_{sample} is the signal observed for the sample and m is the slope of the linear calibration curve. The *minimum detectable concentration*, also called the *detection limit*, is obtained by substituting y_{dl} from Equation 5-2 for y_{sample} in Equation 5-3:

$$\text{Detection limit: minimum detectable concentration} \equiv \frac{3s}{m} \quad (5-4)$$

Example Detection Limit

A procedure was used in which electric current in a detector is proportional to analyte concentration. From previous measurements of a low concentration of analyte, it was estimated that the signal detection limit was in the low nanoampere range. Signals from seven replicate samples with a concentration about three times



the detection limit were 5.0, 5.0, 5.2, 4.2, 4.6, 6.0, and 4.9 nA. Reagent blanks gave values of 1.4, 2.2, 1.7, 0.9, 0.4, 1.5, and 0.7 nA. The slope of the calibration curve for higher concentrations is $m = 0.229 \text{ nA}/\mu\text{M}$. (a) Find the signal detection limit and the minimum detectable concentration. (b) What is the concentration of analyte in a sample that gave a signal of 7.0 nA?

SOLUTION (a) First compute the mean for the blanks and the standard deviation of the samples. Retain extra, insignificant digits to reduce round-off errors.

$$\begin{array}{ll} \text{Blank:} & \text{average} = y_{\text{blank}} = 1.2_6 \text{ nA} \\ \text{Sample:} & \text{standard deviation} = s = 0.5_6 \text{ nA} \end{array}$$

The signal detection limit from Equation 5-2 is

$$y_{\text{dl}} = y_{\text{blank}} + 3s = 1.2_6 \text{ nA} + (3)(0.5_6 \text{ nA}) = 2.9_4 \text{ nA}$$

The minimum detectable concentration is obtained from Equation 5-4:

$$\text{detection limit} = \frac{3s}{m} = \frac{(3)(0.5_6 \text{ nA})}{0.229 \text{ nA}/\mu\text{M}} = 7.3 \mu\text{M}$$

(b) To find the concentration of a sample whose signal is 7.0 nA, use Equation 5-3:

$$\begin{aligned} y_{\text{sample}} - y_{\text{blank}} &= m \times \text{concentration} \\ \Rightarrow \text{concentration} &= \frac{y_{\text{sample}} - y_{\text{blank}}}{m} = \frac{7.0 \text{ nA} - 1.2_6 \text{ nA}}{0.229 \text{ nA}/\mu\text{M}} = 25.1 \mu\text{M} \end{aligned}$$

Test Yourself Suppose that $s = 0.2_8 \text{ nA}$ instead of 0.5_6 nA , but y_{blank} is still 1.2_6 nA . Find the signal detection limit, the minimum detectable concentration, and the concentration of analyte in a sample with a signal of 7.0 nA. (**Answer:** 2.1_0 nA , $3.7 \mu\text{M}$, $25.1 \mu\text{M}$)

The lower limit of detection given in Equation 5-4 is $3s/m$, where s is the standard deviation of a low-concentration sample and m is the slope of the calibration curve. The standard deviation is a measure of the *noise* (random variation) in a blank or a small signal. When the signal is 3 times greater than the noise, it is readily detectable, but still too small for accurate measurement. A signal that is 10 times greater than the noise is defined as the **lower limit of quantitation**, or the smallest amount that can be measured with reasonable accuracy.

$$\text{lower limit of quantitation} \equiv \frac{10s}{m} \quad (5-5)$$

The **reporting limit** is the concentration below which regulations dictate that a given analyte is reported as “not detected.” “Not detected” does not mean that analyte is not observed; it simply means that analyte may be present below a prescribed level. Reporting limits are set at least 5 to 10 times higher than the detection limit so that detecting analyte at the reporting limit is not ambiguous.

Beginning in 2006, labels on U.S. packaged foods must state how much *trans* fat is present. This type of fat is derived mainly from partial hydrogenation of vegetable oil and is a major component of margarine and shortening. Consumption of

Nutrition Facts	
Serving Size 6 Crackers (28g)	
Servings Per Container About 10	
Amount Per Serving	
Calories 120	Calories from Fat 40
	% Daily Value*
Total Fat 4.5g	7%
Saturated Fat 0.5g	3%
Trans Fat 0g	
Polyunsaturated Fat 2.5g	
Monounsaturated Fat 1g	
Cholesterol 0mg	0%
Sodium 150mg	6%
Total Carbohydrate 19g	6%
Dietary Fiber 3g	13%
Sugars 0g	
Protein 3g	

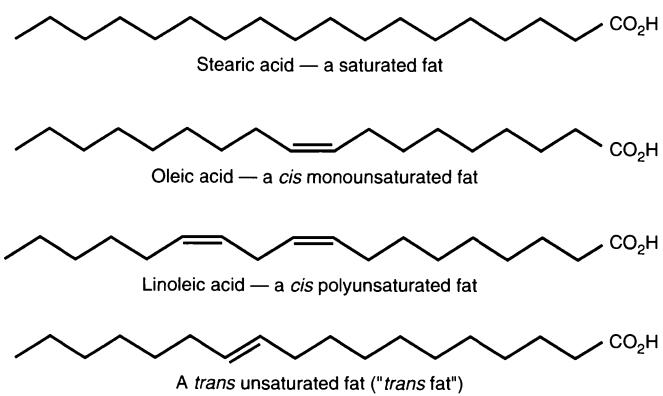


Figure 5-3 Nutritional label from a package of crackers. The reporting limit for *trans* fat is 0.5 g/serving. Any amount less than this is reported as 0. Structures of representative saturated, monounsaturated, polyunsaturated, and *trans* fats are shown. Box 7-1 explains the shorthand used to draw these 18-carbon compounds.

trans fat increases risk of heart disease, stroke, and some types of cancer. However, the *reporting limit* for *trans* fat is 0.5 g per serving. If the concentration is <0.5 g/serving, it is reported as 0, as in Figure 5-3. By reducing the serving size, a manufacturer can state that the *trans* fat content is 0. The reason given by the government for the high reporting limit is that many labs use infrared analysis, whose detection limit is poor. Gas chromatography provides a lower detection limit (Figure 22-4). If your favorite snack food is made with partially hydrogenated oil, it contains *trans* fat even if the label says otherwise.

Ask Yourself

5-B. Method validation includes studies of precision and accuracy of a proposed method. How would you validate precision and accuracy? (*Hint:* Review Section 5-1.)

5-3 Standard Addition

Calibration curves (Section 4-7) are used to determine the relation between signal and concentration in a chemical analysis. In cases where a calibration curve is unreliable, we can use *standard addition* or *internal standards*.

In the method of **standard addition**, a known quantity of analyte is added to a specimen and the increase in signal is measured. The increase in signal allows us to infer how much analyte was in the original specimen. The key assumption is that signal is proportional to the concentration of analyte.

Standard addition is especially appropriate when the sample composition is unknown or complex. In such a case, it is impossible or difficult to create standards and blanks whose composition matches that of the sample. If standards and blanks do

Sections 5-3 and 5-4 could be postponed until you need to use standard addition or internal standards in the lab.

Standard additions are most appropriate when the sample matrix is complex and difficult to reproduce in standard solutions.

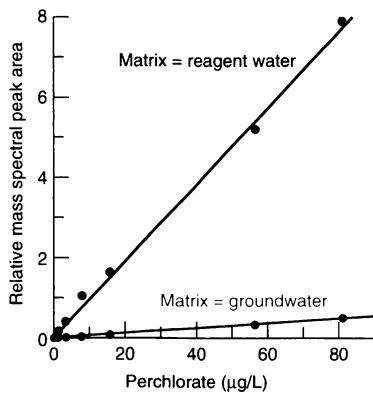


Figure 5-4 Calibration curves for perchlorate in pure water and in groundwater. [Data from C. J. Koester, H. R. Beller, and R. U. Halden, *Environ. Sci. Technol.* **2000**, *34*, 1862.]

Bear in mind that the species X and S are the same.

Equations 5-7 follow from the dilution formula 1-5:

$$[X]_f V_f = [X]_i V_i$$

where f stands for “final” and i stands for “initial.” In Equations 5-7, $V = V_f$ and $V_0 = V_i$.

not match the composition of the unknown sample, a calibration curve is not reliable. The *matrix* is everything in the sample other than analyte. A **matrix effect** is a change in the analytical signal caused by the matrix.

Figure 5-4 shows a matrix effect in the analysis of perchlorate (ClO_4^-) by mass spectrometry. Above 18 µg/L (18 ppb) in drinking water, ClO_4^- can reduce thyroid hormone production. Standard solutions of ClO_4^- in pure water gave the upper calibration curve. The slope in the lower curve for standard ClO_4^- in groundwater was 15 times less. Reduced response to ClO_4^- is a *matrix effect* attributed to other anions in groundwater.

Suppose that a sample with unknown initial concentration $[X]_i$ gives a signal I_X , where I might be peak area in chromatography or detector current of an instrument. Then a known concentration of standard S (a known concentration of analyte) is added to the sample and signal I_{S+X} is observed. Because signal is proportional to analyte concentration,

$$\frac{\text{concentration of analyte in unknown}}{\text{concentration of analyte} + \text{standard in mixture}} = \frac{\text{signal from unknown}}{\text{signal from mixture}}$$

Standard addition equation:
$$\frac{[X]_i}{[X]_f + [S]_f} = \frac{I_X}{I_{S+X}} \quad (5-6)$$

where $[X]_f$ is the final concentration of unknown analyte after adding the standard and $[S]_f$ is the final concentration of standard after addition to the unknown. If we began with an initial volume V_0 of unknown and added the volume V_S of standard with initial concentration $[S]_i$, the total volume is $V = V_0 + V_S$ and the concentrations in Equation 5-6 are

$$[X]_f = [X]_i \underbrace{\left(\frac{V_0}{V} \right)}_{\text{Dilution factor}} \quad [S]_f = [S]_i \underbrace{\left(\frac{V_S}{V} \right)}_{\text{Dilution factor}} \quad (5-7)$$

The *dilution factors* V_0/V and V_S/V relate concentrations before and after dilution.

Example Standard Addition

Ascorbic acid (vitamin C) in a 50.0-mL sample of orange juice was analyzed by an electrochemical method that gave a detector current of 1.78 µA. A standard addition of 0.400 mL of 0.279 M ascorbic acid increased the current to 3.35 µA. Find the concentration of ascorbic acid in the orange juice.

SOLUTION If the initial concentration of ascorbic acid in the juice is $[X]_i$, the concentration after dilution of 50.0 mL of juice with 0.400 mL of standard is

$$\text{final concentration of analyte} = [X]_f = [X]_i \left(\frac{V_0}{V} \right) = [X]_i \left(\frac{50.0}{50.4} \right)$$

The final concentration of the added standard after addition to the orange juice is

$$[S]_f = [S]_i \left(\frac{V_S}{V} \right) = [0.279 \text{ M}] \left(\frac{0.400}{50.4} \right) = 2.214 \text{ mM}$$

The standard addition equation 5-6 therefore becomes

$$\frac{[X]_i}{[X]_f + [S]_f} = \frac{[X]_i}{\left(\frac{50.0}{50.4}\right)[X]_i + 2.214 \text{ mM}} = \frac{1.78 \mu\text{A}}{3.35 \mu\text{A}} \Rightarrow [X]_i = 2.49 \text{ mM}$$

 **Test Yourself** Find the concentration of ascorbic acid in the juice if the standard addition gave a current of 2.50 μA instead of 3.35 μA . (Answer: 5.37 mM)

Graphical Procedure for Standard Addition to a Single Solution

A more accurate procedure is to make a series of standard additions that increase the original signal by a factor of 1.5 to 3 and use all the results together. If you take expressions for $[X]_f$ and $[S]_f$ from Equations 5-7, plug them into the standard addition equation 5-6, and do some rearranging, you would find

Increasing the original signal by more than a factor of 3 reduces the accuracy of the result.

Graphing equation for standard addition:

$$I_{S+x} \left(\frac{V}{V_0} \right) = I_x + \underbrace{\frac{I_x}{[X]_i} [S]_i}_{\text{Function to plot on } y\text{-axis}} \underbrace{\left(\frac{V_s}{V_0} \right)}_{\text{Function to plot on } x\text{-axis}} \quad (5-8)$$

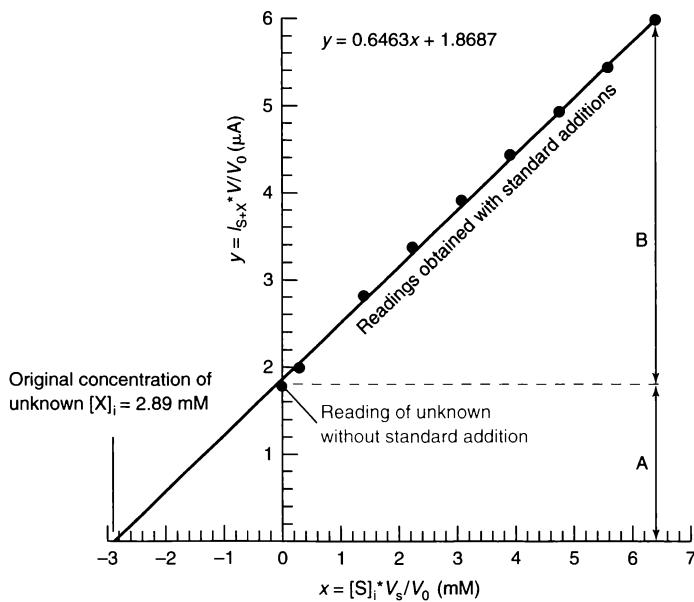
I_{S+x} is the signal measured for a sample containing unknown plus standard. The factor V/V_0 is the final volume divided by the initial volume of the sample. On the right-hand side of Equation 5-8, the function of interest is the product $[S]_i(V_s/V_0)$, where $[S]_i$ is the concentration of standard prior to adding it to the sample, V_s is the volume of standard added, and V_0 is the initial volume of sample. A graph of $I_{S+x}(V/V_0)$ on the y -axis versus $[S]_i(V_s/V_0)$ on the x -axis should be a straight line. The negative x -intercept is the initial concentration of unknown, $[X]_i$. If we started with $V_0 = 10.00 \text{ mL}$ of sample and added 0.50-mL aliquots of standard, $[X]_i$ is the concentration of analyte in the 10.00-mL sample.

In Figure 5-5, the volume of added standard in column B is V_s . Current in column D is the observed detector response, I_{S+x} . Column C gives the x -axis function

	A	B	C	D	E
1	Vitamin C Standard Addition Experiment				
2	Add 0.279 M ascorbic acid to 50.0 mL of orange juice				
3					
4					
5	$V_0 (\text{mL}) =$	mL ascorbic acid added	x-axis function	$I(s+x) =$	y-axis function
6	50		$S_i \cdot V_s / V_0$	signal (μA)	$I(s+x) \cdot V / V_0$
7	$[S]_i (\text{mM}) =$	0.000	0.000	1.78	1.780
8	279	0.050	0.279	2.00	2.002
9		0.250	1.395	2.81	2.824
10		0.400	2.232	3.35	3.377
11		0.550	3.069	3.88	3.923
12		0.700	3.906	4.37	4.431
13		0.850	4.743	4.86	4.943
14		1.000	5.580	5.33	5.437
15		1.150	6.417	5.82	5.954
16					
17	$C7 = \$A\$8*B7/\$A\6	$E7 = D7*(\$A\$6+B7)/\$A\6			

Figure 5-5 Standard addition with increasing final volume to be graphed with Equation 5-8.

Figure 5-6 Graphical treatment of standard addition using Equation 5-8 with increasing final volume. Standard additions should increase the original signal (A) by a factor of 1.5 to 3. That is, $B = 0.5A$ to $2A$.



$[S]_i(V_s/V_0)$. Column E gives the y -axis function $I_{S+X}(V/V_0)$. Equation 5-8 is plotted in Figure 5-6. The negative x -intercept, 2.89 mM, is the *original* concentration $[X]_i$ of ascorbic acid in the undiluted orange juice. In the preceding example, we found $[X]_i = 2.49$ mM from a single standard addition. The 14% difference between the two results is experimental error attributable to using one point instead of several points. Uncertainty in the results from a standard addition graph is discussed in Problem 5-19.

Graphical Procedure for Constant-Volume Standard Addition

In a common procedure that gives a constant final volume for standard addition, equal volumes of unknown are pipetted into several volumetric flasks (Figure 5-7). Different volumes of standard are added, and each flask is diluted to the *same final volume*. In this case, plot the signal I_{S+X} versus the concentration of diluted standard, $[S]_f$ (Figure 5-7). The negative x -intercept is now the *final* concentration of unknown, $[X]_f$. The initial concentration is $[X]_i = [X]_f(V/V_0)$.

If all solutions have *same final volume*:
plot I_{S+X} versus $[S]_f$
 x -intercept is $[X]_f$

Ask Yourself

5-C. Successive standard additions of 1.00 mL of 25.0 mM ascorbic acid were made to 50.0 mL of orange juice. Prepare a graph similar to Figure 5-6 to find the concentration of ascorbic acid in the orange juice.

Total volume of added standard (mL)	Peak current (μA)
-------------------------------------	--------------------------

0	1.66
1.00	2.03
2.00	2.39
3.00	2.79
4.00	3.16
5.00	3.51

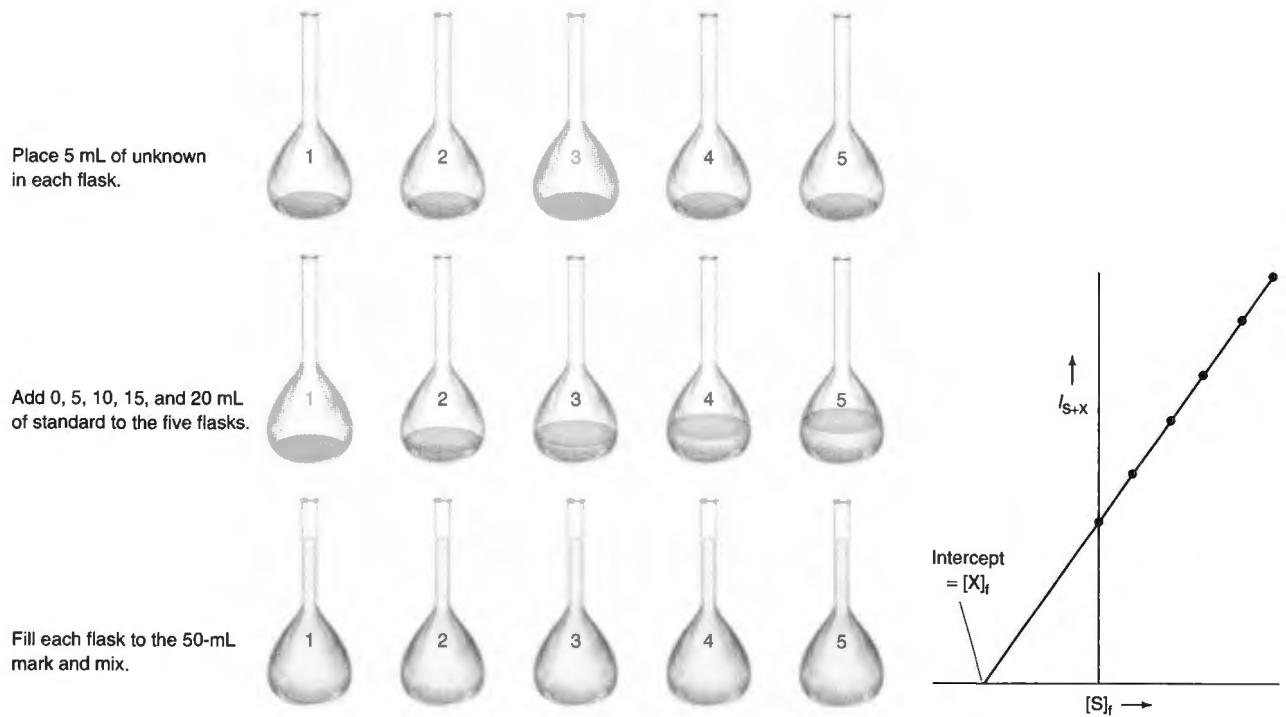


Figure 5-7 Standard addition with *constant volume*. Plot I_{S+x} versus $[S]_f$. The negative x -intercept is the *diluted* analyte concentration, $[X]_f$.

5-4 Internal Standards

An **internal standard** is a known amount of a compound, different from analyte, that is added to an unknown. Signal from analyte is compared with signal from the internal standard to find out how much analyte is present.

Internal standards are especially useful for analyses in which the quantity of sample analyzed or the instrument response varies slightly from run to run for reasons that are difficult to control. For example, gas or liquid flow rates that vary by a few percent in chromatography could change the detector response. A calibration curve is only accurate for the one set of conditions under which it was obtained. However, the *relative* response of the detector to the analyte and standard is usually constant over a wide range of conditions. If signal from the standard increases by 8.4% because of a change in flow rate, signal from the analyte usually increases by 8.4% also. As long as the concentration of standard is known, the correct concentration of analyte can be derived. Internal standards are used in chromatography because the microliter volume of sample injected into the chromatograph is not very reproducible, so a calibration curve would not be accurate.

Internal standards are also desirable when sample loss can occur in sample preparation steps prior to analysis. If a known quantity of standard is added to the unknown prior to any manipulations, the ratio of standard to analyte remains constant because the same fraction of each is lost in any operation.

To use an internal standard, we prepare a known mixture of standard and analyte and measure the relative response of the detector to the two species. In Figure 5-8, the area under each peak is proportional to the concentration of each compound injected into the column. However, the detector generally has a different response to

Section 5-4 can be postponed until you need to use an internal standard in the lab.

An *internal standard* is different from the analyte. In *standard addition*, the standard is the same substance as the analyte.

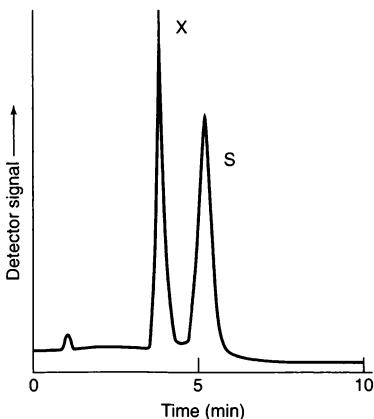


Figure 5-8 Chromatogram illustrating the use of an internal standard. A known amount of standard S is added to unknown X. From the areas of the peaks, we can tell how much X is in the unknown. To do so, we needed to measure the relative response to known amounts of each compound in a separate experiment.

each component. For example, if both the analyte (X) and the internal standard (S) have concentrations of 10.0 mM, the area under the analyte peak might be 2.30 times greater than the area under the standard peak. We say that the **response factor**, F , is 2.30 times greater for X than for S.

$$\text{Internal standard: } \frac{\text{area of analyte signal}}{\text{concentration of analyte}} = F \left(\frac{\text{area of standard signal}}{\text{concentration of standard}} \right) \quad (5-9)$$

$$\frac{A_X}{[X]} = F \left(\frac{A_S}{[S]} \right)$$

[X] and [S] are the concentrations of analyte and standard *after they have been mixed*. Equation 5-9 is predicated on linear response to both the analyte and the standard.

Example Using an Internal Standard

In a chromatography experiment, a solution containing 0.083 7 M X and 0.066 6 M S gave peak areas of $A_X = 423$ and $A_S = 347$. (Areas are measured in arbitrary units by the instrument.) To analyze the unknown, 10.0 mL of 0.146 M S were added to 10.0 mL of unknown, and the mixture was diluted to 25.0 mL in a volumetric flask. This mixture gave the chromatogram in Figure 5-8, with peak areas $A_X = 553$ and $A_S = 582$. Find the concentration of X in the unknown.

SOLUTION First use the standard mixture to find the response factor in Equation 5-9:

$$\text{Standard mixture: } \frac{A_X}{[X]} = F \left(\frac{A_S}{[S]} \right)$$

$$\frac{423}{0.083\,7\,M} = F \left(\frac{347}{0.066\,6\,M} \right) \Rightarrow F = 0.970_0$$

In the mixture of unknown plus standard, the standard has been diluted from 10.0 mL up to 25.0 mL. The concentration of S is

$$[S] = (0.146\,M) \left(\frac{10.0\,mL}{25.0\,mL} \right) = 0.058\,4\,M$$

↓ Initial volume
 Initial concentration Dilution factor Final volume

Using the known response factor, we substitute back into Equation 5-9 to find the concentration of unknown in the mixture:

$$\text{Unknown mixture: } \frac{A_X}{[X]} = F \left(\frac{A_S}{[S]} \right)$$

$$\frac{553}{[X]} = 0.970_0 \left(\frac{582}{0.058\,4\,M} \right) \Rightarrow [X] = 0.057\,2_1\,M$$

Because X was diluted from 10.0 to 25.0 mL when mixed with S, the original concentration of X in the unknown was $(25.0/10.0)(0.057\text{ M}) = 0.143\text{ M}$.

 **Test Yourself** A solution with 0.083 7 M X and 0.050 0 M S' gave areas $A_X = 423$ and $A_{S'} = 372$. Then 10.0 mL of 0.05 0 M S' plus 10.0 mL of unknown were diluted to 25.0 mL. The chromatogram gave $A_X = 553$ and $A_{S'} = 286$. Find [X] in the unknown. (Answer: 0.142 M)

Ask Yourself

5-D. A mixture of 52.4 nM analyte (X) and 38.9 nM standard (S) gave the relative response (area of X)/(area of S) = 0.644/1.000. A second solution containing an unknown quantity of X plus 742 nM S had (area of X)/(area of S) = 1.093/1.000. Find [X] in the second solution.

Key Equations

Detection and quantitation limits

$$\text{Minimum detectable concentration} \equiv \frac{3s}{m}$$

$$\text{Lower limit of quantitation} \equiv \frac{10s}{m}$$

s = standard deviation of sample at 1–5 times detection limit

m = slope of calibration curve

Standard addition

$$\frac{[X]_i}{[X]_f + [S]_f} = \frac{I_X}{I_{S+X}}$$

[X]_i = concentration of analyte in initial unknown

[X]_f = concentration of analyte after standard addition

[S]_f = concentration of standard after addition to unknown

Standard addition graph

For *variable total volume* of sample + standard, prepare the graph in Figure 5-6 from Equation 5-8. The negative x-intercept is the original concentration of analyte.

For *constant total volume* of sample + standard, prepare the graph in Figure 5-7. The negative x-intercept is the final analyte concentration [X]_f in the mixture. The original concentration in unknown is [X]_i = [X]_f (V/V₀).

Internal standard

$$\frac{\text{area of analyte signal}}{\text{concentration of analyte}} = F \left(\frac{\text{area of standard signal}}{\text{concentration of standard}} \right)$$

F = response factor measured in a separate experiment with known concentrations of analyte and standard

Important Terms

assessment
calibration check
control chart
detection limit
dynamic range
field blank
internal standard

linear range
lower limit of quantitation
matrix
matrix effect
method blank
method validation
performance test sample

quality assurance
range
reagent blank
reporting limit
response factor
robustness
selectivity

sensitivity
specifications
spike
standard addition
use objectives

Problems

- 5-1. Distinguish *raw data*, *treated data*, and *results*.
- 5-2. What is the difference between a *calibration check* and a *performance test sample*?
- 5-3. What is a blank and what is its purpose? Distinguish *method blank*, *reagent blank*, and *field blank*.
- 5-4. Distinguish *linear range*, *dynamic range*, and *range*.
- 5-5. What is the difference between a *false positive* and a *false negative*?
- 5-6. Consider a sample that contains analyte at the detection limit defined by Equation 5-4. Refer to Figure 5-2 to explain the following statements: There is approximately a 1% chance of falsely concluding that a sample containing no analyte contains analyte above the detection limit. There is a 50% chance of concluding that a sample that really contains analyte at the detection limit does not contain analyte above the detection limit.
- 5-7. How is a control chart used? State six indications that a process is going out of control.

5-8. Here is a use objective for a chemical analysis to be performed at a drinking water purification plant: "Data and results collected quarterly shall be used to determine whether the concentrations of haloacetates in the treated water demonstrate compliance with the levels set by the Stage 1 Disinfection By-products Rule using Method 552.2" (a specification that sets precision, accuracy, and other requirements). Which of the following questions best summarizes the meaning of the use objective?

- (a) Are haloacetate concentrations known within specified precision and accuracy?
- (b) Are any haloacetates detectable in the water?
- (c) Do any haloacetate concentrations exceed the regulatory limit?

5-9. *False positives and negatives.* Drinking water from wells in Bangladesh and much of Southeast Asia has unsafe levels of naturally occurring arsenic. Colorimetric test kits are used to combat this severe public health problem. If the color response indicates As >50 µg/L, the well is painted red and not used for drinking. If As <50 µg/L, the well is painted green and used for drinking. (By comparison, the allowed level of As in Europe and North America is 10 µg/L). We say that a positive result in the colorimetric test means that As >50 µg/L. A study in 2002 found 50% false positives and 8% false negatives. What percentage of green wells should be red and what percentage of red wells should be green? Would it be better or worse for public health to have a 50% false negative rate?

5-10. *Blind samples* of homogenized beef baby food were provided to three laboratories for analysis. Results from the three labs agreed well for protein, fat, zinc, riboflavin, and palmitic acid. Results for iron were questionable: Lab A:

1.59 ± 0.14 (13); Lab B: 1.65 ± 0.56 (8); Lab C: 2.68 ± 0.78 (3) mg Fe/100 g, with numbers of replicate analyses in parentheses. Lab C produced a higher result than those from Labs A and B. Is the result from Lab C different at the 95% confidence level from that of Lab B? Begin with the *F* test (Section 4-2) to see whether the variances are significantly different and then apply the proper *t* test (Section 4-3).

5-11. *Detection limit.* In spectrophotometry, we measure the concentration of analyte by its absorbance of light. A low-concentration sample was prepared, and nine replicate measurements gave absorbances of 0.004 7, 0.005 4, 0.006 2, 0.006 0, 0.004 6, 0.005 6, 0.005 2, 0.004 4, and 0.005 8 in a 1.000-cm cell. Nine reagent blanks gave values of 0.000 6, 0.001 2, 0.002 2, 0.000 5, 0.001 6, 0.000 8, 0.001 7, 0.001 0, and 0.001 1.

(a) Find the absorbance detection limit with Equation 5-2.

(b) The calibration curve is a graph of absorbance versus concentration in a cell with a pathlength of 1.000 cm. Absorbance is a dimensionless quantity. The slope of the calibration curve is $m = 2.24 \times 10^4 \text{ M}^{-1}$. Find the concentration detection limit with Equation 5-4.

(c) Find the lower limit of quantitation with Equation 5-5.

5-12.  *Control chart.* Volatile compounds in human blood serum were measured by purge and trap gas chromatography-mass spectrometry. For quality control, serum was periodically *spiked* (treated) with a constant amount of 1,2-dichlorobenzene, and the concentration (ng/g = ppb) was measured $n = 1$ time. Find the mean and standard deviation for the following spike data and prepare a control chart. State whether or not the observations meet each of the criteria listed in Box 5-1 for stability in a control chart.

Observed									
Day	ppb								
0	1.05	91	1.13	147	0.83	212	1.03	290	1.04
1	0.70	101	1.64	149	0.88	218	0.90	294	0.85
3	0.42	104	0.79	154	0.89	220	0.86	296	0.59
6	0.95	106	0.66	156	0.72	237	1.05	300	0.83
7	0.55	112	0.88	161	1.18	251	0.79	302	0.67
30	0.68	113	0.79	167	0.75	259	0.94	304	0.66
70	0.83	115	1.07	175	0.76	262	0.77	308	1.04
72	0.97	119	0.60	182	0.93	277	0.85	311	0.86
76	0.60	125	0.80	185	0.72	282	0.72	317	0.88
80	0.87	128	0.81	189	0.87	286	0.68	321	0.67
84	1.03	134	0.84	199	0.85	288	0.86	323	0.68

Data from D. L. Ashley, M. A. Bonin, F. L. Cardinali, J. M. McCraw, J. S. Holler, L. L. Needham, and D. G. Patterson, Jr., *Anal. Chem.* **1992**, *64*, 1021.

5-13. Control chart. The graph in Box 5-1 shows mean values for five replicate quality control samples measured each day. The standard operating procedure calls for stopping work to identify the source of error if the mean daily quality control result is outside the action lines ($\pm 3\sigma/\sqrt{n}$). This condition does not occur in Box 5-1. Are any other rejection conditions from Box 5-1 observed in this data?

5-14. In a murder trial in the 1990s, the defendant's blood was found at the crime scene. The prosecutor argued that blood was left by the defendant during the crime. The defense argued that police "planted" the defendant's blood from a sample collected later. Blood is normally collected in a vial containing the metal-binding compound EDTA (as an anti-coagulant) at a concentration of ~ 4.5 mM after the vial is filled with blood. At the time of the trial, procedures to measure EDTA in blood were not well established. Even though the amount of EDTA found in the crime-scene blood was orders of magnitude below 4.5 mM, the jury acquitted the defendant. This trial motivated the development of a new method to measure EDTA in blood.

(a) *Precision and accuracy.* To measure accuracy and precision of the method, blood was fortified with EDTA to known levels.

$$\text{accuracy} = 100 \times \frac{\text{mean value found} - \text{known value}}{\text{known value}}$$

$$\text{precision} = 100 \times \frac{\text{standard deviation}}{\text{mean}} \equiv \frac{\text{coefficient}}{\text{of variation}}$$

For each of the three spike levels in the table, find the precision and accuracy of the quality control samples.

EDTA measurements (ng/mL) at three fortification levels

Spike:	22.2 ng/mL	88.2 ng/mL	314 ng/mL
Found:	33.3	83.6	322
19.5	69.0	305	
23.9	83.4	282	
20.8	100	329	
20.8	76.4	276	

Data from R. L. Sheppard and J. Henion, *Anal. Chem.* **1997**, 69, 477A, 2901.

(b) *Detection and quantitation limits.* Low concentrations of EDTA near the detection limit gave the following dimensionless instrument readings: 175, 104, 164, 193, 131, 189, 155, 133, 151, and 176. Ten blanks had a mean reading of 45.0. The slope of the calibration curve is $1.75 \times 10^9 \text{ M}^{-1}$. Estimate the signal and concentration detection limits and the lower limit of quantitation for EDTA.

5-15. Spike recovery and detection limit. Species of arsenic found in drinking water include AsO_3^{3-} (arsenite), AsO_4^{3-}

(arsenate), $(\text{CH}_3)_2\text{AsO}_2^-$ (dimethylarsinate), and $(\text{CH}_3)\text{AsO}_3^{2-}$ (methylarsonate). Pure water containing no arsenic was spiked with 0.40 μg arsenate/L. Seven replicate determinations gave 0.39, 0.40, 0.38, 0.41, 0.36, 0.35, and 0.39 $\mu\text{g}/\text{L}$ (J. A. Day, M. Montes-Bayón, A. P. Vonderheide, and J. A. Caruso, *Anal. Bioanal. Chem.* **2002**, 373, 664). Find the mean percent recovery of the spike and the concentration detection limit.

5-16. Standard addition. Vitamin C was measured by an electrochemical method in a 50.0-mL sample of lemon juice. A detector signal of 2.02 μA was observed. A standard addition of 1.00 mL of 29.4 mM vitamin C increased the signal to 3.79 μA . Find the concentration of vitamin C in the juice.

5-17. Standard addition. Analyte in an unknown gave a signal of 10.0 mV. When 1.00 mL of 0.050 0 M standard was added to 100.0 mL of unknown, the signal increased to 14.0 mV. Find the concentration of the original unknown.

5-18. Standard addition graph. Tooth enamel consists mainly of the mineral calcium hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Trace elements in teeth of archeological specimens provide anthropologists with clues about diet and diseases of ancient people. Students at Hamline University measured the trace element strontium in enamel from extracted wisdom teeth by atomic absorption spectroscopy. Solutions were prepared with a *constant total volume* of 10.0 mL containing 0.750 mg dissolved tooth enamel plus variable concentrations of added Sr.

Added Sr (ng/mL = ppb)	Signal (arbitrary units)
0	28.0
2.50	34.3
5.00	42.8
7.50	51.5
10.00	58.6

Data from V. J. Porter, P. M. Sanft, J. C. Dempich, D. D. Dettmer, A. E. Erickson, N. A. Dubauskie, S. T. Myster, E. H. Matts, and E. T. Smith, *J. Chem. Ed.* **2002**, 79, 1114.

(a) Which graph, Figure 5-6 or 5-7, is appropriate for this problem? What is the difference between the two graphs? What does the *x*-intercept tell us in each graph?

(b) Prepare a graph to find the concentration of Sr in the 10-mL sample solution in parts per billion = ng/mL.

(c) Find the concentration of Sr in tooth enamel in parts per million = $\mu\text{g}/\text{g}$.

5-19. *Uncertainty in standard addition.* We now find the uncertainty in the *x*-intercept of the standard addition graph of Problem 5-18 with the formula

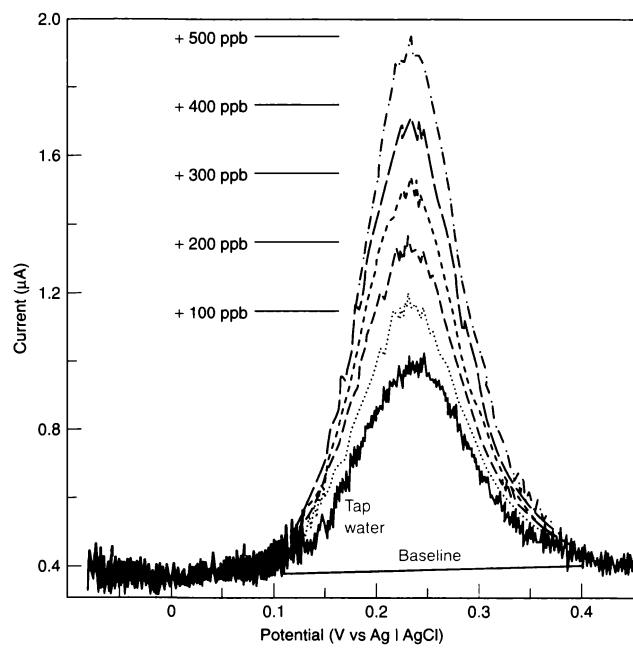
$$\text{standard deviation of } x\text{-intercept} = \frac{s_y}{|m|} \sqrt{\frac{1}{n} + \frac{\bar{y}^2}{m^2 \sum (x_i - \bar{x})^2}}$$

where s_y is the standard deviation of y (Equation 4-15), $|m|$ is the absolute value of the slope of the least-squares line (Equation 4-12), n is the number of data points ($n = 5$), \bar{y} is the mean value of y for the five points, x_i are the individual values of x for the five points, and \bar{x} is the mean value of x for the five points.

(a) Create a spreadsheet like Figure 4-9 to find the straight line for standard additions and include the formula for uncertainty in the x -intercept. Find the uncertainty in the Sr concentration found in Problem 5-18.

(b) If the standard addition intercept is the major source of uncertainty, find the uncertainty in the concentration of Sr in tooth enamel in parts per million.

5-20. Standard addition graph. The figure shows standard additions of Cu^{2+} to acidified tap water measured by an electrochemical method. The standard additions had negligible volume compared with that of the tap water sample, so you can consider all solutions to have the same volume. The signal is the peak height (in μA), which you will need to measure relative to the baseline in the figure. Find the concentration of Cu^{2+} in the tap water.



Five standard additions of 100 ppb Cu^{2+} to tap water. [From M. A. Nolan and S. P. Kounaves, *Anal. Chem.* **1999**, 71, 3567.]

5-21. Standard addition with variable volume. Lead in dry river sediment was extracted with 25 wt% HNO_3 at 35°C for 1 h. Then 1.00 mL of filtered extract was mixed with other reagents to bring the total volume to $V_0 = 4.60 \text{ mL}$. Pb(II) was

measured by an electrochemical method by a series of standard additions of 2.50 ppm Pb(II) .

Pb(II) added (mL)	Signal (arbitrary units)
0	1.10
0.025	1.66
0.050	2.20
0.075	2.81

Data from M. J. Goldcamp, M. N. Underwood, J. L. Cloud, S. Harshman, and K. Ashley, *J. Chem. Ed.* **2008**, 85, 976.

(a) Volume is not constant, so follow the procedure of Figures 5-5 and 5-6 to find ppm Pb(II) in the 1.00-mL extract.

(b) Use the formula in Problem 5-19 to find the uncertainty in the intercept of the graph. Assuming that the uncertainty in intercept is larger than other uncertainties, estimate the uncertainty in ppm Pb(II) in the 1.00-mL extract.

5-22. Internal standard. A mixture containing 12.8 μM analyte (X) and 44.4 μM standard (S) gave chromatographic peak areas of 306 for X and 511 for S. A second solution containing an unknown quantity of X plus 55.5 μM S had peak areas of 251 for X and 563 for S. Find [X] in the second solution.

5-23. Internal standard. A solution was prepared by mixing 10.00 mL of unknown (X) with 5.00 mL of standard (S) containing 8.24 μg S/mL and diluting to 50.0 mL. The measured signal quotient (signal due to X/signal due to S) was 1.69. In a separate experiment, it was found that, for equal concentrations of X and S, the signal due to X was 0.930 times as intense as the signal due to S. Find the concentration of X in the unknown.

5-24. Internal standard. When 1.06 mmol of 1-pentanol and 1.53 mmol of 1-hexanol were separated by gas chromatography, they gave relative peak areas of 922 and 1 570 units, respectively. When 0.57 mmol of pentanol was added to an unknown containing hexanol, the relative chromatographic peak areas were 843:816 (pentanol:hexanol). How much hexanol did the unknown contain?

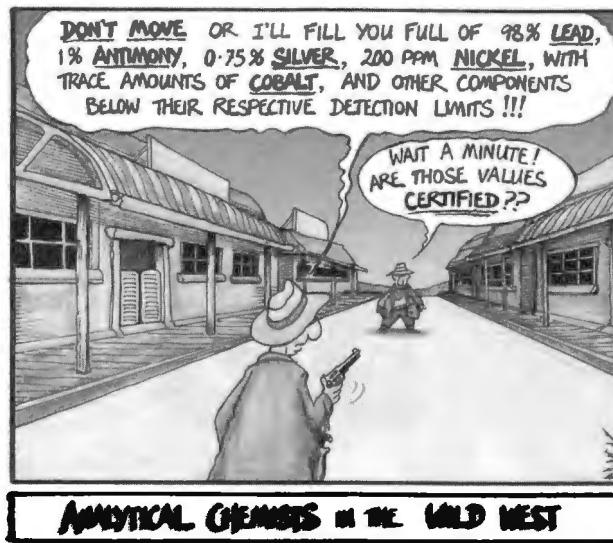
How Would You Do It?

5-25. Olympic athletes are tested to see whether or not they are using illegal performance-enhancing drugs. Suppose that urine samples are taken and analyzed, and the rate of false positive results is 1%. Suppose also that it is too expensive to refine the method to reduce the rate of false positive results. We certainly do not want to accuse innocent people of using illegal drugs. What can you do to reduce the rate of false accusations even though the test always has a false positive rate of 1%?

Further Reading

D. B. Hibbert, *Quality Assurance for the Analytical Chemistry Laboratory* (Oxford: Oxford University Press, 2007).

W. Funk, V. Dammann, and G. Donnevert, *Quality Assurance in Analytical Chemistry* (Hoboken, NJ: Wiley, 2006).

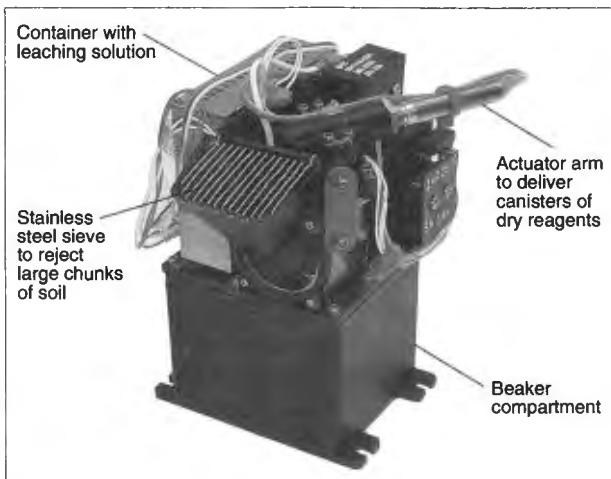


[© Nick Kim, www.linuxgrrls.org]

Titration on Mars



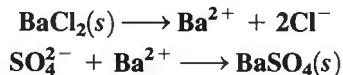
(a)



(b)

(a) Sam Kounaves examines *Phoenix Mars Lander* Wet Chemistry Laboratory cell. (b) Key components of cell.
[Courtesy of S. Kounaves.]

In 2008, Professor Sam Kounaves and his students at Tufts University were filled with excitement as their Wet Chemistry Laboratory experiment aboard the *Phoenix Mars Lander* returned a stream of information about the composition of Martian soil scooped up by a robotic arm. The arm delivered ~ 1 gram of soil through a sieve into a “beaker” fitted with a suite of electrochemical sensors described in Chapter 15. Aqueous solution added to the beaker leached soluble salts from the soil while sensors measured ions appearing in the liquid. Unlike other ions, sulfate was measured by a *precipitation titration* with Ba^{2+} :



As solid BaCl_2 from a reagent canister slowly dissolved in the aqueous liquid, BaSO_4 precipitated. In Problem 6-25, we see that one sensor showed a low level of Ba^{2+} until enough reagent had been added to react with all SO_4^{2-} . Another sensor found steadily increasing Cl^- as BaCl_2 dissolved. The end point of the titration is marked by a sudden increase of Ba^{2+} when the last SO_4^{2-} has precipitated and BaCl_2 continues to dissolve. The increase in Cl^- from the beginning of the titration up to the end point tells how much BaCl_2 was required to consume SO_4^{2-} . Titrations of two soil samples in two cells found ~ 1.3 (± 0.5) wt% sulfate in the soil.¹ Other evidence suggests that the sulfate is mostly from MgSO_4 .

Good Titrations

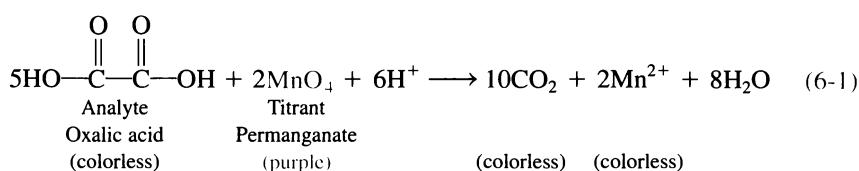
In **volumetric analysis**, the volume of a known reagent required for complete reaction with analyte by a known reaction is measured. From this volume and the stoichiometry of the reaction, we calculate how much analyte is in an unknown substance. In this chapter we discuss general principles that apply to any volumetric procedure, and then we illustrate some analyses based on precipitation reactions. Along the way, we introduce the solubility product as a means of understanding precipitation reactions.

6-1 Principles of Volumetric Analysis

A **titration** is a procedure in which increments of a known reagent—the **titrant**—are added to *analyte* until the reaction between analyte and titrant is complete. Titrant is usually delivered as a solution from a buret (Figure 6-1). Each increment of titrant should be completely and quickly consumed by reaction with analyte until analyte is used up. Common titrations are based on acid-base, oxidation-reduction, complex formation, or precipitation reactions.

Methods of determining when analyte has been consumed include (1) observing an *indicator* color change (Color Plate 1), (2) detecting a sudden change in voltage or current between a pair of electrodes, and (3) monitoring the absorbance of light by species in the reaction. An **indicator** is a compound with a physical property (usually color) that changes abruptly when the titration is complete. The change is caused by the disappearance of analyte or the appearance of excess titrant.

The **equivalence point** is reached when the quantity of titrant added is the exact amount necessary for stoichiometric reaction with the analyte. For example, 5 mol of oxalic acid react with 2 mol of permanganate in hot acidic solution:



If the unknown contains 5.00 mmol of oxalic acid, the equivalence point is reached when 2.00 mmol of MnO_4^- have been added.

The equivalence point is the ideal result that we seek in a titration. What we actually measure is the **end point**, which is marked by a sudden change in a physical property of the solution. For Reaction 6-1, a convenient end point is the abrupt appearance of the purple color of permanganate in the flask. Up to the equivalence

We will study end-point detection methods later:

indicators: Sections 6-6, 9-6, 10-4, 13-3, and 16-2

electrodes: Section 10-4 and Chapter 15

absorbance: Section 19-3

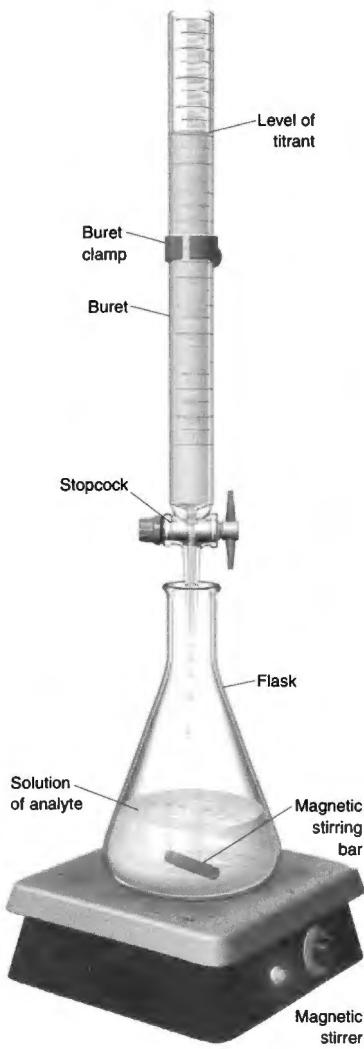


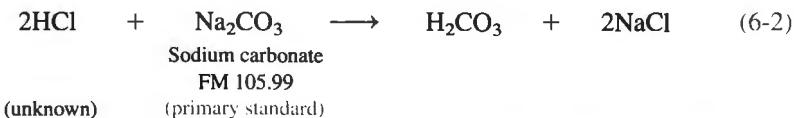
Figure 6-1 Typical setup for a titration. Analyte is contained in the flask and titrant in the buret. The stirring bar is a magnet coated with Teflon, which is inert to most solutions. The bar is spun by a rotating magnet inside the stirrer.

Box 3-1 describes Standard Reference Materials, which allow different laboratories to test the accuracy of their procedures.

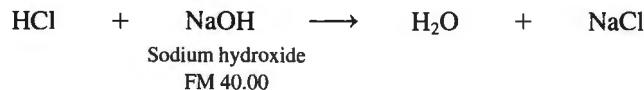
point, all the added permanganate is consumed by oxalic acid, and the titration solution remains colorless. After the equivalence point, unreacted MnO_4^- ion builds up until there is enough to see. The *first trace* of purple color marks the end point. The better your eyes, the closer the measured end point will be to the true equivalence point. The end point cannot exactly equal the equivalence point because extra MnO_4^- , more than that needed to react with oxalic acid, is required to create perceptible color.

The difference between the end point and the equivalence point is an inescapable **titration error**. By choosing an appropriate physical property, in which a change is easily observed (such as indicator color, optical absorbance of a reactant or product, or pH), we can have an end point that is very close to the equivalence point. We can also estimate the titration error with a **blank titration**, in which the same procedure is carried out without analyte. For example, a solution containing no oxalic acid could be titrated with MnO_4^- to see how much titrant is needed to create observable purple color. We subtract this volume of MnO_4^- from the volume observed in the titration of unknown.

The validity of an analytical result depends on knowing the amount of one of the reactants used. A **primary standard** is a reagent that is pure enough to weigh out and use directly to provide a known number of moles. For example, if you wanted to titrate an unknown concentration of hydrochloric acid with base, you could weigh primary-standard-grade sodium carbonate and dissolve it in water to make titrant:



Two moles of HCl react with 1 mol of Na₂CO₃, which has a mass of 105.99 g. You could not carry out the same procedure with solid NaOH because the solid is not pure.



NaOH is normally contaminated with some Na₂CO₃ (from reaction with CO₂ in the air) and H₂O (also from the air). If you weighed out 40.00 g of sodium hydroxide, it would not contain exactly 1 mol.

A primary standard should be 99.9% pure or better. It should not decompose under ordinary storage, and it should be stable when dried by heating or vacuum because drying is required to remove traces of water adsorbed from the atmosphere.

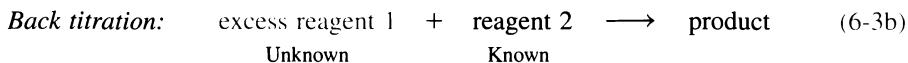
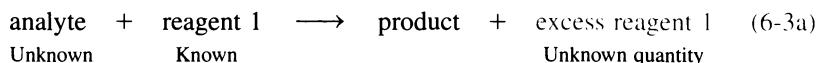
In most cases, titrant is not available as a primary standard. Instead, a solution having approximately the desired concentration is used to titrate a weighed, primary standard. From the volume of titrant required to react with the primary standard, we calculate the concentration of titrant. The process of titrating a standard to determine the concentration of titrant is called **standardization**. We say that a solution whose concentration is known is a **standard solution**. The validity of the analytical result ultimately depends on knowing the composition of some primary standard.

In a **direct titration**, titrant is added to analyte until the end point is observed.



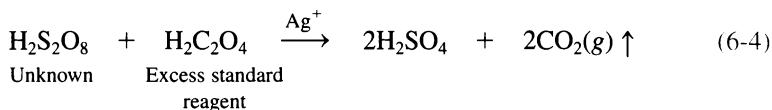
The addition of permanganate titrant to oxalic acid analyte in Reaction 6-1 is a direct titration.

In a **back titration**, a known *excess* of a standard reagent is added to the analyte. Then a second standard reagent is used to titrate the excess of the first reagent.



Back titrations are useful when the end point of the back titration is clearer than the end point of the direct titration or when an excess of the first reagent is required for complete reaction with analyte.

For example, a back titration is used in the determination of peroxydisulfate ($\text{S}_2\text{O}_8^{2-}$). *Determination* is chemists' jargon for "measurement." An unknown such as impure $\text{K}_2\text{S}_2\text{O}_8$ is treated with excess standard $\text{Na}_2\text{C}_2\text{O}_4$ in H_2SO_4 containing Ag_2SO_4 catalyst. We can write the reaction for a strongly acidic solution as



Excess standard reagent ensures complete reaction of the unknown. The mixture is heated until $\text{CO}_2(g)$ evolution is complete. Then the solution is cooled to 40°C, and the excess $\text{H}_2\text{C}_2\text{O}_4$ is back titrated with standard MnO_4^- by Reaction 6-1. The quantity of MnO_4^- required for back titration tells us how much $\text{H}_2\text{C}_2\text{O}_4$ was left over from Reaction 6-4.

In a **gravimetric titration**, titrant is measured by mass, not volume. Titrant concentration is expressed as moles of reagent per kilogram of solution. Precision is improved from 0.3% attainable with a buret to 0.1% with a balance. Experiments by Guenther² and by Butler and Swift³ provide examples. In a gravimetric titration, there is no need for a buret. Titrant can be delivered from a pipet. "Gravimetric titrations should become the gold standard, and volumetric glassware should be seen in museums only."⁴

Peroxydisulfate is a powerful oxidizing agent used to destroy organic matter in environmental analysis. It is also the active ingredient of "cleaning solution" used to destroy grease and organic matter in glassware.

The Book Companion Website www.whfreeman.com/exploringchem5e includes lists of experiments from the *Journal of Chemical Education* keyed to the chapters of this book.

Ask Yourself

- 6-A. (a) Why does the validity of an analytical result ultimately depend on knowing the composition of a primary standard?
(b) How does a blank titration reduce titration error?
(c) What is the difference between a direct titration and a back titration?
(d) Suppose that the uncertainty in locating the equivalence point in a titration is ± 0.04 mL. Why is it more accurate to use enough unknown to require ~ 40 mL in a titration with a 50-mL buret instead of ~ 20 mL?

6-2 Titration Calculations

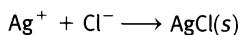
To interpret the results of a direct titration, the key steps are

- From the volume of titrant, calculate the number of moles of titrant consumed.
- From the *stoichiometry* of the titration reaction, relate the unknown moles of analyte to the known moles of titrant.

Stoichiometry is the ratio of substances participating in a chemical reaction.

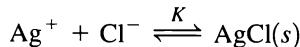
1:1 Stoichiometry

When K is large, we sometimes write \longrightarrow instead of \rightleftharpoons :



Retain an extra, insignificant digit until the end of the calculations to prevent round-off error.

Consider the titration of an unknown chloride solution with standard Ag^+ :



The reaction is rapid and the equilibrium constant is large ($K = 5.6 \times 10^9$), so the reaction essentially goes to completion with each addition of titrant. White AgCl precipitate forms as soon as the two reagents are mixed.

Suppose that 10.00 mL of unknown chloride solution (measured with a transfer pipet) require 22.97 mL of 0.052 74 M AgNO_3 solution (delivered from a buret) for complete reaction. What is the concentration of Cl^- in the unknown?

Following the two-step recipe, we first find the moles of Ag^+ :

$$\text{mol Ag}^+ = \text{volume} \times \text{molarity} = (0.022\ 97 \text{ L}) \left(0.052\ 74 \frac{\text{mol}}{\text{L}} \right) = 0.001\ 211_4 \text{ mol}$$

Next, relate the unknown moles of Cl^- to the known moles of Ag^+ . We know that 1 mol of Cl^- reacts with 1 mol of Ag^+ . If 0.001 211₄ mol of Ag^+ is required, then 0.001 211₄ mol of Cl^- must have been in 10.00 mL of unknown. Therefore

$$[\text{Cl}^-] \text{ in unknown} = \frac{\text{mol Cl}^-}{\text{L of unknown}} = \frac{0.001\ 211_4 \text{ mol}}{0.010\ 00 \text{ L}} = 0.121\ 1_4 \text{ M}$$

The solution that we titrated was made by dissolving 1.004 g of unknown solid in a total volume of 100.0 mL. What is the weight percent of chloride in the solid? We know that 10.00 mL of unknown solution contain 0.001 211₄ mol of Cl^- . Therefore 100.0 mL must contain 10 times as much, or 0.012 11₄ mol of Cl^- . This much Cl^- weighs $(0.012\ 11_4 \text{ mol Cl}^-)(35.453 \text{ g/mol Cl}^-) = 0.429\ 4_8 \text{ g Cl}^-$. The weight percent of Cl^- in the unknown is

$$\text{wt\% Cl}^- = \frac{\text{g Cl}^-}{\text{g unknown}} \times 100 = \frac{0.429\ 4_8 \text{ g Cl}^-}{1.004 \text{ g unknown}} \times 100 = 42.78 \text{ wt\%}$$

Example A Case Involving a Dilution

(a) Standard Ag^+ solution was prepared by dissolving 1.224 3 g of dry AgNO_3 (FM 169.87) in water in a 500.0-mL volumetric flask. A dilution was made by delivering 25.00 mL of solution with a pipet to a second 500.0-mL volumetric flask and diluting to the mark. Find the concentration of Ag^+ in the dilute solution. **(b)** A 25.00-mL aliquot of unknown containing Cl^- was titrated with the dilute Ag^+ solution, and the equivalence point was reached when 37.38 mL of Ag^+ solution had been delivered. Find the concentration of Cl^- in the unknown.

SOLUTION **(a)** The concentration of the initial AgNO_3 solution is

$$[\text{Ag}^+] = \frac{(1.224\ 3 \text{ g})/(169.87 \text{ g/mol})}{0.500\ 0 \text{ L}} = 0.014\ 41_5 \text{ M}$$

To find the concentration of the dilute solution, use the dilution formula 1-5:

$$\begin{aligned} [\text{Ag}^+]_{\text{conc}} \cdot V_{\text{conc}} &= [\text{Ag}^+]_{\text{dil}} \cdot V_{\text{dil}} & (1-5) \\ (0.014\ 41_5 \text{ M}) \cdot (25.00 \text{ mL}) &= [\text{Ag}^+]_{\text{dil}} \cdot (500.0 \text{ mL}) \end{aligned}$$

$$[\text{Ag}^+]_{\text{dil}} = \left(\frac{25.00 \text{ mL}}{500.0 \text{ mL}} \right) (0.014415 \text{ M}) = 7.2073 \times 10^{-4} \text{ M}$$

(b) One mole of Cl^- requires 1 mol of Ag^+ . The number of moles of Ag^+ required to reach the equivalence point is

$$\text{mol Ag}^+ = (7.2073 \times 10^{-4} \text{ M})(0.03738 \text{ L}) = 2.6941 \times 10^{-5} \text{ mol}$$

The concentration of Cl^- in 25.00 mL of unknown is therefore

$$[\text{Cl}^-] = \frac{2.6941 \times 10^{-5} \text{ mol}}{0.02500 \text{ L}} = 1.078 \times 10^{-3} \text{ M} = 1.078 \text{ mM}$$

Notice that the general form of all dilution problems is

$$[\text{X}]_{\text{final}} = \underbrace{\frac{V_{\text{initial}}}{V_{\text{final}}}}_{\text{Dilution factor}} \cdot [\text{X}]_{\text{initial}}$$

mM stands for “millimolar” = $10^{-3} \text{ M} = 10^{-3} \text{ mol/L}$.

 **Test Yourself** Suppose that 25.00 mL of standard AgNO_3 were diluted to 250.0 mL (instead of 500.0 mL). A 10.00-mL aliquot of unknown containing Cl^- required 15.77 mL of Ag^+ solution for titration. Find $[\text{Cl}^-]$ in the unknown. (Answer: 2.273 mM)

Silver ion titrations are nice because AgNO_3 is a primary standard. After drying at 110°C for 1 h to remove moisture, the solid has the exact composition AgNO_3 . Methods for finding the end point in silver titrations are described in Section 6-6. Silver compounds and solutions should be stored in the dark to prevent photodecomposition and should never be exposed to direct sunlight.

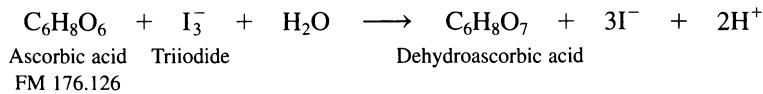
x:y Stoichiometry

In Reaction 6-1, 5 mol of oxalic acid ($\text{H}_2\text{C}_2\text{O}_4$) react with 2 mol of permanganate (MnO_4^-). If an unknown quantity of $\text{H}_2\text{C}_2\text{O}_4$ consumed 2.345×10^{-4} mol of MnO_4^- , there must have been

$$\begin{aligned} \text{mol H}_2\text{C}_2\text{O}_4 &= (\text{mol MnO}_4^-) \left(\frac{5 \text{ mol H}_2\text{C}_2\text{O}_4}{2 \text{ mol MnO}_4^-} \right) \\ &= (2.345 \times 10^{-4} \text{ mol MnO}_4^-) \left(\frac{5 \text{ mol H}_2\text{C}_2\text{O}_4}{2 \text{ mol MnO}_4^-} \right) = 5.862 \times 10^{-4} \text{ mol} \end{aligned}$$

Ask Yourself

6-B. Vitamin C (ascorbic acid) from foods can be measured by titration with I_3^- :



Starch is used as an indicator in the reaction. The end point is marked by the appearance of a deep blue starch-iodine complex when unreacted I_3^- is present.

- (a) If 29.41 mL of I_3^- solution are required to react with 0.1970 g of pure ascorbic acid, what is the molarity of the I_3^- solution?
- (b) A vitamin C tablet containing ascorbic acid plus inert binder was ground to a powder, and 0.4242 g was titrated by 31.63 mL of I_3^- . How many moles of ascorbic acid are present in the 0.4242-g sample?
- (c) Find the weight percent of ascorbic acid in the tablet.

Dilute AgNO_3 solution is an antiseptic. If you spill AgNO_3 solution on yourself, your skin will turn black for a few days until the affected skin is shed.

Photodecomposition of $\text{AgCl}(s)$:



Finely divided $\text{Ag}(s)$ imparts a faint violet color to the white solid.

6-3 Chemistry in a Fishtank



[Hal Van Ryswyk, Harvey Mudd College.]

Students of Hal Van Ryswyk at Harvey Mudd College study analytical chemistry by measuring chemical changes in a saltwater aquarium in their laboratory.⁵ One of the chemicals measured is nitrite, NO_2^- , which is a key species in the natural cycle of nitrogen (Figure 6-2). Box 6-1 shows concentrations of ammonia (NH_3), nitrite, and nitrate (NO_3^-) measured in the aquarium. Concentrations are expressed in parts per million of nitrogen, which means micrograms (μg) of nitrogen per gram of seawater. Because 1 g of water \approx 1 mL, we will consider 1 ppm to be 1 $\mu\text{g}/\text{mL}$.

Nitrite was measured by a spectrophotometric procedure described in Section 18-4. There is no convenient primary standard for nitrite, so a titration is used to standardize a NaNO_2 solution that serves as the standard for the spectrophotometric procedure. As always, the validity of any analytical procedure ultimately depends on knowing the composition of a primary standard, which is sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$) in this case.

Three solutions are required for the measurement of nitrite:

- Prepare $\sim 0.018 \text{ M NaNO}_2$ (FM 68.995) by dissolving 1.25 g of NaNO_2 in 1.00 L of distilled water. We describe the standardization of this solution shortly.
- Prepare $\sim 0.025 \text{ M Na}_2\text{C}_2\text{O}_4$ (FM 134.00) by dissolving $\sim 3.350 \text{ g}$ of primary-standard-grade $\text{Na}_2\text{C}_2\text{O}_4$ in 1.0 M H_2SO_4 and diluting to 1.000 L with 1.0 M H_2SO_4 . It is not necessary to weigh out exactly 3.350 g. What is important is that you know the exact mass so that you can calculate the molarity of the reagent.
- Prepare $\sim 0.010 \text{ M KMnO}_4$ (FM 158.03) by dissolving 1.6 g of KMnO_4 in 1.00 L of distilled water. KMnO_4 is not pure enough to be a primary standard. Also, traces of organic impurities in distilled water consume some of the freshly dissolved MnO_4^- to produce solid manganese dioxide, MnO_2 . Therefore, dissolve KMnO_4 in distilled water to give the approximately desired concentration and boil the solution for 1 h to complete the reaction between MnO_4^- and organic impurities. Filter the mixture

NH_3 (FM 17.031) contains 82.24 wt% N. Therefore a solution with 1.216 mg NH_3/L contains

$$\left(1.216 \frac{\text{mg NH}_3}{\text{L}}\right) \left(0.8224 \frac{\text{mg N}}{\text{mg NH}_3}\right)$$
$$= 1.000 \text{ mg N/L} = 1.000 \mu\text{g N/mL}$$
$$= 1 \text{ ppm N}$$

The concentrations of NaNO_2 and KMnO_4 are only approximate. The solutions will be standardized in subsequent titrations.

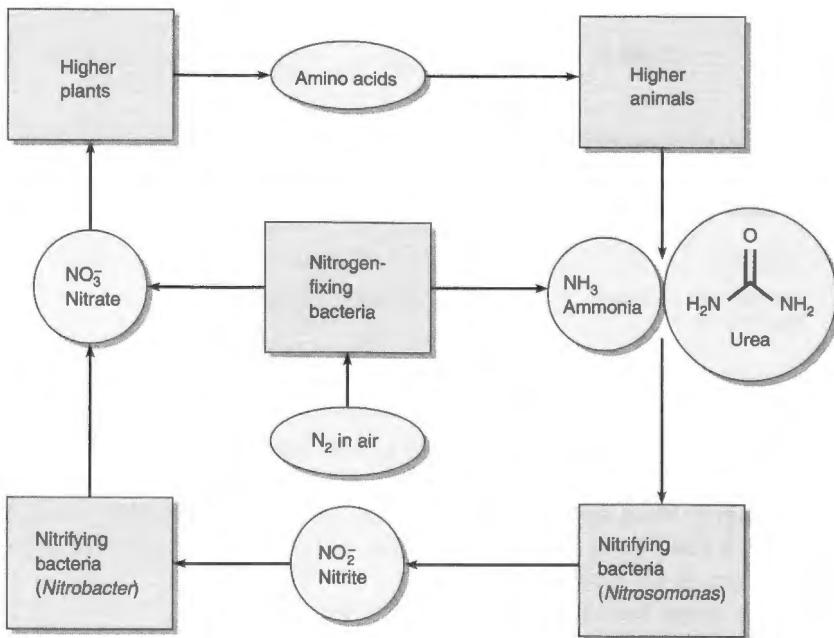
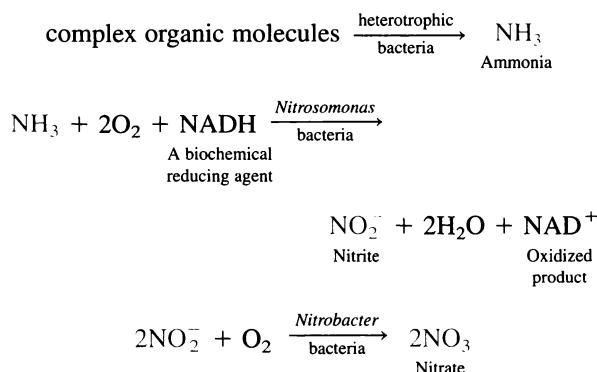


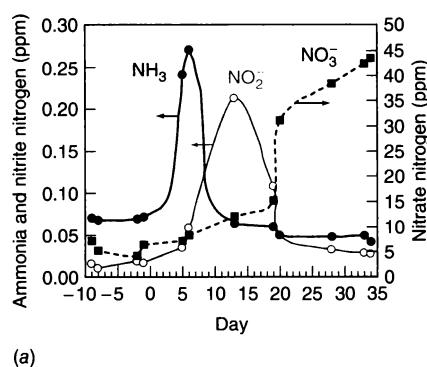
Figure 6-2 Nitrogen is exchanged among different forms of life through the *nitrogen cycle*. Only a few organisms, such as blue-green algae, are able to use N_2 directly from the air. Our existence depends on the health of all organisms in the nitrogen cycle.

Box 6-1 Studying a Marine Ecosystem

Students at Harvey Mudd College monitor a saltwater aquarium to study the chemistry of a marine ecosystem.⁵ When fish and food are introduced into the aquarium on day 0 in panel *a*, organic compounds are metabolized to produce NH₃. Ammonia is toxic to marine animals when the level exceeds 1 ppm; but, fortunately, it is removed by *Nitrosomonas* bacteria, which colonize the aquarium filter and oxidize NH₃ to nitrite (NO₂⁻). Alas, NO₂⁻ is also toxic at levels above 1 ppm, but it is further oxidized to nitrate (NO₃⁻) by a second colonization of *Nitrobacter* bacteria. The natural process of oxidation of NH₃ to NO₂⁻ and NO₃⁻ is called *nitrification*.

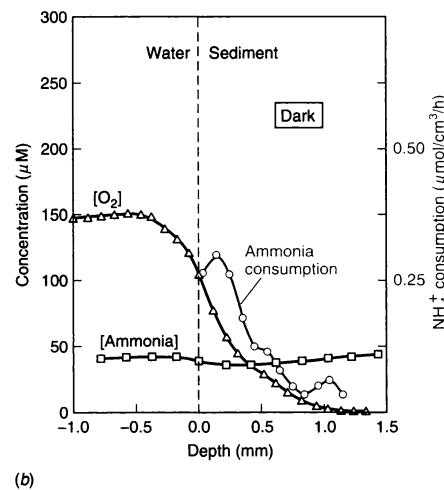


Some actions of nitrogen-metabolizing bacteria. *Heterotrophic* bacteria require complex organic molecules from the breakdown of other organisms for nourishment. By contrast, *autotrophic* bacteria can utilize CO₂ as their carbon source for biosynthesis.

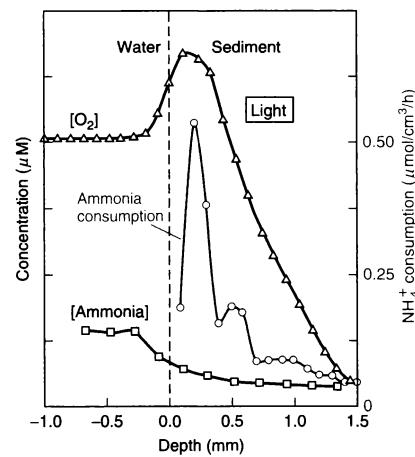


NH₃, NO₂⁻, and NO₃⁻ concentrations in a saltwater aquarium at Harvey Mudd College after fish and food were introduced into the tank on day 0. Concentrations are expressed in parts per million of nitrogen (i.e., μg of N per mL of solution). [Data from Hal Van Ryswyk, Harvey Mudd College.]

Nitrification requires an oxidizing agent to convert NH₃ to NO₂⁻. Panels *b* and *c* show the relationship between photosynthesis and nitrification in river sediment. In the presence of light, photosynthetic bacteria generate O₂, which serves as the oxidizing agent. Ammonia consumption in the top 1 mm of sediment approximately doubles in the presence of light. Ammonia and oxygen were measured with microelectrodes described in Box 15-2 and Section 17-2.



(b)



(c)

Concentrations of O₂ and ammonia and the rate of ammonia consumption in sediment from the Niida River in Japan in the dark (*b*) or exposed to light (*c*). Ammonia was measured as ammonium ion (NH₄⁺), which is the predominant form at pH 7 to 8 in the river. [Data from Y. Nakamura, H. Satoh, T. Kindaichi, and S. Okabe, *Environ. Sci. Technol.* **2006**, *40*, 1532.]

through a sintered-glass filter (not a paper filter, which is organic) to remove $\text{MnO}_2(s)$, cool the solution, and standardize it against primary standard $\text{Na}_2\text{C}_2\text{O}_4$:



For best results, treat the oxalic acid solution at 25°C with 90% to 95% of the expected volume of KMnO_4 . Then heat the solution to 60°C and complete the titration.

Example Standardizing KMnO_4 by a Direct Titration of Oxalate

Standard oxalate solution was made by dissolving 3.299 g of $\text{Na}_2\text{C}_2\text{O}_4$ in 1.000 L of 1 M H_2SO_4 . A 25.00-mL aliquot required 28.39 mL of KMnO_4 for titration, and a blank titration of 25 mL of 1 M H_2SO_4 required 0.03 mL of KMnO_4 . Find the molarity of KMnO_4 .

SOLUTION The number of moles of $\text{Na}_2\text{C}_2\text{O}_4$ dissolved in 1 L is $(3.299 \text{ g})/(134.00 \text{ g/mol}) = 0.02461_9 \text{ mol}$. The $\text{C}_2\text{O}_4^{2-}$ in 25.00 mL is $(0.02461_9 \text{ M}) \times (0.02500 \text{ L}) = 6.154_9 \times 10^{-4} \text{ mol}$. Reaction 6-5 requires 2 mol of permanganate for 5 mol of oxalate. Therefore

$$\begin{aligned} \text{mol MnO}_4^- &= (\text{mol C}_2\text{O}_4^{2-}) \left(\frac{2 \text{ mol MnO}_4^-}{5 \text{ mol C}_2\text{O}_4^{2-}} \right) = (6.154_9 \times 10^{-4} \text{ mol}) \left(\frac{2}{5} \right) \\ &= 2.461_9 \times 10^{-4} \text{ mol} \end{aligned}$$

You can use either ratio

$$\frac{5 \text{ mol C}_2\text{O}_4^{2-}}{2 \text{ mol MnO}_4^-} \quad \text{or} \quad \frac{2 \text{ mol MnO}_4^-}{5 \text{ mol C}_2\text{O}_4^{2-}}$$

whenever you please, as long as the units work out.

The equivalence volume of KMnO_4 is $28.39 - 0.03 = 28.36 \text{ mL}$. The concentration of MnO_4^- titrant is

$$[\text{MnO}_4^-] = \frac{2.461_9 \times 10^{-4} \text{ mol}}{0.02836 \text{ L}} = 8.681_0 \times 10^{-3} \text{ M}$$

 **Test Yourself** A 25.00-mL aliquot of the same standard oxalate solution required 22.05 mL of KMnO_4 for titration, and a blank titration required 0.05 mL of KMnO_4 . Find the molarity of KMnO_4 . (Answer: 0.01119 M)

Here is the procedure for standardizing the NaNO_2 solution prepared in step a:

- Pipet 25.00 mL of standard KMnO_4 into a 500-mL flask, add 300 mL of 0.4 M H_2SO_4 , and warm to 40°C on a hot plate.
- Titrate the KMnO_4 with the NaNO_2 solution whose concentration is to be determined. Add titrant slowly until MnO_4^- is just decolorized. Add titrant very slowly near the end point because the reaction is slow. Best results are obtained with the tip of the buret immersed beneath the surface of the KMnO_4 solution.



Example Standardizing NaNO_2 with KMnO_4

Step 2 required 34.76 mL of NaNO_2 solution from step a. Find the concentration of NaNO_2 .

SOLUTION We required 34.76 mL of NaNO₂ to titrate 25.00 mL of 8.681₀ × 10⁻³ M KMnO₄. The number of moles of KMnO₄ consumed is (0.025 00 L)(8.681₀ × 10⁻³ M) = 2.170₃ × 10⁻⁴ mol. In Reaction 6-6, 2 mol MnO₄⁻ require 5 mol NO₂⁻, so the amount of NaNO₂ that reacted is

mol NaNO₂ reacting with KMnO₄

$$= (2.170_3 \times 10^{-4} \text{ mol KMnO}_4) \left(\frac{5 \text{ mol NaNO}_2}{2 \text{ mol KMnO}_4} \right) = 5.425_6 \times 10^{-4} \text{ mol}$$

The concentration of NaNO₂ reagent is

$$[\text{NaNO}_2] = \frac{5.425_6 \times 10^{-4} \text{ mol}}{0.034\ 76 \text{ L}} = 0.015\ 61 \text{ M}$$

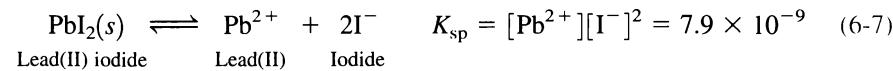
 **Test Yourself** If [KMnO₄] = 6.666 × 10⁻³ M instead of 8.681₀ × 10⁻³ M, what is the concentration of NaNO₂? (**Answer:** 0.011 99 M)

Ask Yourself

- 6-C. (a) Standard oxalate was made by dissolving 3.514 g of Na₂C₂O₄ in 1.000 L of 1 M H₂SO₄. A 25.00-mL aliquot required 24.44 mL of KMnO₄ for titration, and a blank required 0.03 mL of KMnO₄. Find the molarity of KMnO₄.
 (b) To standardize NaNO₂, 25.00 mL of KMnO₄ solution from part (a) of this problem required 38.11 mL of NaNO₂. Find the molarity of the NaNO₂.

6-4 Solubility Product

The **solubility product**, K_{sp} , is the equilibrium constant for the reaction in which a solid **salt** (an ionic compound) dissolves to give its constituent ions in solution. For example,



The solid is omitted from the equilibrium constant because the solid is in its standard state. Values of K_{sp} are listed in Appendix A. We will use the solubility product to discuss precipitation titrations in Section 6-5.

Estimating the Solubility of an Ionic Compound

Consider the dissolution of lead(II) iodide in water in Reaction 6-7. A solution that contains all the solid capable of being dissolved is said to be **saturated**. What is the concentration of Pb²⁺ in a solution saturated with PbI₂?

Reaction 6-7 produces two I⁻ ions for each Pb²⁺ ion. If the concentration of dissolved Pb²⁺ is x M, the concentration of dissolved I⁻ must be $2x$ M. We can display this relation neatly in a little concentration table:

$\text{PbI}_2(s) \rightleftharpoons \text{Pb}^{2+} + 2\text{I}^-$			
Initial concentration	solid	0	0
Final concentration	solid	x	$2x$

Omit pure solid from the equilibrium expression because PbI₂(s) is in its standard state. This is a *really good* time to review Section 1-5 on the equilibrium constant.

Putting these concentrations into the solubility product gives

$$[\text{Pb}^{2+}][\text{I}^-]^2 = (x)(2x)^2 = 7.9 \times 10^{-9}$$

$$4x^3 = 7.9 \times 10^{-9}$$

$$x = \left(\frac{7.9 \times 10^{-9}}{4} \right)^{1/3} = 0.00125 \text{ M}$$

The concentration of Pb^{2+} is 0.00125 M and the concentration of I^- is $2x = (2)(0.00125) = 0.0025 \text{ M}$.

Here is the physical meaning of the solubility product: If water is left in contact with excess solid PbI_2 , solid dissolves until the condition $[\text{Pb}^{2+}][\text{I}^-]^2 = K_{\text{sp}}$ is satisfied. Thereafter, no more solid dissolves. Unless excess solid remains, there is no guarantee that $[\text{Pb}^{2+}][\text{I}^-]^2 = K_{\text{sp}}$. If Pb^{2+} and I^- are mixed together (with appropriate counterions) such that the product $[\text{Pb}^{2+}][\text{I}^-]^2$ exceeds K_{sp} , then $\text{PbI}_2(s)$ precipitates until the condition $[\text{Pb}^{2+}][\text{I}^-]^2 = K_{\text{sp}}$ is satisfied (Figure 6-3).

The solubility product does not tell the entire story of the solubility of ionic compounds. The concentration of *undissociated species* and *complex ions* may be significant. In a solution of calcium sulfate, for example, about two thirds of the dissolved material dissociates to Ca^{2+} and SO_4^{2-} and one third is undissociated $\text{CaSO}_4(aq)$ (a tightly bound *ion pair*). In the PbI_2 case, species such as PbI^+ , $\text{PbI}_2(aq)$, and PbI_3^- also contribute to the total solubility. The species PbI^+ and PbI_3^- are called *complex ions* because they are composed of simpler ions.

The Common Ion Effect

Consider what happens when we add a second source of I^- to a solution saturated with $\text{PbI}_2(s)$. Let's dissolve PbI_2 in 0.030 M NaI , which dissociates completely to Na^+ and I^- . What is the concentration of Pb^{2+} in this solution?

$\text{PbI}_2(s) \rightleftharpoons \text{Pb}^{2+} + 2\text{I}^-$ (6-8)			
Initial concentration	solid	0	0.030
Final concentration	solid	x	$2x + 0.030$

The initial concentration of I^- is from dissolved NaI . The final concentration of I^- has contributions from NaI and PbI_2 .

The solubility product is

$$[\text{Pb}^{2+}][\text{I}^-]^2 = (x)(2x + 0.030)^2 = K_{\text{sp}} = 7.9 \times 10^{-9} \quad (6-9)$$

But think about the size of x . With no added I^- , we found $x = 0.00125 \text{ M}$. Now we anticipate that x will be smaller than 0.00125 M , because of Le Châtelier's principle. Addition of I^- to Reaction 6-8 displaces the reaction in the reverse direction. In the presence of extra I^- , there will be less Pb^{2+} . This application of Le Châtelier's principle is called the **common ion effect**. A salt is less soluble if one of its constituent ions is already present in the solution.

In Equation 6-9, we suspect that $2x$ may be much smaller than 0.030 . As an approximation, ignore $2x$ in comparison with 0.030 . The equation simplifies to

$$\begin{aligned}(x)(0.030)^2 &= K_{\text{sp}} = 7.9 \times 10^{-9} \\ x &= 7.9 \times 10^{-9}/(0.030)^2 = 8.8 \times 10^{-6}\end{aligned}$$

To find the cube root of a number with a calculator, raise the number to the $0.333\ 333\ 33\dots$ power with the y^x key. In Excel, the expression $y^{(1/3)}$ gives the cube root.



Figure 6-3 The yellow solid, lead(II) iodide (PbI_2), precipitates when a colorless solution of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) is added to a colorless solution of potassium iodide (KI).

[Photo by Chip Clark.]

Common ion effect: A salt is less soluble if one of its ions is already present in the solution.

Box 6-2 The Logic of Approximations

Many problems are difficult to solve without judicious approximations. For example, rather than solving the equation

$$(x)(2x + 0.030)^2 = 7.9 \times 10^{-9}$$

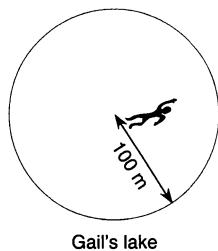
we hope and pray that $2x \ll 0.030$ and therefore solve the much simpler equation

$$(x)(0.030)^2 = 7.9 \times 10^{-9}$$

But how can we be sure that our solution fits the original problem?

When we use an approximation, we assume it is true. *If the assumption is true, it does not create a contradiction. If the assumption is false, it leads to a contradiction.* You can test an assumption by using it and seeing whether you are right or wrong afterward.

You may object to this reasoning, feeling “How can the truth of an assumption be tested by using the assumption?” Suppose you wish to test the statement “Gail can swim 100 meters.” To see whether or not the statement is true, you can assume it is true. If Gail can swim 100 m, then you could dump her in the middle of a lake with a radius of 100 m and expect her to swim to shore. If she comes ashore alive, then your assumption was correct and no contradiction is created. If she does not make it to shore, then there is a contradiction. Either the assumption is correct and using it is correct or the assumption is wrong and leads to a contradiction. (Another possibility in this case is that there are freshwater sharks in the lake.)



Because $2x = 1.8 \times 10^{-5} \ll 0.030$, ignoring $2x$ to solve the problem was justified. The answer also illustrates the common ion effect. In the absence of added I^- , the solubility of Pb^{2+} was 0.0013 M . In the presence of $0.030\text{ M }I^-$, $[Pb^{2+}]$ is reduced to $8.8 \times 10^{-6}\text{ M}$.

What is the maximum I^- concentration at equilibrium in a solution in which $[Pb^{2+}]$ is somehow fixed at $1.0 \times 10^{-4}\text{ M}$? Our concentration table looks like this now:

	$PbI_2(s)$	\rightleftharpoons	Pb^{2+}	+	$2I^-$
Initial concentration	solid		1.0×10^{-4}		0
Final concentration	solid		1.0×10^{-4}		x

Example 1. $(x)(2x + 0.030)^2 = 7.9 \times 10^{-9}$
 $(x)(0.030)^2 = 7.9 \times 10^{-9}$
 (assuming $2x \ll 0.030$)

$$x = (7.9 \times 10^{-9})/(0.030)^2 = 8.8 \times 10^{-6}$$

No contradiction:

$$2x = 1.76 \times 10^{-5} \ll 0.030$$

The assumption is true.

Example 2. $(x)(2x + 0.030)^2 = 7.9 \times 10^{-5}$
 $(x)(0.030)^2 = 7.9 \times 10^{-5}$
 (assuming $2x \ll 0.030$)

$$x = (7.9 \times 10^{-5})/(0.030)^2 = 0.088$$

A contradiction: $2x = 0.176 > 0.030$

The assumption is false.

In Example 2, the assumption leads to a contradiction, so the assumption cannot be correct. When this happens, you must solve the cubic equation $(x)(2x + 0.030)^2 = 7.9 \times 10^{-5}$.

A reasonable way to solve this equation is by trial and error, as shown in the following table. You can create this table by hand or, even more easily, with a spreadsheet. In cell A1, enter a guess for x . In cell A2, enter the formula “=A1*(2*A1+0.030)^2”. When you guess x correctly in cell A1, cell A2 will have the value 7.9×10^{-5} . Problem 6-24 gives an even better way to solve this problem with Excel Goal Seek.

Guess	$x(2x + 0.030)^2$	Result is
$x = 0.01$	2.5×10^{-5}	too low
$x = 0.02$	9.8×10^{-5}	too high
$x = 0.015$	5.4×10^{-5}	too low
$x = 0.018$	7.84×10^{-5}	too low
$x = 0.019$	8.79×10^{-5}	too high
$x = 0.0181$	7.93×10^{-5}	too high
$x = 0.01805$	7.89×10^{-5}	too low
$x = 0.01806$	7.90×10^{-5}	not bad!

It is important to confirm at the end of the calculation that the approximation $2x \ll 0.030$ is valid. Box 6-2 discusses approximations.

$[\text{Pb}^{2+}]$ is not x in this example, so there is no reason to set $[\text{I}^-] = 2x$. The problem is solved by plugging each concentration into the solubility product:

$$\begin{aligned} [\text{Pb}^{2+}][\text{I}^-]^2 &= K_{\text{sp}} \\ (1.0 \times 10^{-4})(x)^2 &= 7.9 \times 10^{-9} \\ x &= [\text{I}^-] = 8.9 \times 10^{-3} \text{ M} \end{aligned}$$

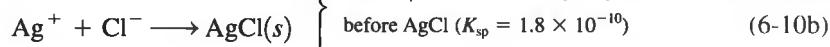
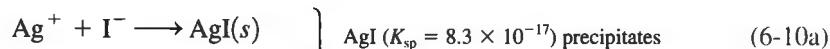
If I^- is added above a concentration of $8.9 \times 10^{-3} \text{ M}$, then $\text{PbI}_2(s)$ will precipitate.

Ask Yourself

6-D. What is the concentration of Pb^{2+} in (a) a saturated solution of PbBr_2 in water or (b) a saturated solution of PbBr_2 in which $[\text{Br}^-]$ is somehow fixed at 0.10 M ?

6-5 Titration of a Mixture

Silver iodide has a smaller solubility product than silver chloride, so AgI is less soluble than AgCl . When Ag^+ is added to a solution of Cl^- and I^- , $\text{AgI}(s)$ precipitates first:



Because the two solubility products are sufficiently different, the first precipitation is nearly complete before the second commences.

Figure 6-4 shows how the reaction is monitored with a silver electrode to find both end points. We will learn how this electrode responds to silver ion concentration in Section 15-1. Figure 6-5 shows experimental curves for the titration of I^- or a mixture of I^- plus Cl^- by Ag^+ .

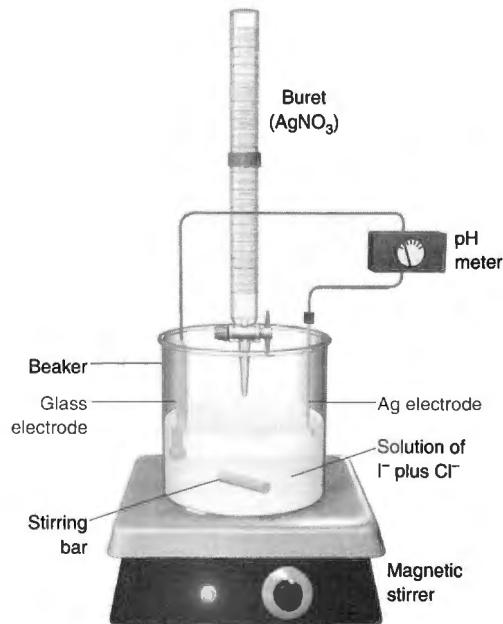


Figure 6-4 Apparatus for measuring the titration curves in Figure 6-5. The silver electrode responds to changes in Ag^+ concentration, and the glass electrode provides a constant reference potential in this experiment. The voltage changes by approximately 59 mV for each factor-of-10 change in $[\text{Ag}^+]$. All solutions, including AgNO_3 , were maintained at pH 2.0 by using 0.010 M sulfate buffer prepared from H_2SO_4 and KOH.

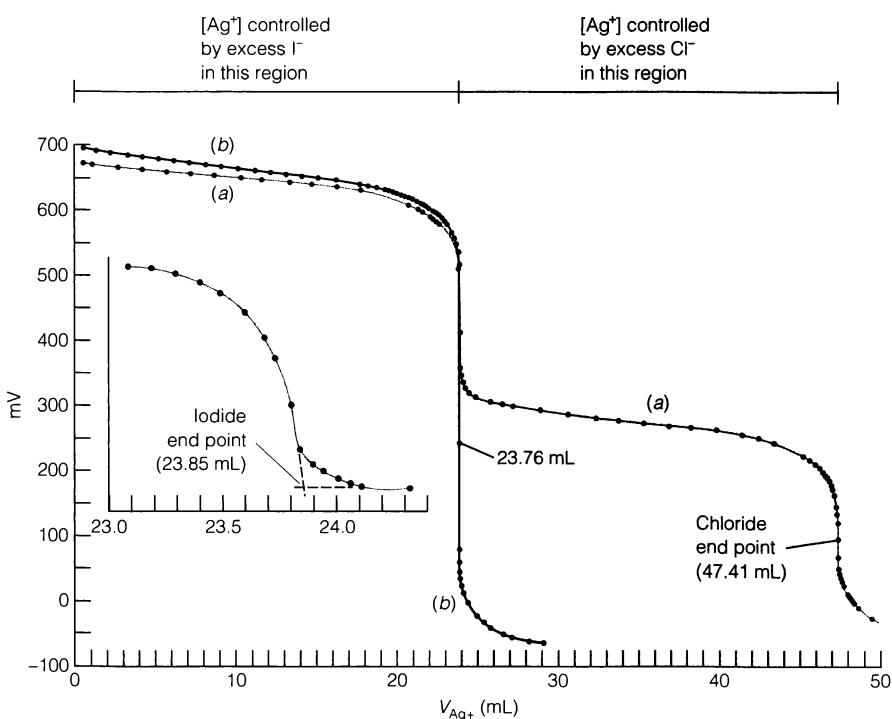


Figure 6-5 Experimental titration curves. The ordinate (*y*-axis) is the electric potential difference (in millivolts) between the two electrodes in Figure 6-4. (a) Titration of KI plus KCl with 0.084 M AgNO_3 . The inset is an expanded view of the region near the first equivalence point. (b) Titration of I^- with 0.084 M Ag^+ .

In the titration of I^- (curve *b* in Figure 6-5), the voltage remains almost constant near 650 mV until the equivalence point (23.76 mL) when I^- is used up. At this point, there is an abrupt decrease in the electrode potential. The abrupt change occurs because the electrode is responding to the concentration of Ag^+ in the solution. Prior to the equivalence point, virtually all the added Ag^+ reacts with I^- to precipitate $\text{AgI}(s)$. The concentration of Ag^+ in solution is very low and nearly constant. When I^- has been consumed, the concentration of Ag^+ suddenly increases because Ag^+ is being added from the buret and is no longer consumed by I^- . This change causes the abrupt decrease in electrode potential.

In the titration of $\text{I}^- + \text{Cl}^-$ (curve *a* in Figure 6-5), there are two abrupt changes in electrode potential. The first occurs when I^- is used up, and the second comes when Cl^- is used up. Prior to the first equivalence point, the very low concentration of Ag^+ is governed by the solubility of AgI . Between the first and the second equivalence points, essentially all I^- has precipitated and Cl^- is in the process of being consumed. The concentration of Ag^+ is still small but governed by the solubility of AgCl , which is greater than that of AgI . After the second equivalence point, the concentration of Ag^+ shoots upward as Ag^+ is added from the buret. Therefore we observe two abrupt changes of electric potential in this experiment.

The I^- end point is taken as the intersection of the steep and nearly horizontal curves shown at 23.85 mL in the inset of Figure 6-5. The reason for using the intersection is that the precipitation of I^- is not quite complete when Cl^- begins to precipitate. Therefore the end of the steep part (the intersection) is a better approximation of the equivalence point than is the middle of the steep section. The Cl^- end point is taken as the midpoint of the second steep section, at 47.41 mL. The moles of Cl^- in the sample correspond to the moles of Ag^+ delivered between the first and the second end points. That is, it requires 23.85 mL of Ag^+ to precipitate I^- , and $(47.41 - 23.85) = 23.56$ mL of Ag^+ to precipitate Cl^- .

Example Extracting Results from Figure 6-5

The elements F, Cl, Br, I, and At are called *halogens*. Their anions are called *halides*.

In curve *a* of Figure 6-5, 40.00 mL of unknown solution containing both I^- and Cl^- were titrated with 0.0845 M Ag^+ . Find the concentrations of each halide ion.

SOLUTION The inset shows the first end point at 23.85 mL. Reaction 6-10a tells us that 1 mol of I^- consumes 1 mol of Ag^+ . The moles of Ag^+ delivered at this point are $(0.0845 \text{ M})(0.02385 \text{ L}) = 2.015 \times 10^{-3}$ mol. The molarity of iodide in the unknown is therefore

$$[\text{I}^-] = \frac{2.015 \times 10^{-3} \text{ mol}}{0.04000 \text{ L}} = 0.0503_8 \text{ M}$$

The second end point is at 47.41 mL. The quantity of Ag^+ titrant required to react with Cl^- is the difference between the two end points: $(47.41 - 23.85) = 23.56 \text{ mL}$. The number of moles of Ag^+ required to react with Cl^- is $(0.0845 \text{ M})(0.02356 \text{ L}) = 1.991 \times 10^{-3}$ mol. The molarity of chloride in the unknown is

$$[\text{Cl}^-] = \frac{1.991 \times 10^{-3} \text{ mol}}{0.04000 \text{ L}} = 0.0497_7 \text{ M}$$

 **Test Yourself** Suppose that the two end points were at 24.85 and 47.41 mL. Find $[\text{I}^-]$ and $[\text{Cl}^-]$ in the unknown. (**Answer:** 0.0525₀ M, 0.0476₆ M)

Ask Yourself

6-E. A 25.00-mL solution containing Br^- and Cl^- was titrated with 0.03333 M AgNO_3 .

- Write the two titration reactions and use solubility products to find which takes place first.
- In an experiment analogous to that in Figures 6-4 and 6-5, the first end point was observed at 15.55 mL. Find the concentration of the first halide that precipitated. Is it Br^- or Cl^- ?
- The second end point was observed at 42.23 mL. Find the concentration of the other halide.

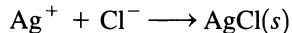
6-6 Titrations Involving Silver Ion

We now introduce two widely used indicator methods for titrations involving Ag^+ , which are called *argentometric titrations*. The methods are

- Volhard titration:** formation of a soluble, colored complex at the end point
- Fajans titration:** adsorption of a colored indicator on the precipitate at the end point

Volhard Titration

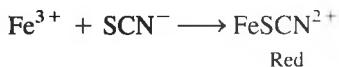
The Volhard procedure ends with a back titration of Ag^+ in 0.5–1.5 M HNO_3 . To determine Cl^- , the unknown Cl^- is precipitated by a known excess of standard AgNO_3 :



The AgCl is isolated, and excess Ag⁺ is titrated with standard KSCN in the presence of Fe³⁺:



When Ag⁺ has been consumed, SCN⁻ reacts with Fe³⁺, which acts as an indicator to form a red complex:



The appearance of red color signals the end point. Knowing how much SCN⁻ was required for the back titration tells us how much Ag⁺ was left over from the reaction with Cl⁻. Because the total amount of Ag⁺ is known, the amount consumed by Cl⁻ can then be calculated.

In the analysis of Cl⁻ by the Volhard method, the end point slowly fades because AgCl is more soluble than AgSCN. The AgCl slowly dissolves and is replaced by AgSCN. To prevent this secondary reaction from happening, we filter off the AgCl and titrate Ag⁺ in the filtrate. Br⁻ and I⁻, whose silver salts are *less* soluble than AgSCN, can be titrated by the Volhard method without isolating the silver halide precipitate.

Fajans Titration

The Fajans titration uses an **adsorption indicator**. To see how this works, consider the electric charge of a precipitate. When Ag⁺ is added to Cl⁻, there is excess Cl⁻ in solution prior to the equivalence point. Some Cl⁻ is selectively adsorbed on the AgCl surface, imparting a negative charge to the crystal surface (Figure 6-6a). After the equivalence point, there is excess Ag⁺ in solution. Adsorption of Ag⁺ cations on the crystal creates a positive charge on the particles of precipitate (Figure 6-6b). The abrupt change from negative charge to positive charge occurs at the equivalence point.

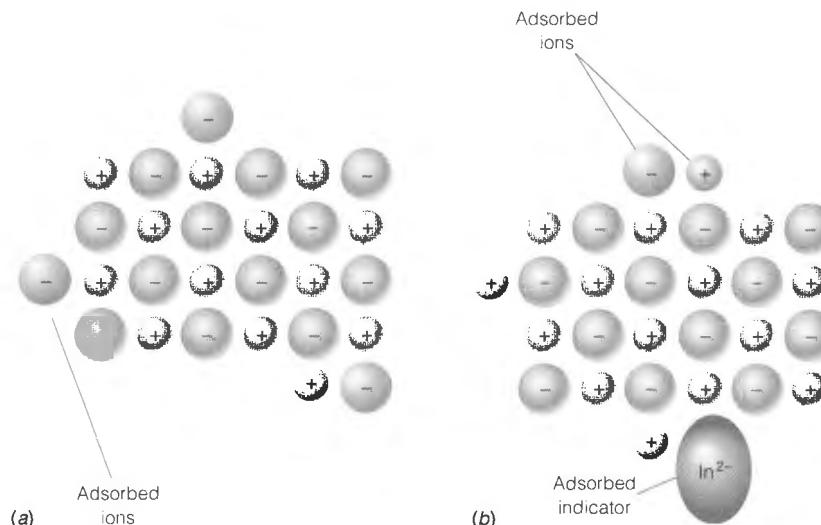
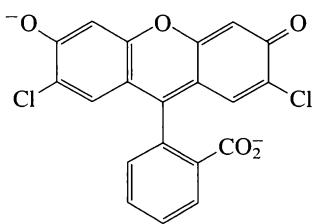


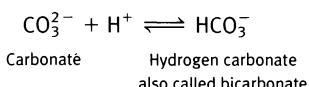
Figure 6-6 Ions from a solution are adsorbed on the surface of a growing crystallite. (a) A crystal growing in the presence of excess lattice anions (anions that belong in the crystal) has a negative charge because anions are predominantly adsorbed. (b) A crystal growing in the presence of excess lattice cations has a positive charge and can therefore adsorb a negative indicator ion. Anions and cations in the solution that do not belong in the crystal lattice are less likely to be adsorbed than are ions belonging to the lattice.



Dichlorofluorescein

Notation for drawing organic compounds is discussed in Box 7-1.

Challenge Consider the equilibrium



Use Le Châtelier's principle to explain why carbonate salts are soluble in acidic solution (which contains a high concentration of H^+).

Common adsorption indicators are anionic (negatively charged) dyes, which are attracted to the positively charged precipitate produced immediately after the equivalence point. Adsorption of the dye on the surface of the solid precipitate changes the color of the dye by interactions that are not well understood. The color change signals the end point in the titration. Because the indicator reacts with the precipitate surface, it is desirable to have as much surface area as possible. The titration is performed under conditions that tend to keep the particles as small as possible, because small particles have more surface area than an equal volume of large particles. Low electrolyte concentration helps prevent coagulation of the precipitate. As in all silver titrations, strong light should be avoided.

The indicator most commonly used for AgCl is dichlorofluorescein, which has a greenish yellow color in solution but turns pink when adsorbed on AgCl (Demonstration 6-1). To maintain the indicator in its anionic form, there must not be too much H^+ in the solution. The dye eosin is useful in the titration of Br^- , I^- , and SCN^- . It gives a sharper end point than dichlorofluorescein and can be used to detect smaller quantities of halide. It does not work for AgCl because eosin anion is more strongly bound than Cl^- to AgCl . Eosin binds to the AgCl crystallites even before the particles become positively charged.

Applications of precipitation titrations are listed in Table 6-1. The Volhard method is an argentometric titration, but the Fajans method has wider application. Because the Volhard titration is carried out in $\sim 1\text{ M}$ acid, it avoids some interference that affects other titrations. Silver salts of anions such as CO_3^{2-} (carbonate), $\text{C}_2\text{O}_4^{2-}$ (oxalate), and AsO_4^{3-} (arsenate) are soluble in acidic solution, so these anions do not interfere with the analysis.

Table 6-1 Applications of precipitation titrations

Species analyzed	Notes
Br^- , I^- , SCN^- , CNO^- , AsO_4^{3-} Cl^- , PO_4^{3-} , CN^- , $\text{C}_2\text{O}_4^{2-}$, CO_3^{2-} , S^{2-} , CrO_4^{2-}	VOLHARD METHOD Precipitate removal is unnecessary. Precipitate removal required.
Cl^- , Br^- , I^- , SCN^- , $\text{Fe}(\text{CN})_6^{4-}$	FAJANS METHOD Titrate with Ag^+ . Detection with dyes such as fluorescein, dichlorofluorescein, eosin, bromophenol blue.
Zn^{2+}	Titrate with $\text{K}_4\text{Fe}(\text{CN})_6$ to produce $\text{K}_2\text{Zn}_3[\text{Fe}(\text{CN})_6]_2$. End point detected with diphenylamine.
SO_4^{2-}	Titrate with $\text{Ba}(\text{OH})_2$ in 50 vol% aqueous methanol; use alizarin red S as indicator.
Hg_2^{2+}	Titrate with NaCl to produce Hg_2Cl_2 . End point detected with bromophenol blue.
PO_4^{3-} , $\text{C}_2\text{O}_4^{2-}$	Titrate with $\text{Pb}(\text{CH}_3\text{CO}_2)_2$ to give $\text{Pb}_3(\text{PO}_4)_2$ or PbC_2O_4 . End point detected with dibromofluorescein (PO_4^{3-}) or fluorescein ($\text{C}_2\text{O}_4^{2-}$).



Demonstration 6-1 Fajans Titration

The Fajans titration of Cl^- with Ag^+ demonstrates indicator end points in precipitation titrations. Dissolve 0.5 g of NaCl plus 0.15 g of dextrin in 400 mL of water. The purpose of the dextrin is to retard coagulation of the AgCl precipitate. Add 1 mL of dichlorofluorescein indicator solution containing 1 mg/mL of dichlorofluorescein in 95 wt% aqueous ethanol or 1 mg/mL of the sodium salt in water. Titrate the NaCl solution with a solution containing 2 g of AgNO_3 in 30 mL of water. About 20 mL are required to reach the end point.

Color Plate 1a shows the yellow color of the indicator in the NaCl solution prior to the titration. Color Plate 1b shows the milky-white appearance of the AgCl suspension during titration, before the end point is reached. The pink suspension in Color Plate 1c appears at the end point, when the anionic indicator becomes adsorbed to the cationic particles of precipitate.



Ask Yourself

- 6-F. (a) Why is precipitated AgCl filtered off in the Volhard titration of chloride?
(b) Why does the surface charge of a precipitate change sign at the equivalence point?
(c) In the Fajans titration of Zn^{2+} in Table 6-1, do you expect the charge on the precipitate to be positive or negative after the equivalence point?

Key Equations

Stoichiometry	For the reaction $a\text{A} + b\text{B} \longrightarrow \text{products}$, use the ratio $\left(\frac{a \text{ mol A}}{b \text{ mol B}}\right)$ for stoichiometry calculations.
Solubility product	$\text{PbI}_2(s) \xrightleftharpoons{K_{\text{sp}}} \text{Pb}^{2+} + 2\text{I}^- \quad K_{\text{sp}} = [\text{Pb}^{2+}][\text{I}^-]^2$ Common ion effect: A salt is less soluble in the presence of one of its constituent ions.

Important Terms

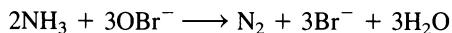
adsorption indicator	equivalence point	solubility product	titration error
back titration	Fajans titration	standardization	Volhard titration
blank titration	gravimetric titration	standard solution	volumetric analysis
common ion effect	indicator	stoichiometry	
direct titration	primary standard	titrant	
end point	saturated solution	titration	

Problems

- 6-1. Distinguish the terms *end point* and *equivalence point*.
6-2. For Reaction 6-1, how many milliliters of 0.165 M KMnO_4 are needed to react with 108.0 mL of 0.165 M oxalic acid? How many milliliters of 0.165 M oxalic acid are required to react with 108.0 mL of 0.165 M KMnO_4 ?

- 6-3. A 10.00-mL aliquot of unknown oxalic acid solution required 15.44 mL of 0.0117 M KMnO_4 solution to reach the purple end point. A blank titration of 10 mL of similar solution containing no oxalic acid required 0.04 mL to exhibit detectable color. Find the concentration of oxalic acid in the unknown.

6-4. Ammonia reacts with hypobromite, OBr^- as follows:



Find the molarity of OBr^- if 1.00 mL of OBr^- solution reacts with 1.69 mg of NH_3 (FM 17.03).

6-5. How many milliliters of 0.100 M KI are needed to react with 40.0 mL of 0.040 0 M $\text{Hg}_2(\text{NO}_3)_2$ if the reaction is $\text{Hg}_2^{2+} + 2\text{I}^- \longrightarrow \text{Hg}_2\text{I}_2(s)$?

6-6. Cl^- in blood serum, cerebrospinal fluid, or urine can be measured by titration with mercuric ion: $\text{Hg}^{2+} + 2\text{Cl}^- \longrightarrow \text{HgCl}_2(aq)$. When the reaction is complete, excess Hg^{2+} reacts with the indicator diphenylcarbazone, which forms a violet-blue color.

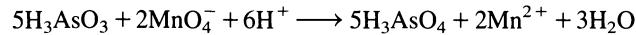
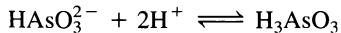
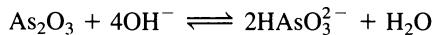
(a) Mercuric nitrate was standardized by titrating a solution containing 147.6 mg of NaCl (FM 58.44), which required 28.06 mL of $\text{Hg}(\text{NO}_3)_2$ solution. Find the molarity of the $\text{Hg}(\text{NO}_3)_2$.

(b) When this same $\text{Hg}(\text{NO}_3)_2$ solution was used to titrate 2.000 mL of urine, 22.83 mL were required. Find the concentration of Cl^- (mg/mL) in the urine.

6-7. Volhard titration. A 30.00-mL solution of unknown I^- was treated with 50.00 mL of 0.365 0 M AgNO_3 . The precipitated AgI was filtered off, and the filtrate (plus Fe^{3+}) was titrated with 0.287 0 M KSCN. When 37.60 mL had been added, the solution turned red. How many milligrams of I^- were present in the original solution?

6-8. How many milligrams of oxalic acid dihydrate, $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ (FM 126.07), will react with 1.00 mL of 0.027 3 M ceric sulfate, $\text{Ce}(\text{SO}_4)_2$, if the reaction is $\text{H}_2\text{C}_2\text{O}_4 + 2\text{Ce}^{4+} \longrightarrow 2\text{CO}_2 + 2\text{Ce}^{3+} + 2\text{H}^+$?

6-9. Arsenic(III) oxide (As_2O_3) is available in pure form and is a useful (and poisonous) primary standard for many oxidizing agents, such as MnO_4^- . As_2O_3 is first dissolved in base and titrated with MnO_4^- in acidic solution. A small amount of iodide (I^-) or iodate (IO_3^-) is used to catalyze the reaction between H_3AsO_3 and MnO_4^- . The reactions are

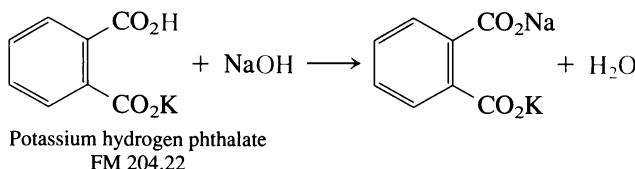


(a) A 3.214-g aliquot of KMnO_4 (FM 158.03) was dissolved in 1.000 L of water, heated to cause any reactions with impurities to occur, cooled, and filtered. What is the theoretical molarity of this solution if no MnO_4^- was consumed by impurities?

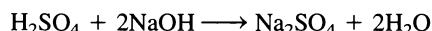
(b) What mass of As_2O_3 (FM 197.84) would be sufficient to react with 25.00 mL of the KMnO_4 solution in part (a)?

(c) It was found that 0.146 8 g of As_2O_3 required 29.98 mL of KMnO_4 solution for the faint color of unreacted MnO_4^- to appear. A blank titration required 0.03 mL of MnO_4^- for color to appear. Find the molarity of the KMnO_4 solution.

6-10. Gravimetric titration. A solution of NaOH was standardized by titration of a known quantity of the primary standard, potassium hydrogen phthalate:



The NaOH was then used to standardize H_2SO_4 :



(a) Titration of 0.824 g of potassium hydrogen phthalate required 38.314 g of NaOH solution to reach the end point detected by phenolphthalein indicator. Find the concentration of NaOH expressed as mol NaOH/kg solution.

(b) A 10.063-g aliquot of H_2SO_4 solution required 57.911 g of NaOH solution to reach the phenolphthalein end point. Find the concentration of H_2SO_4 in mol/kg of solution.

6-11. Uncertainty in volumetric and gravimetric procedures.

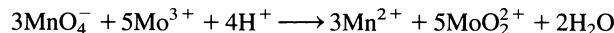
(a) A silver nitrate primary standard for a gravimetric titration of chloride was prepared by dissolving 4.872 \pm 0.003 g AgNO_3 (FM 169.873 1) in 498.633 \pm 0.003 g H_2O . Then 26.207 \pm 0.003 g of the solution were weighed out for titration. Find the moles of Ag^+ (and its relative uncertainty) delivered for titration. The relative uncertainty in formula mass is negligible.

(b) In a volumetric titration, 4.872 \pm 0.003 g AgNO_3 were dissolved in a 500.00 \pm 0.20 mL volumetric flask. Then 25.00 \pm 0.03 mL were withdrawn for titration. Find the moles of Ag^+ (and its relative uncertainty) delivered for titration.

(c) How much greater is the relative uncertainty in the volumetric delivery than the gravimetric delivery? What is the largest source of uncertainty in each method?

6-12. Back titration. Impure $\text{K}_2\text{S}_2\text{O}_8$ (FM 270.32, 0.507 3 g) was analyzed by treatment with excess standard $\text{Na}_2\text{C}_2\text{O}_4$ in H_2SO_4 by Reaction 6-4. After reaction with 50.00 mL of 0.050 06 M $\text{Na}_2\text{C}_2\text{O}_4$, the excess oxalate required 16.52 mL of 0.020 13 M KMnO_4 in Reaction 6-1. Find the weight percent of $\text{K}_2\text{S}_2\text{O}_8$ in the impure reagent.

6-13. An unknown molybdate (MoO_4^{2-}) solution (50.00 mL) was passed through a column containing $\text{Zn}(s)$ to convert molybdate to Mo^{3+} . One mole of MoO_4^{2-} gives one mole of Mo^{3+} . The resulting sample required 22.11 mL of 0.012 34 M KMnO_4 to reach a purple end point from the reaction

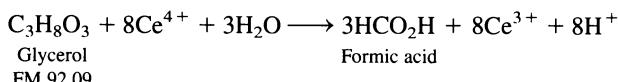


A blank required 0.07 mL. Find the molarity of molybdate in the unknown.

6-14. A 25.00-mL sample of La^{3+} was treated with excess $\text{Na}_2\text{C}_2\text{O}_4$ to precipitate $\text{La}_2(\text{C}_2\text{O}_4)_3$, which was washed to

remove excess $\text{C}_2\text{O}_4^{2-}$ and then dissolved in acid. The oxalate from $\text{La}_2(\text{C}_2\text{O}_4)_3$ required 12.34 mL of 0.004321 M KMnO_4 to reach the purple end point of Reaction 6-1. Find the molarity of La^{3+} in the unknown.

6-15. Back titration. A glycerol solution weighing 153.2 mg was treated with 50.0 mL of 0.0899 M Ce^{4+} in 4 M HClO_4 at 60°C for 15 min to convert glycerol into formic acid:



The excess Ce^{4+} required 10.05 mL of 0.0437 M Fe^{2+} for a back titration by the reaction $\text{Ce}^{4+} + \text{Fe}^{2+} \longrightarrow \text{Ce}^{3+} + \text{Fe}^{3+}$. Find wt% glycerol in the unknown.

6-16. Propagation of uncertainty. Consider the titration of 50.00 (± 0.05) mL of a mixture of I^- and SCN^- with 0.0683 (± 0.0001) M Ag^+ . From the solubility products of AgI and AgSCN , decide which precipitate is formed first. The first equivalence point is observed at 12.6 (± 0.4) mL, and the second occurs at 27.7 (± 0.3) mL. Find the molarity and the uncertainty in molarity of thiocyanate in the original mixture.

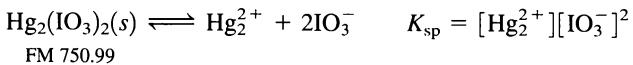
6-17. Calculate the solubility of CuBr (FM 143.45) in water expressed as (a) moles per liter and (b) grams per 100 mL. (This question presumes that Cu^+ and Br^- are the only significant soluble species and that species such as CuBr_2^- and the ion pair $\text{CuBr}(aq)$ are negligible.)

6-18. Find the solubility of silver chromate (FM 331.73) in water. Express your answer as (a) moles of chromate per liter and (b) ppm Ag^+ ($\approx \mu\text{g Ag}^+/\text{mL}$). (This question presumes that other species such as the ion pair AgCrO_4^- are negligible.)

6-19. Consider K_{sp} for $\text{Ag}_4\text{Fe}(\text{CN})_6$ in Appendix A. What concentration of $\text{Fe}(\text{CN})_6^{4-}$ (ferrocyanide) is in equilibrium with 1.0 μM Ag^+ and $\text{Ag}_4\text{Fe}(\text{CN})_6(s)$? Express your answer with a prefix from Table 1-3. Would your answer change if you considered species such as the ion pair $\text{AgFe}(\text{CN})_6^{3-}$?

6-20. Ag^+ at 10–100 ppb (ng/mL) disinfects swimming pools. One way to maintain an appropriate concentration of Ag^+ is to add a slightly soluble silver salt to the pool. Calculate the ppb of Ag^+ in saturated solutions of AgCl , AgBr , and AgI . Which would be best for swimming pool disinfection?

6-21. Mercury(I) is a diatomic ion (Hg_2^{2+} , also called mercurous ion) with a charge of +2. Mercury(I) iodate dissociates as follows:



(a) Find the concentrations of Hg_2^{2+} and IO_3^- in a saturated solution of $\text{Hg}_2(\text{IO}_3)_2(s)$ if there are no other significant species such as the ion pair $\text{Hg}_2^{2+} \cdot \text{IO}_3^-$.

(b) Find $[\text{Hg}_2^{2+}]$ in a 0.010 M solution of KIO_3 saturated with $\text{Hg}_2(\text{IO}_3)_2(s)$.

6-22. If a solution containing 0.10 M Cl^- , Br^- , I^- , and CrO_4^{2-} is treated with Ag^+ , in what order will the anions precipitate?

6-23. Volhard titration. The concentration of Cl^- in “concentrated HCl,” a ~ 12 M solution commonly purchased for laboratories, was determined as follows:

1. Standard Ag^+ was prepared by mixing 25.00 mL of 0.1026 M AgNO_3 , 5 mL of 6 M HNO_3 , and 1 mL of Fe^{3+} indicator solution (40 wt% aqueous $(\text{NH}_4)\text{Fe}(\text{SO}_4)_2$ with a few drops of 6 M HNO_3). To standardize a solution of potassium thiocyanate, KSCN is delivered to the standard Ag^+ solution from a buret. The initial precipitate is white and later it is reddish brown. The color disappears on shaking. At the end point, one drop of KSCN solution produces a faint brown color that does not disappear on shaking. A volume of 24.22 mL was required to reach the end point and the blank correction was 0.02 mL.

2. 10.00 mL of concentrated HCl were diluted with water to 1.000 L in a volumetric flask. Then 20.00 mL of dilute HCl were mixed with 5 mL of 6 M HNO_3 and 25.00 mL of standard 0.1026 M AgNO_3 . AgCl precipitate was filtered and washed with 0.16 M HNO_3 , and the washings were combined with the filtrate. Filtrate was treated with 1 mL of Fe^{3+} indicator and titrated with 2.43 mL of KSCN to reach the faint brown end point. The blank correction was 0.02 mL.

Find the concentration of KSCN in step 1 and the concentration of “concentrated HCl” reagent from step 2.

6-24. Solving equations with Excel Goal Seek. Suppose we saturate a solution of 0.0010 M NaI with PbI_2 . From K_{sp} for PbI_2 , we find $[\text{Pb}^{2+}]$ as follows:

$$[\text{Pb}^{2+}][\text{I}^-]^2 = (x)(2x + 0.0010)^2 = K_{\text{sp}} = 7.9 \times 10^{-9}$$

(a) Solution by trial and error. Following the procedure at the end of Box 6-2, solve for x by guessing the value that makes the expression $(x)(2x + 0.0010)^2$ equal to 7.9×10^{-9} . Write any value of x in cell A4. In cell B4, enter the formula “= A4*(2*A4+0.0010)^2”. Now vary the value of x in cell A4 until you get 7.9×10^{-9} in cell B4.

	A	B
1	Guessing the Answer	
2		
3	x	$x(2x+0.0010)^2$
4	0.01	4.41E-06

(b) Using Excel Goal Seek. Set up the spreadsheet in part (a) and guess a value of 0.01 in cell A4. We will use a built-in procedure to vary cell A4 until cell B4 has the desired value. We want cell B4 to be a small number (7.9×10^{-9}), so we need to set a small tolerance for it. Click the Microsoft Office button at the upper left of the spreadsheet. At the bottom of the Office window, click Excel Options. At the left side of the next window, select Formulas. In Calculation Options, set Maximum Change to 1e-15. Click OK. We just told the computer that cell B4 needs to be precise to 10^{-15} . Select the Data

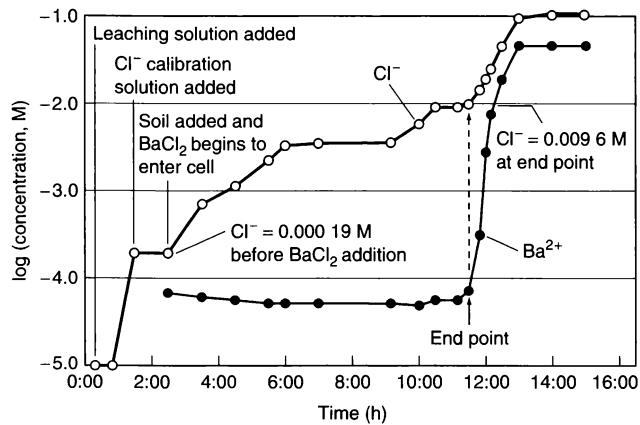
ribbon. In Data Tools, click What-If Analysis and select Goal Seek. In the resulting window, Set cell B4 To value 7.9E-9. By changing cell A4. Click OK and the computer finds $x = 0.000\ 945\ 4$ in cell A4. If you do not see enough digits in cell A4, drag the separator between columns A and B to the right to expand the cell. To select the number of decimal places displayed, highlight cell A4 and select Number from the Home ribbon.

	A	B
1	Using Excel Goal Seek	
2		
3	x	$x(2x+0.0010)^2$
4	0.01	4.41E-06

Before executing Goal Seek

	A	B
1	Using Excel Goal Seek	
2		
3	x	$x(2x+0.0010)^2$
4	0.0009454	7.90E-09

After executing Goal Seek



Barium sulfate precipitation titration from *Phoenix Mars Lander*. [Data from Reference 1, courtesy S. Kounaves, Tufts University.]

How Would You Do It?

- 6-25. Sulfate in soil on Mars.** A barium sulfate precipitation titration described at the opening of this chapter is shown in the figure. The initial concentration of Cl^- before adding BaCl_2 was $0.000\ 19\ \text{M}$ in $25\ \text{mL}$ of aqueous extract of Martian soil. At the end point, when there is a sudden rise in Ba^{2+} , $[\text{Cl}^-] = 0.009\ 6\ \text{M}$.

Notes and References

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Good Titrations—Ode to a Lab Partner

Kurt Wood and Jeff Lederman

(University of California, Davis, 1977)

(Sung to the tune of *Good Vibrations* by the Beach Boys)

Ah! I love the color of pink you get,
And the way the acid drips from your buret.
All the painful things in life seem alien
As I mix in several drops of phenolphthalein.

I'm pickin' up good titrations
She's givin' me neutralizations
Dew drop drop, good titrations . . .

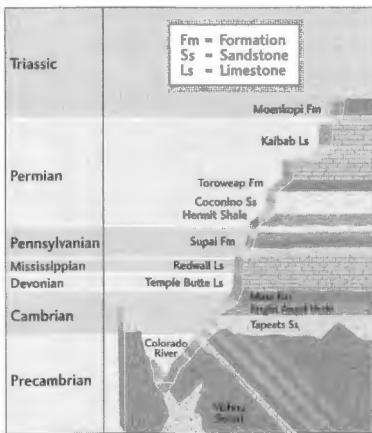
I look at you and drift away;
The ruby red turns slowly to rosé.
You gaze at me and light my fire
As drop on drop falls to the Erlenmeyer.

I'm pickin' up good titrations
She's givin' me neutralizations
Dew drop drop, good titrations . . .

I look longingly to your eyes,
But you stare down at the lab bench in surprise.
Gone our love before it grew much sweeter,
'Cause we passed the end point by a milliliter.

I'm pickin' up back titrations
She's prayin' for neutralizations
Dew drop drop, good titrations . . .

The Geologic Time Scale and Gravimetric Analysis



Layers of rock exposed in the Grand Canyon provide a window on Earth's history. [Left: Adapted from F. Press, R. Siever, J. Gratzinger, and T. H. Jordan. *Understanding Earth*, 4th ed. (New York: W. H. Freeman and Company, 2004). Right: Carol Polich/Lonely Planet Images.]



In the 1800s, geologists understood that new layers (*strata*) of rock are deposited on top of older layers. Characteristic fossils in each layer helped geologists to identify strata from the same geologic era all around the world. However, the age of each layer was unknown.

Work by E. Rutherford, F. Soddy, and B. Boltwood suggested that a uranium atom decays to one atom of Pb and eight atoms of He with a half-life of 4.5 billion years. Rutherford realized that measuring the fraction of U converted to decay products gives the age of a rock. Boltwood and R. Strutt began such measurements.

In 1910, Arthur Holmes was a 20-year-old student of Strutt studying geology at Imperial College in London. Holmes conjectured that when a U-containing mineral crystallized, it should be free of Pb. Once the mineral solidified, Pb would begin to accumulate. The ratio Pb/U is a "clock" giving the age of the mineral.

Holmes isolated U minerals from a "Devonian"-age rock. He measured U by the rate of production of radioactive Rn gas. To measure Pb, he dissolved each mineral in molten borax, poured the fused mass into acid, and quantitatively precipitated milligrams of PbSO_4 . The consistent ratio $\text{Pb}/\text{U} = 0.045 \text{ g/g}$ in 15 minerals supported the hypotheses that Pb is the end product of radioactive decay and that little Pb had been present when the minerals crystallized. The calculated age of the minerals was 370 million years. Previously, the most accepted estimate of Earth's age—from Lord Kelvin—was 100 million years.

Geologic ages deduced by Holmes in 1911

Geologic period	Pb/U (mg/g)	Millions of years	Today's accepted value
Carboniferous	41	340	330–362
Devonian	45	370	362–380
Silurian	53	430	418–443
Precambrian	125–200	1 025–1 640	900–2 500

From C. Lewis, *The Dating Game* (Cambridge: Cambridge University Press, 2000); A. Holmes, *Proc. R. Soc. Lond. A* 1911, 85, 248.

Gravimetric and Combustion Analysis

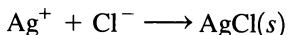
In **gravimetric analysis**, the mass of a product is used to calculate the quantity of original analyte. In the early 1900s, Nobel Prize-winning gravimetric analysis by T. W. Richards and his colleagues measured the atomic mass of Ag, Cl, and N to six-figure accuracy and formed the basis for accurate atomic mass determinations. In **combustion analysis**, a sample is burned in excess oxygen and the products are measured. Combustion is typically used to measure C, H, N, S, and halogens in organic compounds.

Gravimetric analysis has been largely replaced by instrumental methods of analysis, which are faster and less labor intensive. However, gravimetric determinations executed by a skilled analyst remain among the most accurate methods available to produce standards for instrumental analysis. Students are still exposed to some gravimetric analysis early in their laboratory career because gravimetric procedures demand excellent laboratory technique to produce accurate and precise results.

7-1 Examples of Gravimetric Analysis

An important industrial gravimetric analysis is the Rose-Gottlieb method for measuring fat in food. First, a weighed sample is dissolved in an appropriate manner to solubilize protein. Ammonia and ethanol are then added to break up microscopic droplets of fat, which are extracted into an organic solvent. Proteins and carbohydrates remain in the aqueous phase. After the organic phase has been separated from the aqueous phase, the organic phase is evaporated to dryness at 102°C and the dried residue is weighed. The residue consists of fats from the food.

A simple gravimetric analysis that you might encounter is the determination of Cl^- by precipitation with Ag^+ :

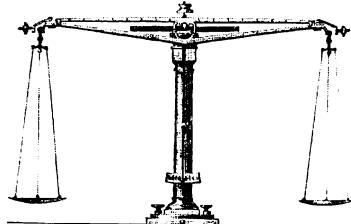


The mass of AgCl product tells us how many moles of AgCl were produced. For every mole of AgCl produced, there must have been 1 mol of Cl^- in the unknown solution.

Example A Simple Gravimetric Calculation

A 10.00-mL solution containing Cl^- was treated with excess AgNO_3 to precipitate 0.436 8 g of AgCl (FM 143.321). What was the molarity of Cl^- in the unknown?

Gravimetric procedures were the mainstay of chemical analyses of ores and industrial materials in the eighteenth and nineteenth centuries, long before the chemical basis for the procedures was understood.



Nineteenth-century balance reproduced from Fresenius' *Quantitative Chemical Analysis*, 2nd American ed., 1881.

SOLUTION A precipitate weighing 0.463 g contains

$$\frac{0.463 \text{ g AgCl}}{143.321 \text{ g AgCl/mol AgCl}} = 3.048 \times 10^{-3} \text{ mol AgCl}$$

Because 1 mol of AgCl contains 1 mol of Cl⁻, there must have been 3.048×10^{-3} mol of Cl⁻ in the unknown. The molarity of Cl⁻ in the unknown is therefore

$$[\text{Cl}^-] = \frac{3.048 \times 10^{-3} \text{ mol}}{0.010 \text{ L}} = 0.304 \text{ M}$$

 **Test Yourself** A 25.00-mL solution containing NaCl plus KCl was treated with excess AgNO₃ to precipitate 0.436 g AgCl. What was the molarity of Cl⁻? (Answer: 0.121 9 M)

Table 7-1 Representative gravimetric analyses

Species analyzed	Precipitated form	Form weighed	Some interfering species
K ⁺	KB(C ₆ H ₅) ₄	KB(C ₆ H ₅) ₄	NH ₄ ⁺ , Ag ⁺ , Hg ²⁺ , Tl ⁺ , Rb ⁺ , Cs ⁺
Mg ²⁺	Mg(NH ₄)PO ₄ · 6H ₂ O	Mg ₂ P ₂ O ₇	Many metals except Na ⁺ and K ⁺
Ca ²⁺	CaC ₂ O ₄ · H ₂ O	CaCO ₃ or CaO	Many metals except Mg ²⁺ , Na ⁺ , K ⁺
Ba ²⁺	BaSO ₄	BaSO ₄	Na ⁺ , K ⁺ , Li ⁺ , Ca ²⁺ , Al ³⁺ , Cr ³⁺ Fe ³⁺ , Sr ²⁺ , Pb ²⁺ , NO ₃ ⁻
Cr ³⁺	PbCrO ₄	PbCrO ₄	Ag ⁺ , NH ₄ ⁺
Mn ²⁺	Mn(NH ₄)PO ₄ · H ₂ O	Mn ₂ P ₂ O ₇	Many metals
Fe ³⁺	Fe(HCO ₃) ₃	Fe ₂ O ₃	Many metals
Co ²⁺	Co(1-nitroso-2-naphthoate) ₂	CoSO ₄ (by reaction with H ₂ SO ₄)	Fe ³⁺ , Pd ²⁺ , Zr ⁴⁺
Ni ²⁺	Ni(dimethylglyoximate) ₂	Same	Pd ²⁺ , Pt ²⁺ , Bi ³⁺ , Au ³⁺
Cu ²⁺	CuSCN (after reduction to Cu ⁺)	CuSCN	NH ₄ ⁺ , Pb ²⁺ , Hg ²⁺ , Ag ⁺
Zn ²⁺	Zn(NH ₄)PO ₄ · H ₂ O	Zn ₂ P ₂ O ₇	Many metals
Al ³⁺	Al(8-hydroxyquinolate) ₃	Same	Many metals
Sn ⁴⁺	Sn(cupferron) ₄	SnO ₂	Cu ²⁺ , Pb ²⁺ , As(III)
Pb ²⁺	PbSO ₄	PbSO ₄	Ca ²⁺ , Sr ²⁺ , Ba ²⁺ , Hg ²⁺ , Ag ⁺ , HCl, HNO ₃
NH ₄ ⁺	NH ₄ B(C ₆ H ₅) ₄	NH ₄ B(C ₆ H ₅) ₄	K ⁺ , Rb ⁺ , Cs ⁺
Cl ⁻	AgCl	AgCl	Br ⁻ , I ⁻ , SCN ⁻ , S ²⁻ , S ₂ O ₃ ²⁻ , CN ⁻
Br ⁻	AgBr	AgBr	Cl ⁻ , I ⁻ , SCN ⁻ , S ²⁻ , S ₂ O ₃ ²⁻ , CN ⁻
I ⁻	AgI	AgI	Cl ⁻ , Br ⁻ , SCN ⁻ , S ²⁻ , S ₂ O ₃ ²⁻ , CN ⁻
SCN ⁻	CuSCN	CuSCN	NH ₄ ⁺ , Pb ²⁺ , Hg ²⁺ , Ag ⁺
CN ⁻	AgCN	AgCN	Cl ⁻ , Br ⁻ , I ⁻ , SCN ⁻ , S ²⁻ , S ₂ O ₃ ²⁻
F ⁻	(C ₆ H ₅) ₃ SnF	(C ₆ H ₅) ₃ SnF	Many metals (except alkali metals), SiO ₄ ⁴⁻ , CO ₃ ²⁻
ClO ₄ ⁻	KClO ₄	KClO ₄	
SO ₄ ²⁻	BaSO ₄	BaSO ₄	Na ⁺ , K ⁺ , Li ⁺ , Ca ²⁺ , Al ³⁺ , Cr ³⁺ , Fe ³⁺ , Sr ²⁺ , Pb ²⁺ , NO ₃ ⁻
PO ₄ ³⁻	Mg(NH ₄)PO ₄ · 6H ₂ O	Mg ₂ P ₂ O ₇	Many metals except Na ⁺ , K ⁺
NO ₃ ⁻	Nitron nitrate	Nitron nitrate	ClO ₄ ⁻ , I ⁻ , SCN ⁻ , CrO ₄ ²⁻ , ClO ₃ ⁻ , NO ₂ ⁻ , Br ⁻ , C ₂ O ₄ ²⁻

Table 7-2 Common organic precipitating agents

Name	Structure	Some ions precipitated
Dimethylglyoxime		Ni^{2+} , Pd^{2+} , Pt^{2+}
Cupferron		Fe^{3+} , VO_2^+ , Ti^{4+} , Zr^{4+} , Ce^{4+} , Ga^{3+} , Sn^{4+}
8-Hydroxyquinoline (oxine)		Mg^{2+} , Zn^{2+} , Cu^{2+} , Cd^{2+} , Pb^{2+} , Al^{3+} , Fe^{3+} , Bi^{3+} , Ga^{3+} , Th^{4+} , Zr^{4+} , UO_2^{2+} , TiO^{2+}
1-Nitroso-2-naphthol		Co^{2+} , Fe^{3+} , Pd^{2+} , Zr^{4+}
Nitron		NO_3^- , ClO_4^- , BF_4^- , WO_4^{2-}
Sodium tetraphenylborate	$\text{Na}^+\text{B}(\text{C}_6\text{H}_5)_4^-$	K^+ , Rb^+ , Cs^+ , NH_4^+ , Ag^+ , organic ammonium ions
Tetr phenyl arsonium chloride	$(\text{C}_6\text{H}_5)_4\text{As}^+\text{Cl}^-$	$\text{Cr}_2\text{O}_7^{2-}$, MnO_4^- , ReO_4^- , MoO_4^{2-} , WO_4^{2-} , ClO_4^- , I_3^-

Representative analytical precipitations are listed in Table 7-1. Potentially interfering substances listed in the table may need to be removed prior to analysis. A few common organic **precipitants** (agents that cause precipitation) are listed in Table 7-2. For the determination of Cl^- , Ag^+ is the precipitant.

For those who are not familiar with drawing structures of organic compounds, Box 7-1 provides a primer.

Ask Yourself

7-A. A 50.00-mL solution containing NaBr was treated with excess AgNO_3 to precipitate 0.214 g of AgBr (FM 187.772).

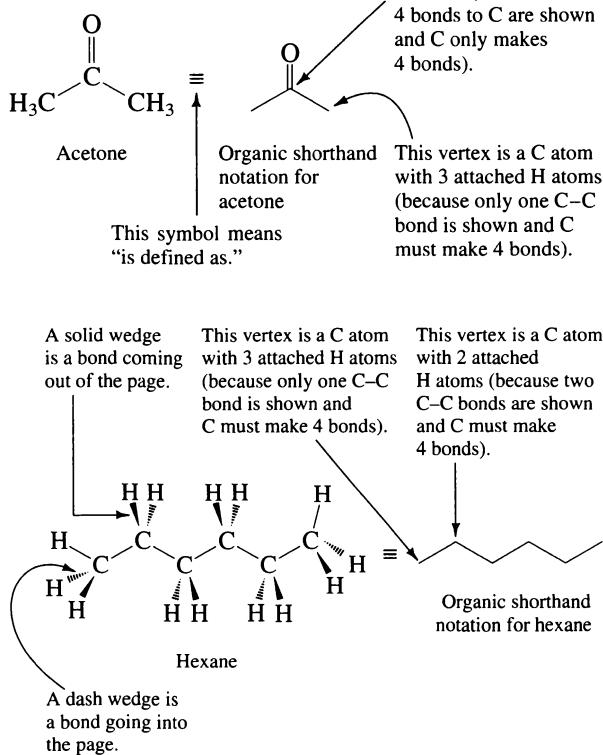
- (a) How many moles of AgBr product were isolated?
- (b) What was the molarity of NaBr in the solution?

7-2 Precipitation

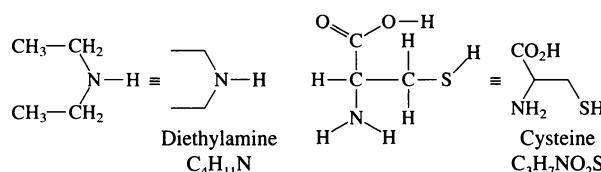
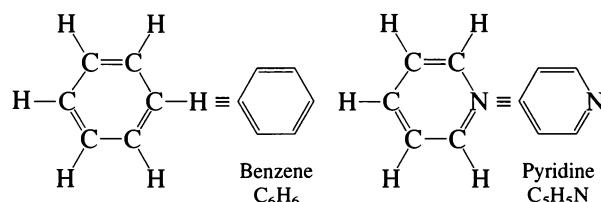
The ideal gravimetric precipitate should be insoluble, be easily filtered, and possess a known, constant composition. The precipitate should be stable when you heat it to remove the last traces of solvent. Although few substances meet these requirements, techniques described in this section help optimize the properties of precipitates.

Box 7-1 Shorthand for Organic Structures

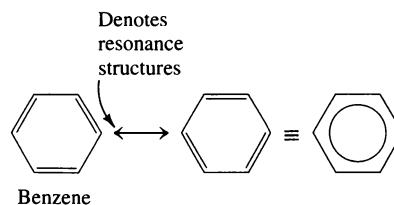
Chemists and biochemists use simple conventions for drawing structures of carbon-containing compounds to avoid drawing every atom. Each vertex of a structure is understood to be a carbon atom, unless otherwise labeled. In the shorthand, we usually omit bonds from carbon to hydrogen. Carbon forms four chemical bonds. If you see carbon forming fewer than four bonds, the remaining bonds are assumed to go to hydrogen atoms that are not written. Here are some examples:



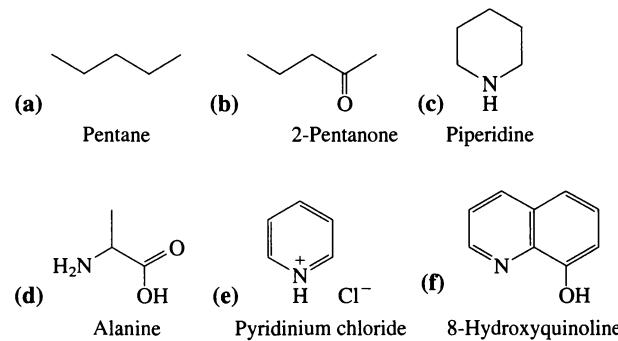
Atoms other than carbon and hydrogen are always shown. Hydrogen atoms attached to atoms other than carbon are always shown. Oxygen and sulfur normally make two bonds. Nitrogen makes three bonds if it is neutral and four bonds if it is a cation. Here are some examples:



Because of the two equivalent resonance structures of a benzene ring, the alternating single and double bonds are often replaced by a circle:



Exercise: Write the chemical formula (such as $\text{C}_4\text{H}_8\text{O}$) for each structure below.



Answers: (a) C_5H_{12} ; (b) $\text{C}_5\text{H}_{10}\text{O}$; (c) $\text{C}_7\text{H}_{11}\text{N}$; (d) $\text{C}_3\text{H}_7\text{NO}_2$; (e) $\text{C}_5\text{H}_6\text{NCl}$; (f) $\text{C}_9\text{H}_7\text{NO}$

Precipitate particles should be large enough to be collected by filtration; they should not be so small that they either clog or pass through the filter. Large crystals also have less surface area to which foreign species may become attached. At the other extreme is a *colloid*, whose particles are so small ($\sim 1\text{--}500\text{ nm}$) that they pass through most filters (Figure 7-1 and Demonstration 7-1).

Crystal Growth

Crystallization occurs in two phases: *nucleation* and *particle growth*. In **nucleation**, dissolved molecules or ions form small crystalline aggregates capable of growing into larger particles. Nucleation tends to occur on preexisting surfaces that attract and hold solutes. Traces of insoluble particles in a liquid or scratches on a glass surface are potentially capable of initiating nucleation. In **particle growth**, solute molecules or ions add to an existing aggregate to form a crystal.

A solution containing more dissolved solute than should be present at equilibrium is said to be **supersaturated**. For example, the solubility product for BaSO_4 is 1.1×10^{-10} . A saturated solution of BaSO_4 contains $[\text{Ba}^{2+}] = [\text{SO}_4^{2-}] = 1.05 \times 10^{-5}\text{ M}$. A supersaturated solution containing $[\text{Ba}^{2+}] = [\text{SO}_4^{2-}] = 2.1 \times 10^{-5}\text{ M}$ has twice as much of each ion as would be present at equilibrium. It is possible for supersaturated solutions to have many times the equilibrium concentrations (for example, 100 times). Over time, ions come together to form nuclei of a critical size, and crystal growth occurs on these nuclei to create larger crystals. Eventually, the concentrations of dissolved ions are reduced to their equilibrium values.

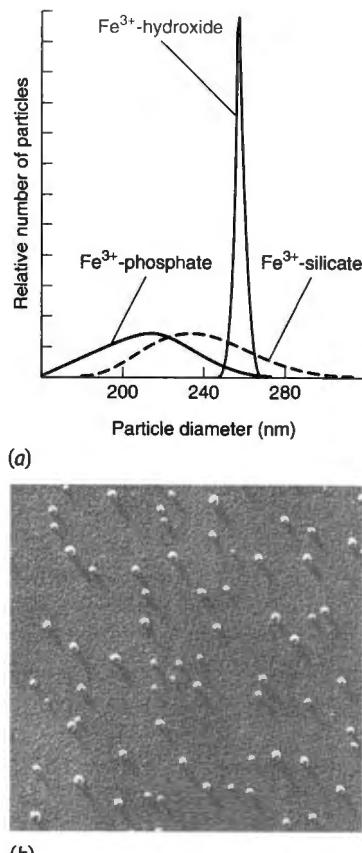
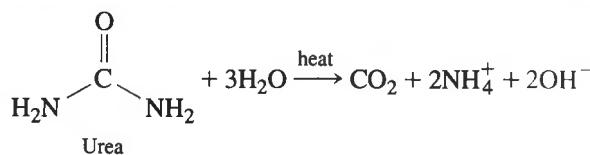
In a highly supersaturated solution, nucleation proceeds faster than particle growth. Many nuclei form before there is time for crystal growth to create larger particles. Therefore highly supersaturated solutions create tiny particles. In less concentrated solution, nucleation is slower, so nuclei have a chance to grow into larger, more tractable particles. Techniques that promote particle growth include

1. Raising the temperature to increase solubility and thereby decrease supersaturation
2. Adding precipitant slowly with vigorous mixing to avoid high local supersaturation where the stream of precipitant first enters the analyte
3. Keeping the volume of solution large so that the concentrations of analyte and precipitant are low

Homogeneous Precipitation

In the discussion so far, precipitation is carried out by mixing a solution of precipitant with a solution of analyte. In **homogeneous precipitation**, precipitant is generated slowly from within an initially homogeneous solution by a chemical reaction. When precipitation is slow, particle growth dominates over nucleation to give larger, purer particles that are easier to filter. When precipitation is rapid, nucleation tends to dominate over crystallization and the resulting particles are small and hard to filter.

An example of homogeneous precipitation is the slow formation of Fe(III) formate from a solution of Fe(III) plus formic acid. Precipitation is initiated by decomposing urea in boiling water to slowly produce OH^- :



(b)

Figure 7-1 (a) Particle-size distribution of colloids formed when FeSO_4 was oxidized to Fe^{3+} in 10^{-4} M OH^- in the presence of phosphate (PO_4^{3-}), silicate (SiO_4^{4-}), or no added anions. [From M. L. Magnuson, D. A. Lytle, C. M. Frietch, and C. A. Kelty, *Anal. Chem.* **2001**, 73, 4815.]

(b) Electron micrograph of 7-nm-diameter colloidal particles of $[\text{Fe}(\text{OH})_{2.5}(\text{NO}_3)_{0.5}]_{1000}$ made by treating ferric nitrate with 2HCO_3^- per Fe^{3+} . [From T. G. Spiro, S. E. Allerton, J. Renner, A. Terzis, R. Bils, and P. Saltman, *J. Am. Chem. Soc.* **1966**, 88, 2721.]



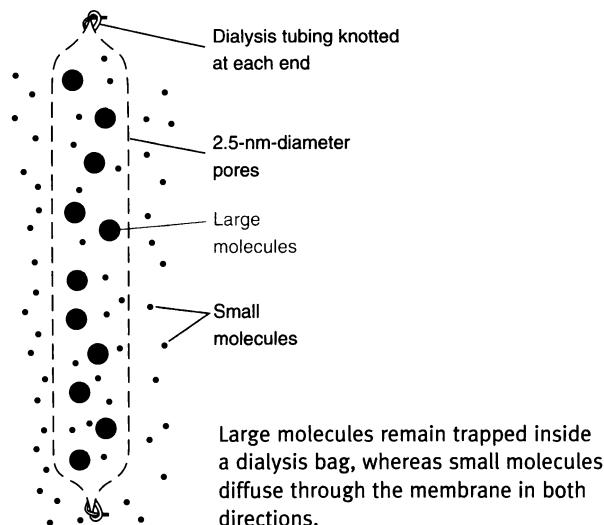
Demonstration 7-1 Colloids, Dialysis, and Microdialysis

Colloids are particles with diameters in the approximate range 1–500 nm. They are larger than molecules, but too small to precipitate. Colloids remain in solution indefinitely, suspended by the Brownian motion (random movement) of solvent molecules.

To make a colloid, heat a beaker containing 200 mL of distilled water to 70°–90°C and leave an identical beaker of water at room temperature. Add 1 mL of 1 M FeCl_3 to each beaker and stir. The warm solution turns brown-red in a few seconds, whereas the cold solution remains yellow (Color Plate 2a). The yellow color is characteristic of low-molecular-mass Fe^{3+} compounds. The red color results from colloidal aggregates of Fe^{3+} ions held together by hydroxide, oxide, and some chloride ions. These particles have a molecular mass of $\sim 10^5$ and a diameter of ~ 10 nm and contain $\sim 10^3$ atoms of Fe.

To demonstrate the size of colloidal particles, we perform a **dialysis** experiment in which two solutions are separated by a *semipermeable membrane*. The membrane has pores through which small molecules, but not large molecules and colloids, can diffuse. Celulose dialysis tubing (such as catalog number 3787 from A. H. Thomas Co.) has 1- to 5-nm pores.

Pour some of the brown-red colloidal Fe solution into a dialysis tube knotted at one end; then tie off the other end. Drop the tube into a flask of distilled water to show that the color remains entirely within the bag even after several days (Color Plates 2b and 2c). For



comparison, leave an identical bag containing a dark blue solution of 1 M $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in another flask. Cu^{2+} diffuses out of the bag, and the solution in the flask becomes light blue in 24 h. Alternatively, the yellow food coloring, tartrazine, can be used in place of Cu^{2+} . If dialysis is conducted in hot water, it is completed in one class period.

Dialysis is used to treat patients suffering from kidney failure. Blood is run over a dialysis membrane having a very large surface area. Small metabolic waste products in the blood diffuse across the membrane and are diluted

The OH^- reacts with formic acid to produce formate, which precipitates Fe(III):

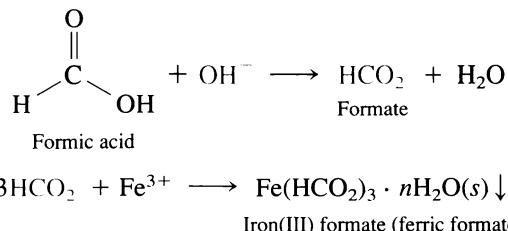


Table 7-3 lists several common reagents for homogeneous precipitation.

Precipitation in the Presence of Electrolyte

An **electrolyte** is a compound that dissociates into ions when it dissolves. We say that the electrolyte ionizes when it dissolves.

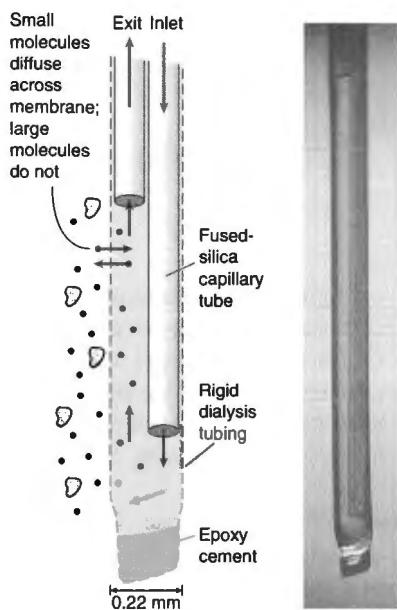
Ionic compounds are usually precipitated in the presence of added **electrolyte**. To understand why, consider how tiny crystallites *coagulate* (come together) into larger crystals. We will illustrate the case of AgCl , which is commonly formed in the presence of 0.1 M HNO_3 .

into a large volume of liquid going out as waste. Large proteins, which are a necessary part of the blood plasma, cannot cross the membrane and are retained in the blood.

Microdialysis

A *microdialysis probe* is used in biology to sample small molecules in fluids without contamination by large molecules, such as proteins. For example, a probe

made of a thin, rigid semipermeable tube can be inserted into the brain of an anesthetized rat to collect neurotransmitter molecules. Fluid pumped through the probe at a rate of 3 $\mu\text{L}/\text{min}$ transports small molecules that diffused into the probe. Small molecules in the fluid exiting the probe (*dialysate*) are monitored by liquid chromatography (Chapter 22) or capillary electrophoresis (Chapter 23).



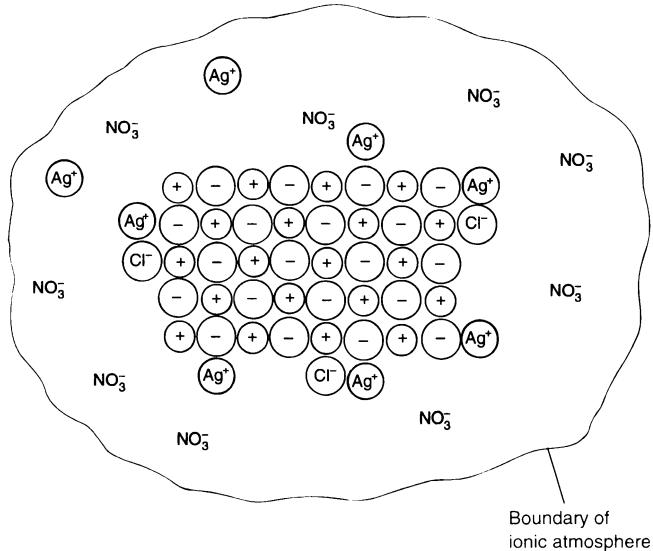
Microdialysis probe. Small molecules pass through the semipermeable membrane, but large molecules cannot. [Courtesy R. T. Kennedy and Z. D. Sandlin, University of Michigan.]

Table 7-3 Common reagents for homogeneous precipitation

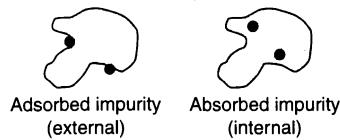
Precipitant	Reagent	Reaction	Some elements precipitated
OH	Urea	$(\text{H}_2\text{N})_2\text{CO} + 3\text{H}_2\text{O} \longrightarrow \text{CO}_2 + 2\text{NH}_4^+ + 2\text{OH}^-$	Al, Ga, Th, Bi, Fe, Sn
S^2-	Thioacetamide ^a	$\text{CH}_3\overset{\text{S}}{\underset{\parallel}{\text{C}}} \text{NH}_2 + \text{H}_2\text{O} \longrightarrow \text{CH}_3\overset{\text{O}}{\underset{\parallel}{\text{C}}} \text{NH}_2 + \text{H}_2\text{S}$	Sb, Mo, Cu, Cd
SO_4^{2-}	Sulfamic acid	$\text{H}_3\overset{+}{\text{NSO}_3^-} + \text{H}_2\text{O} \longrightarrow \text{NH}_4^+ + \text{SO}_4^{2-} + \text{H}^+$	Ba, Ca, Sr, Pb
$\text{C}_2\text{O}_4^{2-}$	Dimethyl oxalate	$\text{CH}_3\overset{\text{OO}}{\underset{\parallel}{\text{C}}} \text{COCH}_3 + 2\text{H}_2\text{O} \longrightarrow 2\text{CH}_3\text{OH} + \text{C}_2\text{O}_4^{2-} + 2\text{H}^+$	Ca, Mg, Zn
PO_4^{3-}	Trimethyl phosphate	$(\text{CH}_3\text{O})_3\text{P}=\text{O} + 3\text{H}_2\text{O} \longrightarrow 3\text{CH}_3\text{OH} + \text{PO}_4^{3-} + 3\text{H}^+$	Zr, Hf

a. Hydrogen sulfide is volatile and toxic; it should be handled only in a well-ventilated hood. Thioacetamide is a carcinogen that should be handled with gloves. If thioacetamide contacts your skin, wash yourself thoroughly immediately. Leftover reagent is destroyed by heating at 50°C with 5 mol of NaOCl per mole of thioacetamide and then washing the products down the drain.

Figure 7-2 Colloidal particle of AgCl in a solution containing excess Ag^+ , H^+ , and NO_3^- . The particle has a net positive charge because of adsorbed Ag^+ ions. The region of solution surrounding the particle is called the *ionic atmosphere*. It has a net negative charge, because the particle attracts anions and repels cations.



Although it is common to find the excess common ion adsorbed on the crystal surface, it is also possible to find other ions selectively adsorbed. In the presence of citrate and sulfate, there is more citrate than sulfate adsorbed on $\text{BaSO}_4(s)$.



Adsorbed impurity (external)

Adsorbed impurity (internal)

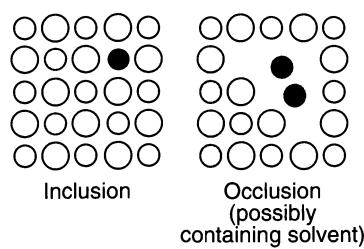
Figure 7-2 shows a colloidal particle of AgCl growing in a solution containing excess Ag^+ , H^+ , and NO_3^- . The particle has an excess positive charge due to **adsorption** of extra silver ions on exposed chloride ions. (To be adsorbed means to be attached to the surface. In contrast, **absorption** entails penetration beyond the surface, to the inside.) The positively charged surface of the solid attracts anions and repels cations from the liquid surrounding the particle, thereby creating a negatively charged *ionic atmosphere* around the particle.

Colloidal particles must collide with one another to coalesce. However, the negatively charged ionic atmospheres of the particles repel one another. Particles must have enough kinetic energy to overcome electrostatic repulsion before they can coalesce. Heating promotes coalescence by increasing the particles' kinetic energy.

Increasing electrolyte concentration (HNO_3 for AgCl) decreases the thickness of the ionic atmosphere and allows particles to approach closer together before repulsion becomes significant. Therefore most gravimetric precipitations are done in the presence of electrolyte.

Digestion

Mother liquor is the solution from which a substance crystallized.



Digestion is the process of allowing a precipitate to stand in contact with the *mother liquor* for some period of time, usually with heating. Digestion promotes slow recrystallization of the precipitate. Particle size increases and impurities tend to be expelled from the crystal.

Purity

Adsorbed impurities are bound to the surface of a crystal. *Absorbed* impurities (within the crystal) are classified as *inclusions* or *occlusions*. Inclusions are impurity ions that randomly occupy sites in the crystal lattice normally occupied by ions that belong in the crystal. Inclusions are more likely when the impurity ion

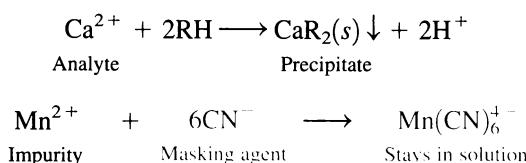
has a size and charge similar to those of one of the ions that belongs to the product. Occlusions are pockets of impurity that are literally trapped inside the growing crystal.

Adsorbed, occluded, and included impurities are said to be **coprecipitated**. That is, the impurity is precipitated along with the desired product, even though the solubility of the impurity has not been exceeded. Coprecipitation tends to be worst in colloidal precipitates (which have a large surface area), such as BaSO_4 , $\text{Al}(\text{OH})_3$, and $\text{Fe}(\text{OH})_3$. Figure 7-3 shows that phosphate coprecipitated with calcium carbonate in coral is proportional to the concentration of phosphate in seawater. By measuring P/Ca in ancient coral, we can infer the concentration of phosphate in the sea at the time the coral lived.

Some procedures call for washing away the mother liquor, redissolving the precipitate, and *reprecipitating* the product. In the second precipitation, the concentration of impurities is lower than in the first precipitation, and the degree of coprecipitation therefore tends to be lower (Table 7-4).

Occasionally, a trace component that is too dilute to be measured is intentionally concentrated by coprecipitation with a major component of the solution. The procedure is called **gathering**, and the precipitate used to collect the trace component is said to be a *gathering agent*. When the precipitate is dissolved in a small volume of solvent, the concentration of the trace component is high enough for accurate analysis.

Some impurities can be treated with a **masking agent**, which prevents them from reacting with the precipitant. In the gravimetric analysis of Be^{2+} , Mg^{2+} , Ca^{2+} , or Ba^{2+} with the reagent *N-p-chlorophenylcinnamohydroxamic acid* (designated RH), impurities such as Ag^+ , Mn^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Fe^{2+} , and Ga^{3+} are kept in solution by excess KCN.



Impurities might collect on the product while it is standing in the mother liquor. This process is called *postprecipitation* and usually entails a supersaturated impurity that does not readily crystallize. An example is the crystallization of magnesium oxalate (MgC_2O_4) on calcium oxalate (CaC_2O_4).

Washing precipitate on a filter removes droplets of liquid containing excess solute. Some precipitates can be washed with water, but many require electrolyte to maintain coherence. For these precipitates, the ions in solution are required to

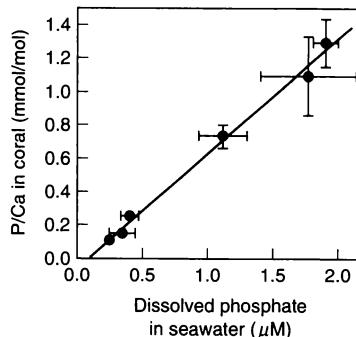
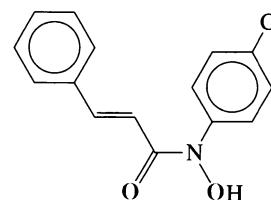


Figure 7-3 Coprecipitation of phosphate with calcium carbonate in coral. By measuring P/Ca in ancient coral, we can infer that the concentration of phosphate in the western Mediterranean Sea 11 200 years ago was more than twice as high as it is today. [Data from P. Montagna, M. McCulloch, M. Taviani, C. Mazzoli, and B. Vendrell, *Science* **2006**, 312, 1788.]

Example of *gathering*: Se(IV) at concentrations as low as 25 ng/L is gathered by coprecipitation with $\text{Fe}(\text{OH})_3$. Precipitate is then dissolved in a small volume of concentrated acid to obtain more concentrated Se(IV) for analysis.



N-p-Chlorophenylcinnamohydroxamic acid (RH)
(The two oxygen atoms that bind to metal ions are bold.)

Table 7-4 Removal of occluded NO_3^- from BaSO_4 by reprecipitation^a

	$[\text{NO}_3^-]/[\text{SO}_4^{2-}]$ in precipitate
Initial precipitate	0.279
First reprecipitation	0.028
Second reprecipitation	0.001

^a Data from H. Bao, *Anal. Chem.* **2006**, 78, 304.

Ammonium chloride, for example, decomposes when it is heated:



neutralize the surface charge of the particles. If electrolyte is washed away with water, charged solid particles repel one another and the product breaks up. This breaking up, called **peptization**, results in loss of product through the filter. Silver chloride peptizes if washed with water, so it is washed with dilute HNO_3 instead. Volatile electrolytes including HNO_3 , HCl , NH_4NO_3 , NH_4Cl , and $(\text{NH}_4)_2\text{CO}_3$ are used for washing because they evaporate during drying.

Product Composition

The final product must have a known, stable composition. A **hygroscopic substance** is one that picks up water from the air and is therefore difficult to weigh accurately. Many precipitates contain a variable quantity of water and must be dried under conditions that give a known (possibly zero) stoichiometry of H_2O .

Ignition (strong heating) is used to change the chemical form of some precipitates that do not have a constant composition after drying at moderate temperatures. For example, $\text{Fe}(\text{HCO}_3)_3 \cdot n\text{H}_2\text{O}$ is ignited at 850°C for 1 h to give Fe_2O_3 , and $\text{Mg}(\text{NH}_4)\text{PO}_4 \cdot 6\text{H}_2\text{O}$ is ignited at 1100°C to give $\text{Mg}_2\text{P}_2\text{O}_7$.

In **thermogravimetric analysis** conducted with commonly available commercial instruments, a sample is heated and its mass is measured as a function of temperature. Figure 7-4 shows how the composition of calcium salicylate changes in four stages:

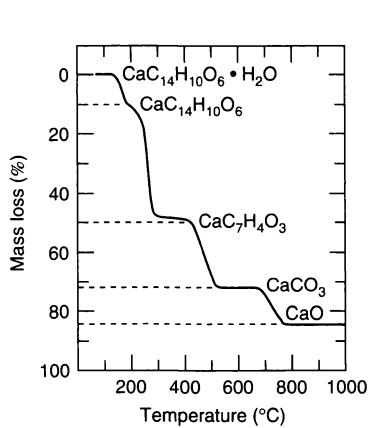
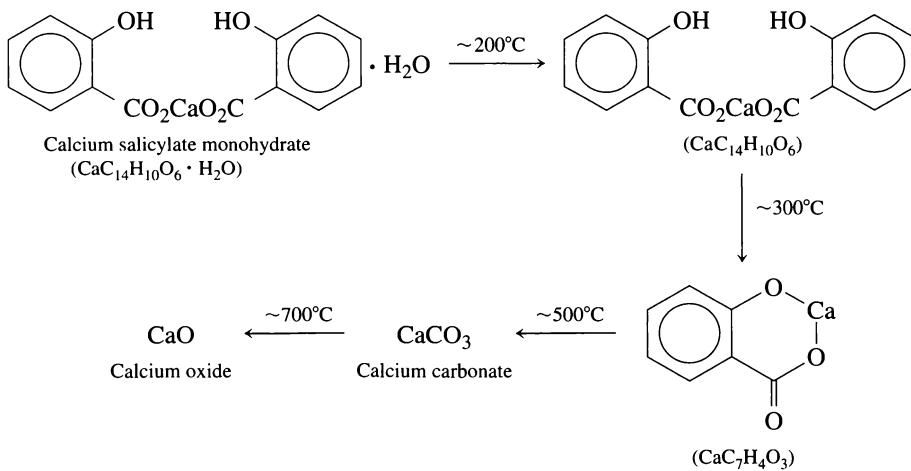


Figure 7-4 Thermogravimetric curve for calcium salicylate. [From G. Liptay, ed., *Atlas of Thermoanalytical Curves* (London: Heyden and Son, 1976).]



The composition of the product depends on the temperature and duration of heating.

Ask Yourself

- 7-B. See if you have digested this section by answering the following questions:
- What is the difference between absorption and adsorption?
 - How is an inclusion different from an occlusion?
 - What are desirable properties of a gravimetric precipitate?

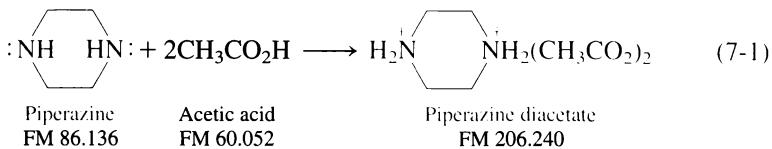
- (d) Why is high supersaturation undesirable in a gravimetric precipitation?
 - (e) How can you decrease supersaturation during a precipitation?
 - (f) Why are many ionic precipitates washed with electrolyte solution instead of pure water?
 - (g) Why is it less desirable to wash AgCl precipitate with aqueous NaNO₃ than with HNO₃ solution?
 - (h) Why would a reprecipitation be employed in a gravimetric analysis?
 - (i) What is done in thermogravimetric analysis?
-

7-3 Examples of Gravimetric Calculations

We now illustrate how to relate the mass of a gravimetric precipitate to the quantity of original analyte. *The general approach is to relate the moles of product to the moles of reactant.*

Example Relating Mass of Product to Mass of Reactant

The piperazine content of an impure commercial material can be determined by precipitating and weighing piperazine diacetate:



If you were performing this analysis, it would be important to determine that impurities in the piperazine are not also precipitated.

In one experiment, 0.312 6 g of sample was dissolved in 25 mL of acetone, and 1 mL of acetic acid was added. After 5 min, the precipitate was filtered, washed with acetone, dried at 110°C, and found to be 0.712 1 g. Find wt% piperazine in the commercial material.

SOLUTION We are asked to find wt% piperazine, which is

$$\text{wt\% piperazine} = \frac{\text{mass of piperazine}}{\text{mass of sample}} \times 100$$

Reminder:

$$\text{wt\%} = \frac{\text{mass of analyte}}{\text{mass of unknown}} \times 100$$

We know the mass of sample (0.312 6 g), so we need to find the mass of piperazine. The experiment gives us the mass of the product piperazine diacetate (0.712 1 g) made from piperazine. How much piperazine is contained in 0.712 1 g of piperazine diacetate? Each mole of piperazine diacetate contains one mole of piperazine in Reaction 7-1. If we compute the moles of piperazine diacetate product, we can find the moles of piperazine in the sample. From the moles of piperazine, we can calculate the grams of piperazine and its weight percent.

$$\begin{aligned} \text{moles of piperazine} &= \text{moles of product} = \frac{0.712 \text{ g product}}{206.240 \frac{\text{g product}}{\text{mol product}}} \\ &= 3.453 \times 10^{-3} \text{ mol} \end{aligned}$$

This many moles of piperazine corresponds to

grams of piperazine

$$= (3.453 \times 10^{-3} \text{ mol piperazine}) \left(86.136 \frac{\text{g piperazine}}{\text{mol piperazine}} \right) = 0.2974 \text{ g}$$

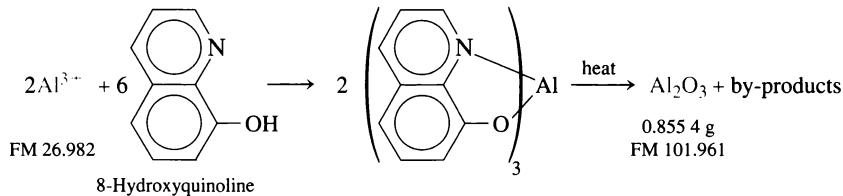
So, of the 0.3126 g sample, 0.2974 g is piperazine. Therefore

$$\text{wt\% piperazine in analyte} = \frac{0.2974 \text{ g piperazine}}{0.3126 \text{ g unknown}} \times 100 = 95.14\%$$

 **Test Yourself** Find wt% piperazine if 0.288 g of commercial product gave 0.555 g of precipitate. (Answer: 80.5%)

Example When the Stoichiometry Is Not 1:1

Solid residue weighing 8.4448 g from an aluminum refining process was dissolved in acid to give Al(III) in solution. The solution was treated with 8-hydroxyquinoline to precipitate $(8\text{-hydroxyquinoline})_3\text{Al}$, which was ignited to give Al_2O_3 weighing 0.8554 g. Find the weight percent of Al in the original mixture.



SOLUTION We are asked to find wt% Al, which is

$$\text{wt\% Al} = \frac{\text{mass of Al}}{\text{mass of sample}} \times 100$$

The mass of sample is 8.4448 g, so we need to find the mass of Al in this sample. Each mole of product (Al_2O_3) contains two moles of Al. The mass of product tells us the moles of product, and from this we can find the moles of Al in the sample. The moles of product are

$$\text{moles of product} = \frac{0.8554 \text{ g } \text{Al}_2\text{O}_3}{101.961 \frac{\text{g } \text{Al}_2\text{O}_3}{\text{mol } \text{Al}_2\text{O}_3}} = 0.0083895 \text{ mol } \text{Al}_2\text{O}_3$$

Because each mole of product contains two moles of Al, there must have been

$$\begin{aligned} \text{moles of Al in unknown} &= \frac{2 \text{ mol Al}}{\text{mol } \text{Al}_2\text{O}_3} \times 0.0083895 \text{ mol } \text{Al}_2\text{O}_3 \\ &= 0.016779 \text{ mol Al} \end{aligned}$$

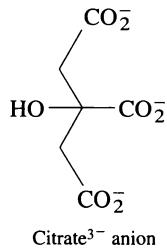
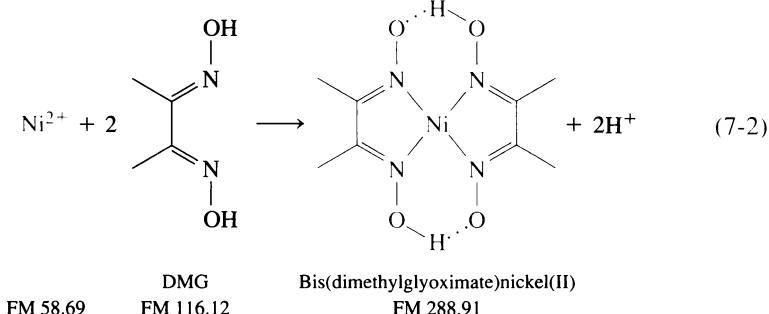
The mass of Al is $(0.016779 \text{ mol})(26.982 \text{ g/mol}) = 0.45273 \text{ g Al}$. The weight percent of Al in the unknown is

$$\text{wt\% Al} = \frac{0.4527 \text{ g Al}}{8.4448 \text{ g unknown}} \times 100 = 5.361\%$$

 **Test Yourself** Residue weighing 10.232 g gave 1.023 g Al_2O_3 after ignition. Find wt% Al in the original residue. (Answer: 5.292%)

Example Calculating How Much Precipitant to Use

(a) To measure the nickel content in steel, the steel is dissolved in 12 M HCl and neutralized in the presence of citrate ion, which binds iron and keeps it in solution. The slightly basic solution is warmed, and dimethylglyoxime (DMG) is added to precipitate the red DMG-nickel complex. The product is filtered, washed with cold water, and dried at 110°C.



If the nickel content is known to be near 3 wt% and we wish to analyze 1.0 g of the steel, what volume of 1.0 wt% DMG in alcohol solution should be used to give a 50% excess of DMG for the analysis? Assume that the density of the alcohol solution is 0.79 g/mL.

Density means

$$\frac{\text{grams of solution}}{\text{milliliters of solution}}$$

SOLUTION Our strategy is to estimate the moles of Ni in 1.0 g of steel. Equation 7-2 tells us that 2 mol of DMG are required for each mole of Ni. After finding the required number of moles of DMG, we will multiply it by 1.5 to get a 50% excess to be sure we have enough.

The Ni content of the steel is about 3%, so 1.0 g of steel contains about $(0.03)(1.0 \text{ g}) = 0.03 \text{ g}$ of Ni, which corresponds to $(0.03 \text{ g Ni})/(58.69 \text{ g/mol Ni}) = 5.1 \times 10^{-4} \text{ mol Ni}$. This amount of Ni requires

$$2 \left(\frac{\text{mol DMG}}{\text{mol Ni}} \right) (5.1 \times 10^{-4} \text{ mol Ni}) \left(116.12 \frac{\text{g DMG}}{\text{mol DMG}} \right) = 0.12 \text{ g DMG}$$

A 50% excess of DMG would be $(1.5)(0.12 \text{ g}) = 0.18 \text{ g}$.

The DMG solution is 1.0 wt%, which means that there are 0.010 g of DMG per gram of solution. The required mass of solution is

1.0 wt% DMG means

$$\frac{1.0 \text{ g DMG}}{100 \text{ g solution}}$$

$$\left(\frac{0.18 \text{ g DMG}}{0.010 \text{ g DMG/g solution}} \right) = 18 \text{ g solution}$$

$$\text{density} = \frac{\text{mass}}{\text{volume}}$$

The volume of solution is found from the mass of solution and the density:

$$\text{volume} = \frac{\text{mass}}{\text{density}} = \frac{18 \text{ g solution}}{0.79 \text{ g solution/mL}} = 23 \text{ mL}$$

(b) If 1.163 4 g of steel gave 0.179 5 g of $\text{Ni}(\text{DMG})_2$ precipitate, what is the weight percent of Ni in the steel?

Strategy:

1. Write balanced reaction.
2. Calculate moles of product from mass of pure product.
3. From balanced reaction, relate moles of unknown (reactant) to moles of product.
4. From moles of unknown, compute mass or wt% or whatever is asked about the unknown.

SOLUTION Here is the strategy: From the mass of precipitate, we find the moles of precipitate. We know that 1 mol of precipitate comes from 1 mol of Ni in Equation 7-2. From the moles of Ni, we compute the mass of Ni and its weight percent in the steel.

First, find the moles of precipitate in 0.179 5 g of precipitate:

$$\frac{0.1795 \text{ g } \text{Ni}(\text{DMG})_2}{288.91 \text{ g } \text{Ni}(\text{DMG})_2/\text{mol } \text{Ni}(\text{DMG})_2} = 6.213 \times 10^{-4} \text{ mol } \text{Ni}(\text{DMG})_2$$

There must have been 6.213×10^{-4} mol of Ni in the steel. The mass of Ni in the steel is $(6.213 \times 10^{-4} \text{ mol Ni})(58.69 \text{ g/mol Ni}) = 0.03646 \text{ g}$, and the wt% Ni in steel is

$$\text{wt\% Ni} = \frac{0.03646 \text{ g Ni}}{1.1634 \text{ g steel}} \times 100 = 3.134\%$$

Figure 7-5 reviews the problem-solving procedure, which should make sense to you now. The point is not to memorize an algorithm, but to assimilate the general approach of working backward from what is known to what is unknown.

 **Test Yourself** If 2.376 g of steel gave 0.402 g of $\text{Ni}(\text{DMG})_2$, what is the wt% Ni in the steel? (Answer: 3.44%)

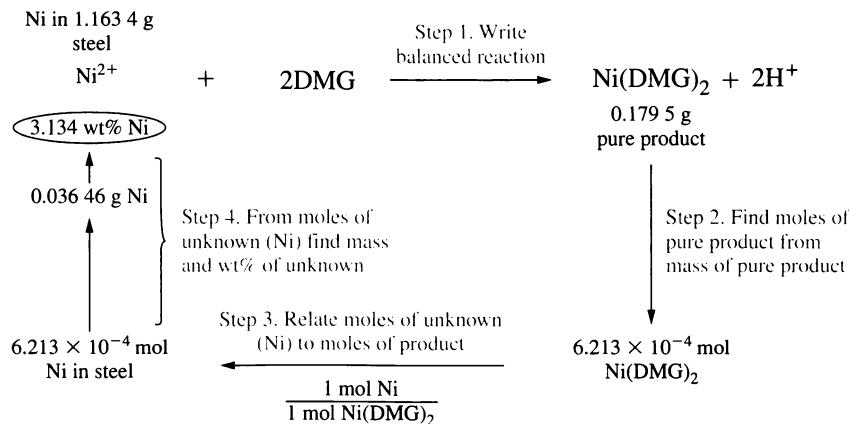
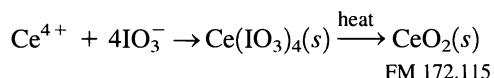


Figure 7-5 Steps taken to find wt% Ni in steel from mass of pure $\text{Ni}(\text{DMG})_2$ product.

Ask Yourself

7-C. The element cerium, discovered in 1839 and named for the asteroid Ceres, is a major component of flint lighters. To find the Ce⁴⁺ content of a solid, an analyst dissolved 4.37 g of the solid and treated it with excess iodate to precipitate Ce(IO₃)₄. The precipitate was collected, washed, dried, and ignited to produce 0.104 g of CeO₂.



- (a) How much cerium is contained in 0.104 g of CeO₂?
- (b) What was the weight percent of Ce in the original solid?

7-4 Combustion Analysis

A historically important form of gravimetric analysis is *combustion analysis*, used to determine the carbon and hydrogen content of organic compounds burned in excess O₂. Modern combustion analyzers use thermal conductivity, infrared absorption, or electrochemical methods to measure the products.

Gravimetric Combustion Analysis

In gravimetric combustion analysis (Figure 7-6), partly combusted product is passed through catalysts such as Pt gauze, CuO, PbO₂, or MnO₂ at elevated temperature to complete the oxidation to CO₂ and H₂O. The products are flushed through a chamber containing P₄O₁₀ (“phosphorus pentoxide”), which absorbs water, and then through a chamber of Ascarite*, which absorbs CO₂. The increase in mass of each chamber

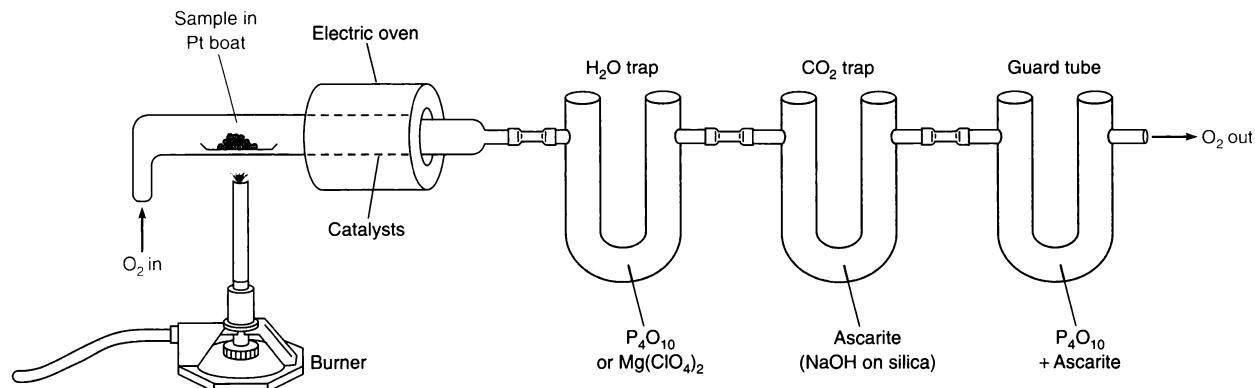


Figure 7-6 Gravimetric combustion analysis for carbon and hydrogen.

*The original Ascarite was NaOH coated on asbestos. Asbestos is no longer used because breathing particles of asbestos can cause a fatal lung disease. In modern products such as Ascarite II®, asbestos is replaced by an inert silica (SiO₂) carrier.

tells how much hydrogen and carbon, respectively, were initially present. A guard tube prevents atmospheric H₂O or CO₂ from entering the chambers from the reverse direction.

Example Combustion Analysis Calculations

A compound weighing 5.714 mg produced 14.414 mg of CO₂ and 2.529 mg of H₂O upon combustion. Find wt% C and H in the sample.

SOLUTION One mole of CO₂ contains one mole of carbon. Therefore

$$\text{moles of C in sample} = \text{moles of CO}_2 \text{ produced}$$

$$= \frac{14.414 \times 10^{-3} \text{ g CO}_2}{44.010 \text{ g CO}_2/\text{mol}} = 3.275 \times 10^{-4} \text{ mol}$$

$$\text{mass of C in sample} = (3.275 \times 10^{-4} \text{ mol C}) \left(12.0107 \frac{\text{g}}{\text{mol C}} \right) = 3.934 \text{ mg}$$

$$\text{wt\% C} = \frac{3.934 \text{ mg C}}{5.714 \text{ mg sample}} \times 100 = 68.84\%$$

One mole of H₂O contains two moles of H. Therefore

$$\text{moles of H in sample} = 2(\text{moles of H}_2\text{O produced})$$

$$= 2 \left(\frac{2.529 \times 10^{-3} \text{ g H}_2\text{O}}{18.015 \text{ g H}_2\text{O/mol}} \right) = 2.808 \times 10^{-4} \text{ mol}$$

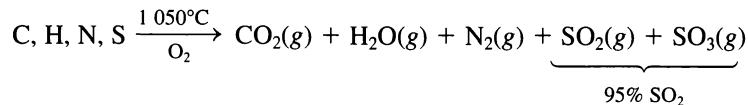
$$\begin{aligned} \text{mass of H in sample} &= (2.808 \times 10^{-4} \text{ mol H}) \left(1.00794 \frac{\text{g}}{\text{mol H}} \right) \\ &= 2.830 \times 10^{-4} \text{ g} \end{aligned}$$

$$\text{wt\% H} = \frac{0.2830 \text{ mg H}}{5.714 \text{ mg sample}} \times 100 = 4.952\%$$

Solution A sample weighing 6.603 mg produced 2.603 mg of H₂O by combustion. Find the wt% H in the sample. (Answer: 4.411%)

Combustion Analysis Today

Figure 7-7 shows how C, H, N, and S are measured in a single operation. An accurately weighed 2-mg sample is sealed in a tin or silver capsule. The analyzer is swept with He gas that has been treated to remove traces of O₂, H₂O, and CO₂. At the start of a run, a measured excess volume of O₂ is added to the He stream. Then the sample capsule is dropped into a preheated ceramic crucible, where the capsule melts and the sample is rapidly oxidized.



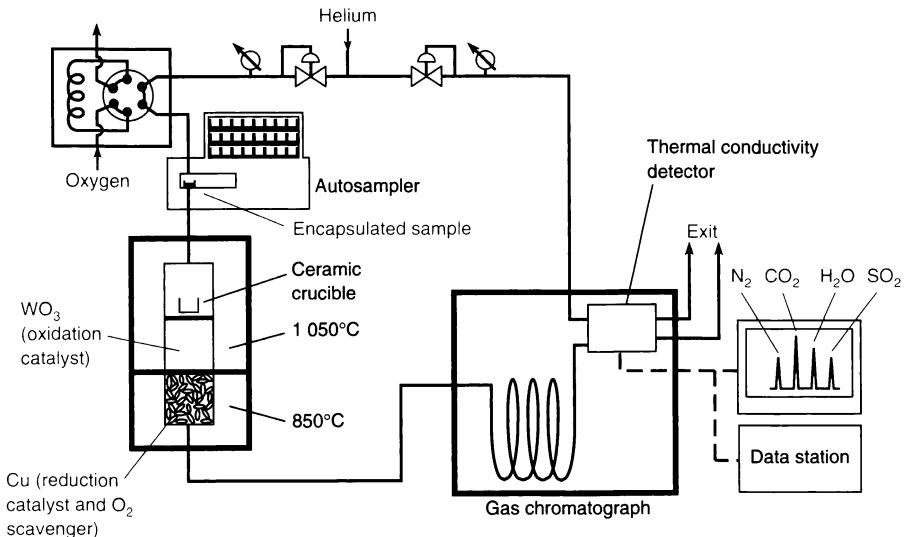
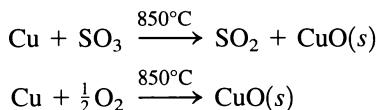


Figure 7-7 Diagram of C, H, N, S elemental analyzer that uses gas chromatographic separation and thermal conductivity detection.
[From E. Pella, *Am. Lab.*, August 1990, p. 28.]

Products pass through a hot WO₃ catalyst to complete the combustion of carbon to CO₂. In the next zone, metallic Cu at 850°C reduces SO₃ to SO₂ and removes excess O₂:



The mixture of CO₂, H₂O, N₂, and SO₂ is separated by gas chromatography, and each component is measured with a thermal conductivity detector described in Section 22-1. Another common instrument uses infrared absorbance to measure CO₂, H₂O, and SO₂ and thermal conductivity to measure N₂.

A key to successful analysis in Figure 7-7 is *dynamic flash combustion*, which creates a short burst of gaseous products, instead of slowly bleeding products out over several minutes. Chromatographic separation requires that the whole sample be injected at once. If a mixture is injected over too long a time, products cannot be separated.

In dynamic flash combustion, the sample is encapsulated in tin and dropped into the preheated furnace shortly after the flow of a 50 vol% O₂/50 vol% He mixture is started. The Sn capsule melts at 235°C and is instantly oxidized to SnO₂, liberating 594 kJ/mol and heating the sample to 1 700°–1 800°C. Because the sample is dropped in before very much O₂ is present, the sample decomposes prior to oxidation, which minimizes the formation of nitrogen oxides. Diversion of nitrogen to form nitrogen oxides instead of N₂ would lead to a low value of nitrogen in the analysis.

Oxygen analysis requires a different strategy. The sample is thermally decomposed (a process called **pyrolysis**) without adding oxygen. The gaseous products are passed through nickel-coated carbon at 1 075°C to convert oxygen from the analyte into CO (not CO₂). Other products include N₂, H₂, CH₄, and hydrogen halides. Acidic products are absorbed by Ascarite II, and the remaining gases are separated and measured by gas chromatography with a thermal conductivity detector.

Elemental analyzers use an *oxidation catalyst* to complete the oxidation of sample and a *reduction catalyst* to carry out any required reduction and to remove excess O₂.

The Sn capsule is oxidized to SnO₂, which

1. Liberates heat to vaporize and crack (decompose) sample
2. Uses available oxygen immediately
3. Ensures that sample oxidation occurs in gas phase
4. Acts as an oxidation catalyst

Table 7-5 Accuracy and precision of combustion analysis of pure compounds^{a,b}

Substance	C	H	N	S
$\text{C}_7\text{H}_9\text{NO}_2\text{S}$ theoretical wt%	49.10	5.30	8.18	18.73
Toluene-4-sulfonamide	49.1 ± 0.63	5.3 ± 0.31	8.2 ± 0.38	18.7 ± 0.89
$\text{C}_4\text{H}_7\text{NO}_2\text{S}$ theoretical wt%	36.07	5.30	10.52	24.08
4-Thiazolidinecarboxylic acid	36.0 ± 0.33	5.3 ± 0.16	10.5 ± 0.16	24.0 ± 0.53
Mean uncertainty for 7 different compounds	± 0.47	± 0.24	± 0.31	± 0.76

a. From R. Companyó, R. Rubio, A. Sahuquillo, R. Boqué, Á Maroto, and J. Riu, *Anal. Bioanal. Chem.* **2008**, 392, 1497.

b. Compounds were analyzed by 33–45 labs each year over six years. First row is theoretical wt% and second row is measured wt%. Uncertainties are 95% confidence intervals.

For halogen analysis, the combustion product contains HX ($\text{X} = \text{Cl}, \text{Br}, \text{I}$). HX is trapped in water and titrated with Ag^+ ions by an automated electrochemical process.

Table 7-5 shows results for two of seven compounds sent to many labs to compare their performance in combustion analysis. For each compound, the first row gives the theoretical wt% for each element and the second row shows the measured wt%. Accuracy is excellent: Mean wt% C, H, N, and S are usually within 0.1 wt% of theoretical values. The 95% confidence intervals for uncertainty for C for the first compound is ± 0.63 wt% and the uncertainty for the second compound is ± 0.33 wt%. The mean uncertainty for C listed in the bottom row of the table for all seven compounds in the study was ± 0.47 wt%. Mean 95% confidence intervals for H, N, and S are ± 0.24 , ± 0.31 , and ± 0.76 wt%, respectively. Chemists consider a result within ± 0.3 wt% of theoretical to be good evidence that the compound has the expected formula. This criterion can be difficult to meet for C and S with a single analysis because the 95% confidence intervals are larger than ± 0.3 .

Ask Yourself

- 7-D. (a) What is the difference between combustion and pyrolysis?
 (b) What is the purpose of the WO_3 and Cu in Figure 7-7?
 (c) Why is tin used to encapsulate a sample for combustion analysis?
 (d) Why is sample dropped into the preheated furnace before the oxygen concentration reaches its peak in dynamic flash combustion?
 (e) What is the balanced equation for the combustion of $\text{C}_8\text{H}_7\text{NO}_2\text{SBrCl}$ in a C,H,N,S elemental analyzer?

Important Terms

absorption	dialysis	hygroscopic substance	peptization
adsorption	digestion	ignition	precipitant
colloid	gathering	masking agent	pyrolysis
combustion analysis	gravimetric analysis	nucleation	supersaturated solution
coprecipitation	homogeneous precipitation	particle growth	thermogravimetric analysis

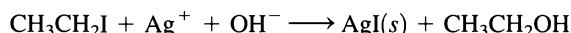
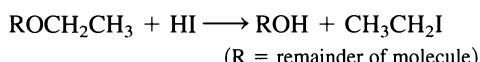
Problems

7-1. BaSO₄ precipitate in Table 7-4 contains occluded nitrate impurity.

(a) What is the difference between occluded, included, and adsorbed impurity?

(b) Why does the ratio $[NO_3^-]/[SO_4^{2-}]$ in the precipitate decrease with each reprecipitation?

7-2. An organic compound with a molecular mass of 417 was analyzed for ethoxyl ($\text{CH}_2\text{CH}_2\text{O}-$) groups by the reactions



A 25.42-mg sample of compound produced 29.03 mg of AgI (FM 234.77). How many ethoxyl groups are there in each molecule?

7-3. A 0.050 02-g sample of impure piperazine contained 71.29 wt% piperazine. How many grams of product will be formed if this sample is analyzed by Reaction 7-1?

7-4. A 1.000-g sample of unknown analyzed by Reaction 7-2 gave 2.500 g of bis(dimethylglyoximate)nickel(II). Find wt% Ni in the unknown.

7-5. How many milliliters of 2.15 wt% dimethylglyoxime solution should be used to provide a 50.0% excess for Reaction 7-2 with 0.9984 g of steel containing 2.07 wt% Ni? The density of the dimethylglyoxime solution is 0.790 g/mL.

7-6. A solution containing 1.263 g of unknown potassium compound was dissolved in water and treated with excess sodium tetraphenylborate, $\text{Na}^+ \text{B}(\text{C}_6\text{H}_5)_4^-$ solution to precipitate 1.003 g of insoluble $\text{K}^+ \text{B}(\text{C}_6\text{H}_5)_4^-$ (FM 358.33). Find wt% K in the unknown.

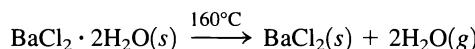
7-7. Twenty dietary iron tablets with a total mass of 22.131 g were ground and mixed thoroughly. Then 2.998 g of the powder were dissolved in HNO_3 and heated to convert all the iron to Fe^{3+} . Addition of NH_3 precipitated $\text{Fe}_2\text{O}_3 \cdot x\text{H}_2\text{O}$, which was ignited to give 0.264 g of Fe_2O_3 (FM 159.69). What is the average mass of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (FM 278.01) in each tablet?

7-8. *Man in the vat.* Once upon a time, a worker at a dye factory fell into a vat containing hot concentrated sulfuric and nitric acids, and he dissolved! Nobody witnessed the accident, so it was necessary to prove that he fell in for his wife to collect insurance money. The man weighed 70 kg, and a human body contains \sim 6.3 parts per thousand phosphorus. Liquid in the vat was analyzed for P to see if it contained a dissolved human.

(a) The vat had 8.00×10^3 L of liquid, and 100.0 mL were analyzed. If the man did fall into the vat, what is the expected quantity of phosphorus in 100.0 mL?

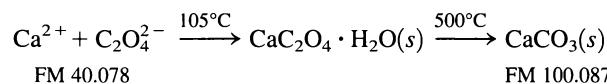
(b) The 100.0-mL sample was treated with molybdate reagent to precipitate ammonium phosphomolybdate, $(\text{NH}_4)_3[\text{P}(\text{Mo}_{12}\text{O}_{40})] \cdot 12\text{H}_2\text{O}$. This substance was dried at 110°C and heated to 400°C to give $\text{P}_2\text{O}_5 \cdot 24\text{MoO}_3$ (FM 3 596.46) weighing 0.371 8 g. A fresh mixture of acids (not from the vat) treated in the same manner gave 0.033 1 g of $\text{P}_2\text{O}_5 \cdot 24\text{MoO}_3$. This *blank determination* tells us the amount of P in starting reagents. $\text{P}_2\text{O}_5 \cdot 24\text{MoO}_3$ that could have come from the dissolved man is therefore $0.371\ 8 - 0.033\ 1 = 0.338\ 7\ \text{g}$. How much P was in the 100.0-mL sample? Is this quantity consistent with a dissolved man?

7-9. Consider a mixture of the two solids, $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ (FM 244.26) and KCl (FM 74.551). When the mixture is heated to 160°C for 1 h, the water of crystallization is driven off:



A sample originally weighing 1.783 g weighed 1.562 g after heating. Calculate wt% Ba, K, and Cl in the original sample. (*Hint:* The mass loss tells how much water was lost, which tells how much $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ was present. The remainder of the sample is KCl.)

7-10. Finely ground mineral (0.632 g) was dissolved in 25 mL of boiling 4 M HCl and diluted with 175 mL H₂O containing two drops of methyl red indicator. The solution was heated to 100°C and 50 mL of warm solution containing 2.0 g (NH₄)₂C₂O₄ were slowly added to precipitate CaC₂O₄. Then 6 M NH₃ was added until the indicator changed from red to yellow, thus showing that the liquid was neutral or slightly basic. After cooling, the liquid was decanted and the solid transferred to a filter crucible and washed with cold 0.1 wt% (NH₄)₂C₂O₄ solution five times until no Cl⁻ was detected in the filtrate on addition of AgNO₃ solution. The crucible was dried at 105°C for 1 h and then at 500°C in a furnace for 2 h.



The empty crucible weighed 18.231 1 g and the crucible with $\text{CaCO}_3(s)$ weighed 18.546 7 g.

(a) Find the wt% Ca in the mineral.

(b) Why is the unknown solution heated to boiling and the precipitant solution, $(\text{NH}_4)_2\text{C}_2\text{O}_4$, also heated before slowly mixing the two?

(c) What is the purpose of washing the precipitate with 0.1 wt% $(\text{NH}_4)_2\text{C}_2\text{O}_4$?

(d) What is the purpose of testing the filtrate with AgNO_3 solution?

7-11. Write a balanced equation for combustion of benzoic acid, $C_6H_5CO_2H$, to give CO_2 and H_2O . How many milligrams

of CO_2 and of H_2O will be produced from 4.635 mg of benzoic acid?

7-12. Combustion of 8.732 mg of an unknown organic compound gave 16.432 mg of CO_2 and 2.840 mg of H_2O .

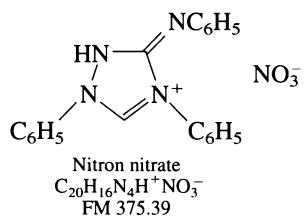
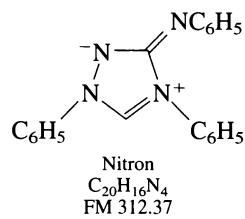
(a) Find the wt% C and H in the substance.

(b) Find the smallest reasonable integer mole ratio of C:H in the compound.

7-13. Combustion of a compound containing just C, H, N, and O showed that it contains 46.21 wt% C, 9.02 wt% H, 13.74 wt% N, and, by difference, $100 - 46.21 - 9.02 - 13.74 = 31.03$ wt% O. That is, 100 g of unknown contain 46.21 g of C, 9.02 g of H, and so on. Find the atomic ratio C:H:N:O. Divide each stoichiometry coefficient by the smallest one and express the composition in the lowest reasonable integer ratio ($\text{C}_x\text{H}_y\text{N}_z\text{O}_w$, where x, y, z, and w are integers and one of them is 1).

7-14. Organic carbon in seawater can be measured by oxidation to CO_2 with $\text{K}_2\text{S}_2\text{O}_8$, followed by gravimetric determination of CO_2 trapped by a column of Ascarite. Water weighing 6.234 g produced 2.378 mg of CO_2 (FM 44.010). Find ppm carbon in the seawater.

7-15. Nitron forms a fairly insoluble salt with nitrate, the product having a solubility of 0.99 g/L near 20°C. SO_4^{2-} and CH_3CO_2^- do not precipitate with nitron, but ClO_4^- , ClO_3^- , I^- , SCN^- , and $\text{C}_2\text{O}_4^{2-}$ do precipitate and interfere with the analysis. A 50.00-mL unknown solution containing KNO_3 and NaNO_3 was treated with 1 mL of acetic acid and heated to near boiling; 10 mL of solution containing excess nitron were added with stirring. After cooling to 0°C for 2 h, the crystalline product was filtered, washed with three 5-mL portions of ice-cold, saturated nitron nitrate solution, followed by two 3-mL portions of ice-cold water. The product weighed 0.513 g after drying at 105°C for 1 h.



- (a) What was the molarity of nitrate in the unknown solution?
 (b) Some of the nitron nitrate dissolves in the final ice-cold wash water. Does this dissolution lead to a random or a systematic error in the analysis?

7-16. A mixture of $\text{Al}_2\text{O}_3(s)$ and $\text{CuO}(s)$ weighing 18.371 mg was heated under $\text{H}_2(g)$ at 1 000°C to give 17.462 mg of $\text{Al}_2\text{O}_3(s) + \text{Cu}(s)$. The other product is $\text{H}_2\text{O}(g)$. Find wt% Al_2O_3 in the original mixture.

7-17. Acetanilide, $\text{C}_6\text{H}_5\text{NHC}(=\text{O})\text{CH}_3$, was found by combustion analysis to contain 71.17 ± 0.41 wt% C, 6.76 ± 0.12 wt% H, and 10.34 ± 0.08 wt% N. Find the uncertainties in the formula $\text{C}_8\text{H}_{h\pm x}\text{N}_{n\pm y}$.

7-18. Sulfur from combustion produces a mixture of SO_2 and SO_3 that can be passed through H_2O_2 to convert both into H_2SO_4 . When 6.123 mg of a substance were burned, the H_2SO_4 required 3.01 mL of 0.015 76 M NaOH for titration by the reaction $\text{H}_2\text{SO}_4 + 2\text{NaOH} \longrightarrow \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}$. Find wt% S in the sample.

7-19. Sulfur in coal that is burned to generate electricity is a major source of acid rain and SO_2 air pollution. In *Eschka's method* to measure S, coal is fused with 5 times its mass of a 2:1 (by mass) mixture of MgO and anhydrous Na_2CO_3 at 800°C for 4 h in air. Sulfur ends up as sulfate (SO_4^{2-}) and sulfite (SO_3^{2-}) salts. The fused mass is dissolved in 6 M HCl and boiled with aqueous Br_2 to oxidize SO_3^{2-} to SO_4^{2-} . Excess Br_2 is evaporated. The solution is adjusted to pH 3 and aqueous BaCl_2 is added to precipitate BaSO_4 , which is filtered, washed, dried by ignition, and weighed.

(a) Fusion of 2.136 g of coal with 10 g of Eschka's mixture of $\text{MgO} + \text{Na}_2\text{CO}_3$ gave 0.352 g BaSO_4 . Find wt% S in the coal.

(b) A power plant burns 8 million metric tons (metric ton = 1 000 kg) of coal annually. If the coal contains an average of 2 wt% S, which is all converted to $\text{SO}_2(g)$, how many tons of SO_2 are produced annually?

(c) The coal leaves 7 wt% ash containing metal oxides. Ash is hazardous because it contains some toxic metals. How many tons of ash are produced annually?

7-20. Some analyte ions can be gathered by LaPO_4 at pH 3 ($[\text{H}^+] = 10^{-3}$ M) (S. Kagaya, M. Saiki, Z. A. Malek, Y. Araki, and K. Hasegawa, *Fresenius J. Anal. Chem.* **2001**, 371, 391.) To 100.0 mL of aqueous sample were added 2 mL of La^{3+} solution (containing 5 mg La^{3+} /mL in 0.6 M HCl) and 0.3 mL of 0.5 M H_3PO_4 . pH was adjusted to 3.0 with NH_3 . Precipitate was collected on a filter with 0.2-μm pore size. The filter, whose volume is <0.01 mL, was placed in a 10-mL volumetric flask and treated with 1 mL of 16 M HNO_3 to dissolve precipitate. The flask was made up to 10 mL with H_2O .

(a) A 100.0-mL distilled water sample was spiked with 10.0 μg of each of the following elements: Fe^{3+} , Pb^{2+} , Cd^{2+} , In^{3+} , Cr^{3+} , Mn^{2+} , Co^{2+} , Ni^{2+} , and Cu^{2+} . Analysis of the final solution in the 10-mL volumetric flask by atomic spectroscopy gave the following concentrations: $[\text{Fe}^{3+}] = 17.6 \mu\text{M}$, $[\text{Pb}^{2+}] = 5.02 \mu\text{M}$, $[\text{Cd}^{2+}] = 8.77 \mu\text{M}$, $[\text{In}^{3+}] = 8.50 \mu\text{M}$, $[\text{Cr}^{3+}] < 0.05 \mu\text{M}$, $[\text{Mn}^{2+}] = 6.64 \mu\text{M}$, $[\text{Co}^{2+}] = 1.09 \mu\text{M}$, $[\text{Ni}^{2+}] < 0.05 \mu\text{M}$, $[\text{Cu}^{2+}] = 6.96 \mu\text{M}$. Find the percent recovery of each element, defined as

$$\% \text{ recovery} = \frac{\mu\text{g found}}{\mu\text{g added}} \times 100$$

(b) Which elements are quantitatively gathered (recovery = 95–105%)?

(c) Analyte is *preconcentrated* by gathering from a dilute sample into a more concentrated solution that is analyzed. How much more concentrated do the analytes become in this procedure?

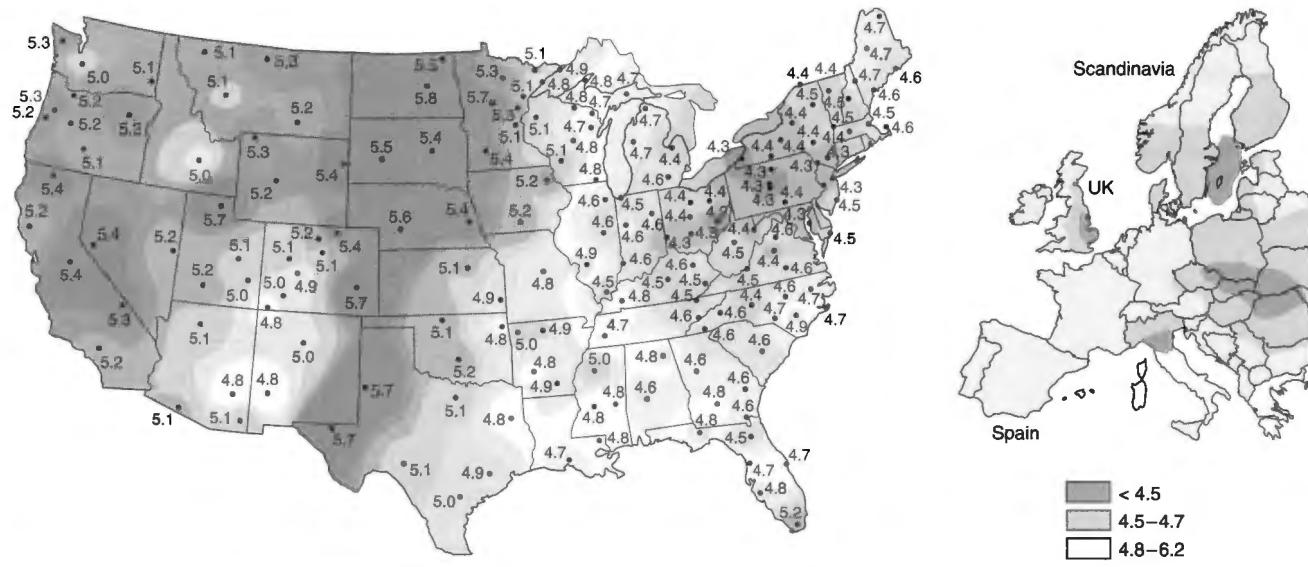
How Would You Do It?

7-21. (a) Fat in homogenized whole milk was measured by the Rose-Gottlieb method described at the beginning of Section 7-1. Are results from the manual and automated methods statistically the same or different?

Weight percent fat in milk		
Manual method		Automated method
2.934	2.925	2.967
2.981	2.948	2.958
2.906	2.981	3.022
2.976	2.913	2.983
2.958	2.881	2.966
2.945	2.847	2.992
2.893	2.880	2.950
		3.006
		2.982
		2.979
		2.951
		3.047

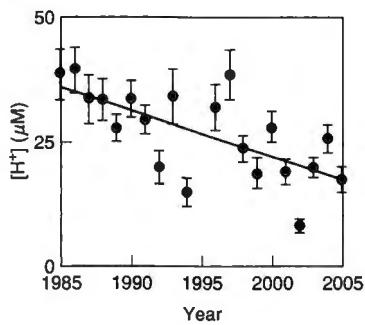
(b) Show that you get the same answer with Excel's built-in *t* test in Section 4-4.

Acid Rain



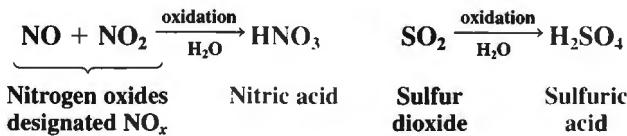
pH of precipitation in the United States in 2001. The lower the pH, the more acidic the water.
[Redrawn from National Atmospheric Deposition Program (NRSP-3)/National Trends Network (2002). Illinois State Water Survey, 2204 Griffith Dr., Champaign, IL 61820.]

pH of rain in Europe. [From H. Rodhe, F. Dentener, and M. Schulz, *Environ. Sci. Technol.* **2002**, 36, 4382.]



Volume-weighted H⁺ concentration in precipitation in Wilmington, NC, decreased by a factor of 2 between 1985 and 2005, typical of the nationwide decrease of rainwater acidity. Error bars are ± 1 standard deviation. [From J. D. Willey, R. J. Kieber, and G. B. Avery, Jr., *Environ. Sci. Technol.* **2006**, 40, 5675.] In volume weighting, [H⁺] in each rainfall is multiplied by the volume of that rainfall to get moles. Total moles for the year are divided by total volume for the year.

Combustion products from automobiles and power plants include nitrogen oxides and sulfur dioxide, which react with oxidizing agents in the atmosphere to produce acids and fall to the Earth as acid rain.



Acid rain in the U.S. is worst in the Northeast, downwind from coal-burning power plants and factories. Rain in portions of Europe and Asia is similarly acidic. Acid rain kills fish and large stands of evergreen forest. Half of the essential nutrients Ca²⁺ and Mg²⁺ have been leached from the soil in Sweden since 1950. Acid increases the solubility of toxic Al³⁺ and other metals in groundwater. U.S. legislation in 1990 to reduce sulfur and nitrogen emissions has decreased the acidity of precipitation, as shown in the graph. However, much damage has been done, and 16 of 50 states increased their SO₂ emissions between 1990 and 2000. Demonstration 8-2 illustrates the chemistry of acid rain.

Introducing Acids and Bases

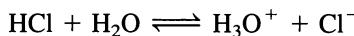
The chemistry of acids and bases is probably the most important topic you will study in chemical equilibrium. It is difficult to have a meaningful discussion of subjects ranging from protein folding to the weathering of rocks without understanding acids and bases. It will take us several chapters to provide meaningful detail to the study of acid-base chemistry.

8-1 What Are Acids and Bases?

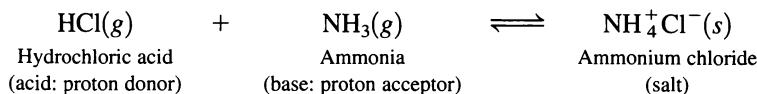
In aqueous chemistry, an **acid** is a substance that increases the concentration of H_3O^+ (**hydronium ion**). Conversely, a **base** decreases the concentration of H_3O^+ in aqueous solution. As we shall see shortly, a decrease in H_3O^+ concentration necessarily requires an increase in OH^- (**hydroxide**) concentration. Therefore a base is also a substance that increases the concentration of OH^- in aqueous solution.

The species H^+ is called a *proton* because a proton is all that remains when a hydrogen atom loses its electron. Hydronium ion, H_3O^+ , is a combination of H^+ with H_2O (Figure 8-1). Although H_3O^+ is a more accurate representation than H^+ for the hydrogen ion in aqueous solution, we will use H_3O^+ and H^+ interchangeably in this book.

A more general definition of acids and bases given by Brønsted and Lowry is that an **acid** is a *proton donor* and a **base** is a *proton acceptor*. This definition includes the one already stated. For example, HCl is an acid because it donates a proton to H_2O to form H_3O^+ :



The Brønsted-Lowry definition can be extended to nonaqueous solvents and to the gas phase:



Salts

Any ionic solid, such as ammonium chloride, is called a **salt**. In a formal sense, a salt can be thought of as the product of an acid-base reaction. When an acid and a base react, they are said to **neutralize** each other. Most salts are **strong electrolytes**,

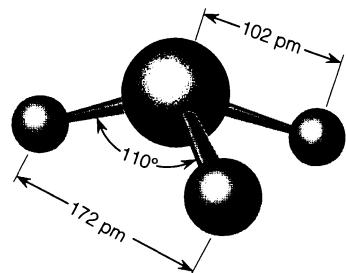


Figure 8-1 Structure of hydronium ion, H_3O^+ .

Brønsted-Lowry acid: proton donor
Brønsted-Lowry base: proton acceptor

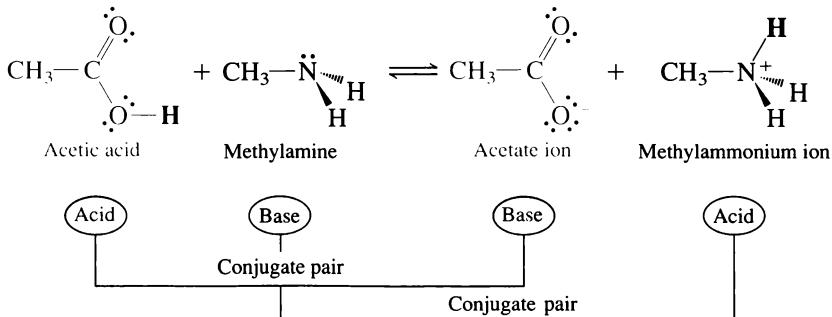
meaning that they dissociate almost completely into their component ions when dissolved in water. Thus, ammonium chloride gives NH_4^+ and Cl^- in aqueous solution:



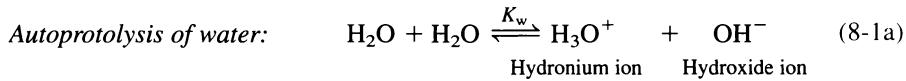
Conjugate Acids and Bases

The products of a reaction between an acid and a base also are acids and bases:

A solid wedge is a bond coming out of the page toward you. A dash wedge is a bond going behind the page. Methylammonium acetate is a *salt* that dissociates to methyl ammonium and acetate ions.



In **autoprolysis** (also called *self-ionization*), one substance acts as both an acid and a base:



We abbreviate Reaction 8-1a in the following manner:



and we designate its equilibrium constant as K_w .

Autoprotolysis constant for water: $K_w = [H^+][OH^-] = 1.0 \times 10^{-14}$ at 25°C (8-2)

Equation 8-2 provides a tool with which we can find the concentration of H^+ and OH^- in pure water. Also, given that the product $[\text{H}^+][\text{OH}^-]$ is constant, we can always find the concentration of either species if the concentration of the other is known. Because the product is a constant, *as the concentration of H^+ increases, the concentration of OH^- necessarily decreases, and vice versa.*

Example Concentration of H^+ and OH^- in Pure Water at 25°C

Calculate the concentrations of H^+ and OH^- in pure water at 25°C.

SOLUTION H^+ and OH^- are produced in a 1:1 mole ratio in Reaction 8-1b. Calling each concentration x , we write

$$K_w = 1.0 \times 10^{-14} = [\text{H}^+][\text{OH}^-] = [x][x] \Rightarrow x = \sqrt{1.0 \times 10^{-14}} \\ = 1.0 \times 10^{-7} \text{ M}$$

The concentrations of H^+ and OH^- are both $1.0 \times 10^{-7} \text{ M}$.

 **Test Yourself** At 0°C, the equilibrium constant K_w has the value 1.2×10^{-15} . Find $[\text{H}^+]$ and $[\text{OH}^-]$ in pure water at 0°C. (**Answer:** both $3.5 \times 10^{-8} \text{ M}$)

Example Finding $[\text{OH}^-]$ When $[\text{H}^+]$ Is Known

What is the concentration of OH^- if $[\text{H}^+] = 1.0 \times 10^{-3} \text{ M}$ at 25°C?

SOLUTION Setting $[\text{H}^+] = 1.0 \times 10^{-3} \text{ M}$ gives

$$K_w = [\text{H}^+][\text{OH}^-] \Rightarrow [\text{OH}^-] = \frac{K_w}{[\text{H}^+]} = \frac{1.0 \times 10^{-14}}{1.0 \times 10^{-3}} = 1.0 \times 10^{-11} \text{ M}$$

When $[\text{H}^+] = 1.0 \times 10^{-3} \text{ M}$, $[\text{OH}^-] = 1.0 \times 10^{-11} \text{ M}$. If $[\text{OH}^-] = 1.0 \times 10^{-3} \text{ M}$, then $[\text{H}^+] = 1.0 \times 10^{-11} \text{ M}$. As one concentration increases, the other decreases.

 **Test Yourself** Find $[\text{H}^+]$ in water when $[\text{OH}^-] = 1.0 \times 10^{-4} \text{ M}$. (**Answer:** $1.0 \times 10^{-10} \text{ M}$)

To simplify the writing of H^+ concentration, we define **pH** as

$$\text{Approximate definition of pH:} \quad \text{pH} = -\log[\text{H}^+] \quad (8-3)$$

pH is really defined in terms of the activity of H^+ , which is related to concentration. Section 12-2 discusses activity.

Here are some examples:

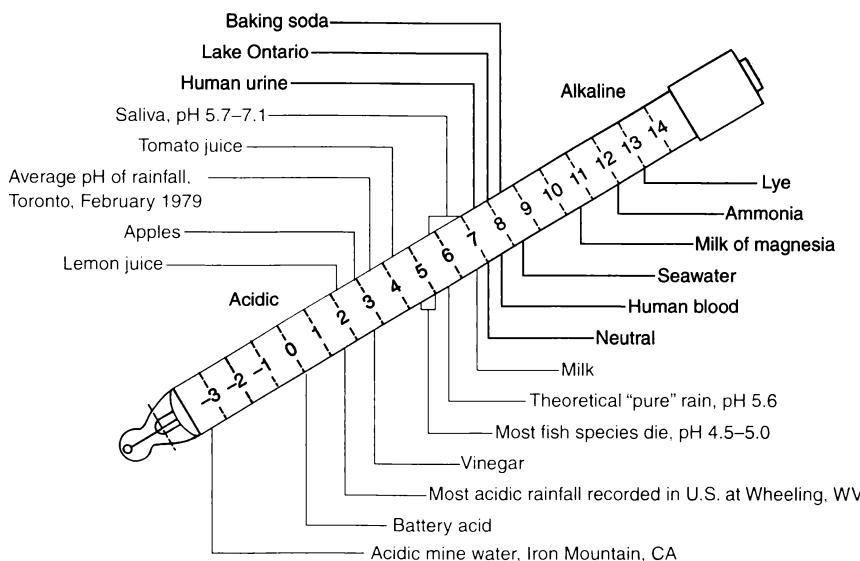
$$[\text{H}^+] = 10^{-3} \text{ M} \Rightarrow \text{pH} = -\log(10^{-3}) = 3$$

$$[\text{H}^+] = 10^{-10} \text{ M} \Rightarrow \text{pH} = -\log(10^{-10}) = 10$$

$$[\text{H}^+] = 3.8 \times 10^{-8} \text{ M} \Rightarrow \text{pH} = -\log(3.8 \times 10^{-8}) = 7.42$$

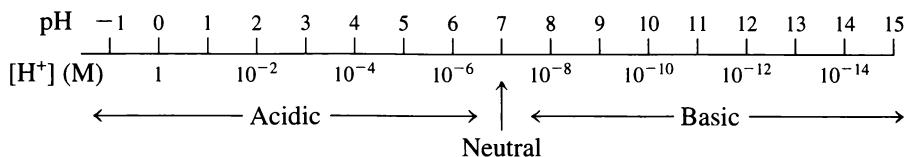
Changing the pH by 1 unit changes $[\text{H}^+]$ by a factor of 10. When the pH changes from 3 to 4, $[\text{H}^+]$ changes from 10^{-3} to 10^{-4} M .

Figure 8-2 pH values of various substances. The most acidic rainfall in the United States is more acidic than lemon juice.



Example: An acidic solution has $\text{pH} = 4$, which means $[\text{H}^+] = 10^{-4} \text{ M}$ and $[\text{OH}^-] = K_w/[\text{H}^+] = 10^{-10} \text{ M}$. Therefore $[\text{H}^+] > [\text{OH}^-]$.

A solution is **acidic** if $[\text{H}^+] > [\text{OH}^-]$. A solution is **basic** if $[\text{H}^+] < [\text{OH}^-]$. In pure water (which is neither acidic nor basic and is said to be *neutral*), $[\text{H}^+] = [\text{OH}^-] = 10^{-7} \text{ M}$, so the pH is $-\log(10^{-7}) = 7$. At 25°C , an acidic solution has a pH below 7, and a basic solution has a pH above 7 (Figure 8-2).



Although pH generally falls in the range 0 to 14, these are not limits. A pH of -1 , for example, means $-\log[\text{H}^+] = -1$, or $[\text{H}^+] = 10^{+1} = 10 \text{ M}$. This pH is attained in a concentrated solution of a strong acid such as HCl.



Ask Yourself

- 8-B. A solution of 0.050 M Mg^{2+} is treated with NaOH until Mg(OH)_2 precipitates.
- At what concentration of OH^- does this occur? (Remember the solubility product in Section 6-4. Use K_{sp} for brucite Mg(OH)_2 in Appendix A.)
 - At what pH does this occur?

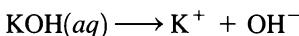
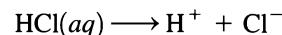
8-3 Strengths of Acids and Bases

Acids and bases are classified as strong or weak, depending on whether they react “completely” or only “partly” to produce H^+ or OH^- . Because there is a continuous range for a “partial” reaction, there is no sharp distinction between weak and strong. However, some compounds react so completely that they are unquestionably strong acids or bases—and everything else is defined as weak.

Strong Acids and Bases

Common strong acids and bases are listed in Table 8-1, which you must memorize. Note that even though HCl, HBr, and HI are strong acids, HF is *not*. A **strong acid**

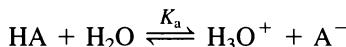
or **strong base** is completely dissociated in aqueous solution. That is, the equilibrium constants for the following reactions are large:



Virtually no undissociated HCl or KOH exists in aqueous solution. Demonstration 8-1 shows one consequence of the strong-acid behavior of HCl.

Weak Acids and Bases

All **weak acids**, HA, react with water by donating a proton to H₂O:

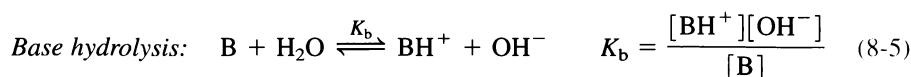


which means exactly the same as



The equilibrium constant, K_a , is called the **acid dissociation constant**. A weak acid is only partly dissociated in water, which means that some undissociated HA remains.

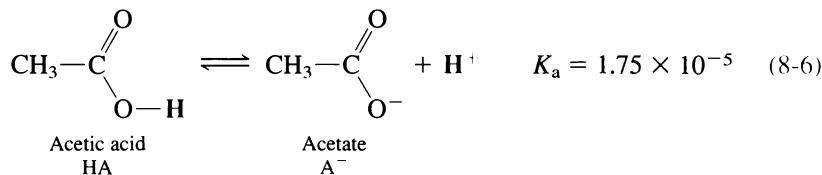
Weak bases, B, react with water by abstracting (grabbing) a proton from H₂O:



The equilibrium constant K_b is called the **base hydrolysis constant**. A weak base is one for which some unreacted B remains.

Carboxylic Acids Are Weak Acids and Amines Are Weak Bases

Acetic acid is a typical weak acid:



Acetic acid is representative of carboxylic acids, which have the general structure shown below, where R is an organic substituent. **Most carboxylic acids are weak acids, and most carboxylate anions are weak bases.**

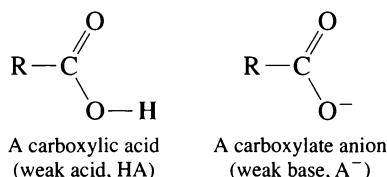


Table 8-1 Common strong acids and bases

Formula	Name
ACIDS	
HCl	Hydrochloric acid (hydrogen chloride)
HBr	Hydrogen bromide
HI	Hydrogen iodide
H ₂ SO ₄ ^a	Sulfuric acid
HNO ₃	Nitric acid
HClO ₄	Perchloric acid
BASES	
LiOH	Lithium hydroxide
NaOH	Sodium hydroxide
KOH	Potassium hydroxide
RbOH	Rubidium hydroxide
CsOH	Cesium hydroxide
R ₄ NOH ^b	Quaternary ammonium hydroxide

a. For H₂SO₄, only the first proton ionization is complete. Dissociation of the second proton has an equilibrium constant of 1.0×10^{-2} .

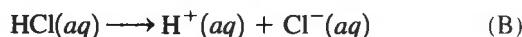
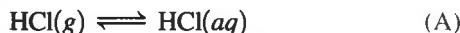
b. This is a general formula for any hydroxide salt of an ammonium cation containing four organic groups. An example is tetrabutylammonium hydroxide: (CH₃CH₂CH₂CH₂)₄N⁺OH⁻.

Roughly speaking, an acid is weak if $K_a < 1$ and a base is weak if $K_b < 1$.



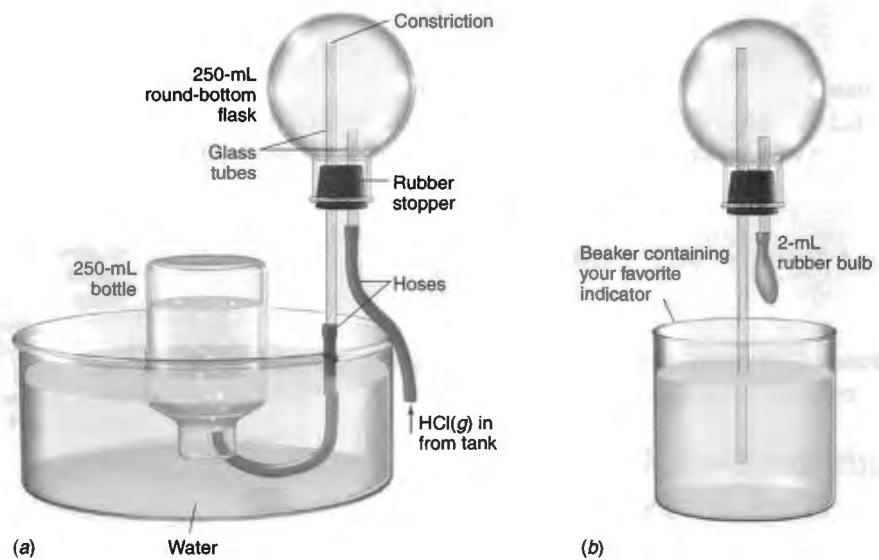
Demonstration 8-1 HCl Fountain

The complete dissociation of HCl into H⁺ and Cl⁻ makes HCl(g) extremely soluble in water.



Reaction B consumes the product of reaction A, thereby pulling reaction A to the right.

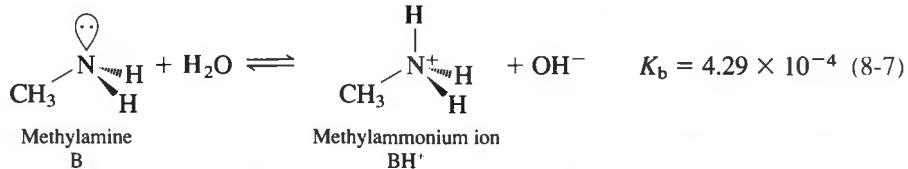
Assemble the HCl fountain¹ in panel *a* with the inverted 250-mL round-bottom flask containing air. The inlet tube leads to a tank of HCl(g) and the outlet tube goes to an inverted bottle of water. Admit HCl to the flask until the bottle is filled with air. At this point, the flask contains mostly HCl(g).



Carboxylic acids (RCO₂H) and ammonium ions (R₃NH⁺) are weak acids.

Carboxylate anions (RCO₂⁻) and amines (R₃N) are weak bases.

Methylamine is a typical weak base. It forms a bond to H⁺ by sharing the lone pair of electrons from the nitrogen atom of the *amine*:



Methylamine is a representative **amine**, a nitrogen-containing compound:

RNH ₂	a primary amine	RNH ₃ ⁺
R ₂ NH	a secondary amine	R ₂ NH ₂ ⁺
R ₃ N	a tertiary amine	R ₃ NH ⁺

ammonium ions

Replace the inlet hose of the flask with a rubber bulb containing water (panel *b*). Insert the glass outlet tube of the flask into a beaker of indicator such as slightly alkaline, commercial methyl purple solution, which is green above pH 5.4 and purple below pH 4.8. Squirt 1 mL of water from the rubber bulb into the flask to create a vacuum and draw a colorful fountain of indicator solution into the flask (Color Plate 3).

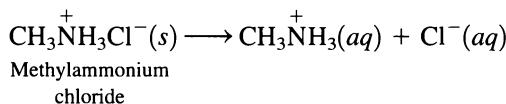
Questions Why is vacuum created when water is squirted into the flask? Why does the indicator change color when it enters the flask?

Amines are weak bases, and ammonium ions are weak acids. The “parent” of all amines is ammonia, NH₃. When methylamine reacts with water, the product is the conjugate acid. That is, the methylammonium ion produced in Reaction 8-7 is a weak acid:



The methylammonium (BH⁺) ion is the conjugate acid of methylamine (B).

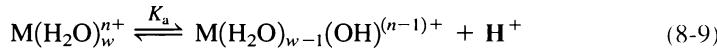
You should learn to recognize whether a compound is acidic or basic. For example, the salt methylammonium chloride dissociates completely in water to give methylammonium cation and chloride anion:



The methylammonium ion, being the conjugate acid of methylamine, is a weak acid (Reaction 8-8). The chloride ion is neither an acid nor a base. It is the conjugate base of HCl, a strong acid. In other words, Cl⁻ has virtually no tendency to associate with H⁺; otherwise, HCl would not be classified as a strong acid. We predict that methylammonium chloride solution is acidic, because the methylammonium ion is an acid and Cl⁻ is not a base.

Metal Ions with Charge ≥ 2 Are Weak Acids

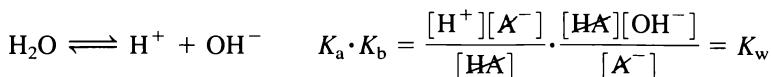
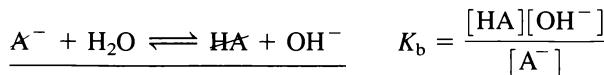
Metal ions with a charge of +2 or higher are acidic. In aqueous solution, metal ions bind several water molecules to form M(H₂O)_wⁿ⁺ in which electrons from oxygen are shared with the metal ion. Many metal ions bind w = 6 water molecules, but large metal ions can bind more water. A proton can dissociate from M(H₂O)_wⁿ⁺ to reduce the positive charge on the metal complex.



The higher the charge on the metal, the more acidic it tends to be. For example, K_a for Fe²⁺ is 4×10^{-10} , but K_a for Fe³⁺ is 6.5×10^{-3} . Cations with a charge of +1 have negligible acidity. Now you should understand why solutions of metal salts such as Fe(NO₃)₃ are acidic.

Relation Between K_a and K_b

An important relation exists between K_a and K_b of a conjugate acid-base pair in aqueous solution. We can derive this result with the acid HA and its conjugate base A⁻.



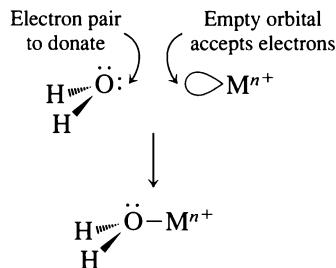
Weak acids: HA and BH⁺

Weak bases: A⁻ and B

Methylammonium chloride is a weak acid because

1. it dissociates into CH₃NH₃⁺ and Cl⁻.
2. CH₃NH₃⁺ is a weak acid, being conjugate to CH₃NH₂, a weak base.
3. Cl⁻ has no basic properties (it is conjugate to HCl, a strong acid; that is, HCl dissociates completely)

Challenge Phenol (C₆H₅OH) is a weak acid. Explain why a solution of the ionic compound potassium phenolate (C₆H₅O⁻K⁺) is basic.



When reactions are added, their equilibrium constants must be multiplied, thereby giving a most useful result:

Relation between K_a and K_b for a conjugate pair:

$$K_a \cdot K_b = K_w \quad (8-10)$$

$K_a \cdot K_b = K_w$ for a conjugate acid-base pair in aqueous solution.

Equation 8-10 applies to any acid and its conjugate base in aqueous solution.

Example Finding K_b for the Conjugate Base

The value of K_a for acetic acid is 1.75×10^{-5} (Reaction 8-6). Find K_b for acetate ion.

SOLUTION
$$K_b = \frac{K_w}{K_a} = \frac{1.0 \times 10^{-14}}{1.75 \times 10^{-5}} = 5.7 \times 10^{-10}$$

 **Test Yourself** K_a for ammonium ion (NH_4^+) is 5.7×10^{-10} . Find K_b for ammonia (NH_3). (Answer: 1.8×10^{-5} M)

Example Finding K_a for the Conjugate Acid

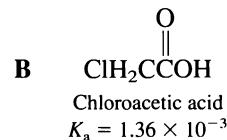
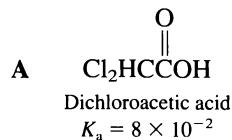
K_b for methylamine is 4.29×10^{-4} (Reaction 8-7). Find K_a for methylammonium ion.

SOLUTION
$$K_a = \frac{K_w}{K_b} = \frac{1.0 \times 10^{-14}}{4.29 \times 10^{-4}} = 2.33 \times 10^{-11}$$

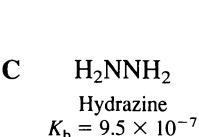
 **Test Yourself** K_b for formate (HCO_2^-) is 5.6×10^{-11} . Find K_a for formic acid (HCO_2H). (Answer: 1.8×10^{-4} M)

 **Ask Yourself**

8-C. Which is a stronger acid, **A** or **B**? Write the K_a reaction for each.

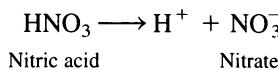


Which is a stronger base, **C** or **D**? Write the K_b reaction for each.

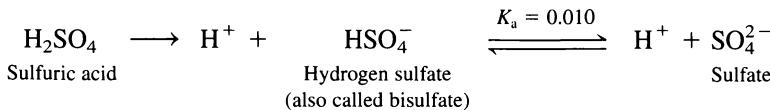


8-4 pH of Strong Acids and Bases

The principal components that make rainwater acidic are nitric and sulfuric acids, which are strong acids. Each molecule of *strong acid* or *strong base* in aqueous solution dissociates completely to provide one molecule of H^+ or OH^- . Nitric acid is a strong acid, so the reaction



goes to completion. In the case of sulfuric acid, one proton is completely dissociated, but the second is only partly dissociated (depending on conditions):



pH of a Strong Acid

Because HBr is completely dissociated, the pH of 0.010 M HBr is

$$\text{pH} = -\log[\text{H}^+] = -\log(0.010) = 2.00$$

Is pH 2 sensible? (Always ask yourself that question at the end of a calculation.) Yes—because pH values below 7 are acidic and pH values above 7 are basic.

Acid: pH < 7
Base: pH > 7

Example pH of a Strong Acid

Find the pH of 4.2×10^{-3} M HClO_4 .

SOLUTION HClO_4 is completely dissociated, so $[\text{H}^+] = 4.2 \times 10^{-3}$ M.

$$\text{pH} = -\log[\text{H}^+] = -\log(4.2 \times 10^{-3}) = 2.38$$

2 significant figures 2 digits in mantissa

How about significant figures? Two significant figures in the mantissa (the decimal part) of the logarithm correspond to two significant figures in the number 4.2×10^{-3} .



Test Yourself What is the pH of 0.055 M HBr? (Answer: 1.26)

For consistency in working problems in this book, we are generally going to express pH values to the 0.01 decimal place regardless of what is justified by significant figures. Real pH measurements are rarely more accurate than ± 0.02 , although differences in pH between two solutions can be accurate to ± 0.002 pH units.

pH of a Strong Base

Now we ask, “What is the pH of 4.2×10^{-3} M KOH?” The concentration of OH^- is 4.2×10^{-3} M, and we can calculate $[\text{H}^+]$ from the K_w equation, 8-2:

$$[\text{H}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.0 \times 10^{-14}}{4.2 \times 10^{-3}} = 2.38 \times 10^{-12} \text{ M}$$

$$\text{pH} = -\log[\text{H}^+] = -\log(2.38 \times 10^{-12}) = 11.62$$

Keep at least one extra insignificant figure (or all the digits in your calculator) in the middle of a calculation to avoid round-off errors in the final answer.

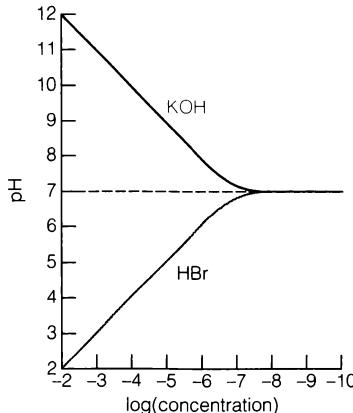


Figure 8-3 Calculated pH as a function of the concentration of a strong acid or strong base dissolved in water.

Acids and bases suppress water ionization, as predicted by Le Châtelier's principle.

Question What concentrations of H^+ and OH^- are produced by H_2O dissociation in 10^{-2} M NaOH?

Here is a trick question: What is the pH of 4.2×10^{-9} M KOH? By our previous reasoning, we might first say

$$[\text{H}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.0 \times 10^{-14}}{4.2 \times 10^{-9}} = 2.38 \times 10^{-6} \text{ M} \Rightarrow \text{pH} = 5.62$$

Is this reasonable? Can we dissolve base in water and obtain an acidic pH (< 7)? No way!

The fallacy is that we neglected the contribution of the reaction $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ to the concentration of OH^- . Pure water creates 10^{-7} M OH^- , which is more OH^- than the KOH that we added. The pH of water plus added KOH cannot fall below 7. The pH of 4.2×10^{-9} M KOH is very close to 7. Similarly, the pH of 10^{-10} M HNO₃ is very close to 7, not 10. Figure 8-3 shows how pH depends on concentration for a strong acid and a strong base. In a very dilute solution exposed to air, the acid-base chemistry of dissolved carbon dioxide ($\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+$) would overwhelm the effect of the added acid or base.

Water Almost Never Produces 10^{-7} M H^+ and 10^{-7} M OH^-

The ion concentrations 10^{-7} M H^+ and 10^{-7} M OH^- occur *only* in extremely pure water with no added acid or base. In a 10^{-4} M solution of HBr, for example, the pH is 4. The concentration of OH^- is $K_w/[\text{H}^+] = 10^{-10}$ M. But the source of $[\text{OH}^-]$ is dissociation of water. If water produces only 10^{-10} M OH^- , it must also produce only 10^{-10} M H^+ , because it makes one H^+ for every OH^- . In a 10^{-4} M HBr solution, water dissociation produces only 10^{-10} M OH^- and 10^{-10} M H^+ .



Ask Yourself

- 8-D. (a) What is the pH of (i) 1.0×10^{-3} M HBr and (ii) 1.0×10^{-2} M KOH?
 (b) Calculate the pH of (i) 3.2×10^{-5} M HI and (ii) 7.7 mM LiOH.
 (c) Find the concentration of H^+ in a solution whose pH is 4.44.
 (d) Find $[\text{H}^+]$ in 7.7 mM LiOH solution. What is the source of this H^+ ?
 (e) Find the pH of 3.2×10^{-9} M tetramethylammonium hydroxide, $(\text{CH}_3)_4\text{N}^+\text{OH}^-$.

8-5 Tools for Dealing with Weak Acids and Bases

By analogy to the definition of pH, we define **pK** as the negative logarithm of an equilibrium constant. For the acid dissociation constant in Equation 8-4 and the base hydrolysis constant in Equation 8-5, we can write

$$\text{p}K_a = -\log K_a \quad \text{p}K_b = -\log K_b \quad (8-11)$$

The *stronger* an acid, the *smaller* its $\text{p}K_a$.

Stronger acid	Weaker acid
$K_a = 10^{-4}$	$K_a = 10^{-8}$
$\text{p}K_a = 4$	$\text{p}K_a = 8$

However, both $K_a = 10^{-4}$ and $K_a = 10^{-8}$ are classified as weak acids.

Remember from Equation 8-10 the very important relation between K_a and K_b for a conjugate acid-base pair, which are related by gain or loss of a single proton: $K_a \cdot K_b = K_w$.

Weak Is Conjugate to Weak

The conjugate base of a weak acid is a weak base. The conjugate acid of a weak base is a weak acid. Consider a weak acid, HA, with $K_a = 10^{-4}$. The conjugate base, A^- , has $K_b = K_w/K_a = 10^{-10}$. That is, if HA is a weak acid, A^- is a weak base. If the K_a value were 10^{-5} , then the K_b value would be 10^{-9} . As HA becomes a weaker acid, A^- becomes a stronger base (but never a strong base). Conversely, the greater the acid strength of HA, the less the base strength of A^- . However, if either A^- or HA is weak, so is its conjugate. If HA is strong (such as HCl), its conjugate base (Cl^-) is so weak that it is not a base at all in water.

Using Appendix B

Acid dissociation constants appear in Appendix B. Each compound is shown in its *fully protonated form*. Methylamine, for example, is shown as CH_3NH_3^+ , which is really the methylammonium ion. The value of K_a (2.33×10^{-11}) given for methylamine is actually K_a for the methylammonium ion. To find K_b for methylamine, we write $K_b = K_w/K_a = (1.0 \times 10^{-14})/(2.33 \times 10^{-11}) = 4.29 \times 10^{-4}$.

For polyprotic acids and bases, several K_a values are given, beginning with the most acidic group. Pyridoxal phosphate is given in its fully protonated form as follows:

Phosphate	O	O	H	Hydroxyl	pK _a	Group	K _a
					1.4	POH	0.04
					3.44	OH	3.6×10^{-4}
					6.01	POH	9.8×10^{-7}
					8.45	NH	3.5×10^{-9}

pK₁ (1.4) is for dissociation of one of the phosphate protons, and pK₂ (3.44) is for the hydroxyl proton. The third most acidic proton is the other phosphate proton, for which pK₃ = 6.01, and the NH⁺ group is the least acidic (pK₄ = 8.45).

Ask Yourself

- Which acid is stronger, pK_a = 3 or pK_a = 4?
- Which base is stronger, pK_b = 3 or pK_b = 4?
- Write the acid dissociation reaction for formic acid, HCO₂H.
- What is the conjugate base of formic acid?
- Write the K_a equilibrium expression for formic acid and look up its value.
- Write the K_b equilibrium expression for formate ion, HCO₂⁻.
- Find the base hydrolysis constant for formate.

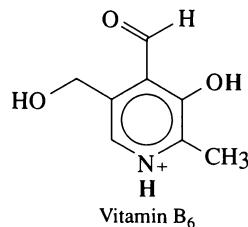
The conjugate base of a weak acid is a weak base.

The conjugate acid of a weak base is a weak acid.

Weak is conjugate to weak.

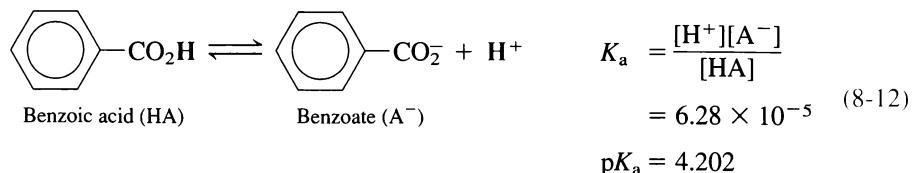


Pyridoxal phosphate is derived from vitamin B₆, which is essential in amino acid metabolism in your body. Box 7-1 discussed the drawing of organic structures.



8-6 Weak-Acid Equilibrium

Let's find the pH and composition of a solution containing 0.020 0 mol benzoic acid in 1.00 L of water.



For every mole of A^- formed by dissociation of HA, 1 mole of H^+ is created. That is, $[A^-] = [H^+]$. (For reasonable concentrations of weak acids of reasonable strength, the contribution of H^+ from the acid is much greater than the contribution from $H_2O \rightleftharpoons H^+ + OH^-$.) Abbreviating the formal concentration of HA as F and using x for the concentration of H^+ , we can make a table showing concentrations before and after dissociation of the weak acid:

	HA	\rightleftharpoons	A^-	+	H^+
Initial concentration	F		0		0
Final concentration	$F - x$		x		x

Putting these values into the K_a expression in Equation 8-12 gives

$$K_a = \frac{[H^+][A^-]}{[HA]} = \frac{(x)(x)}{F - x} \quad (8-13)$$

Setting $F = 0.020\ 0\ M$ and $K_a = 6.28 \times 10^{-5}$ gives

$$\frac{x^2}{0.020\ 0 - x} = 6.28 \times 10^{-5} \quad (8-14)$$

The first step in solving Equation 8-14 for x is to multiply both sides by $(0.020\ 0 - x)$:

$$\begin{aligned} \left(\frac{x^2}{0.020\ 0 - x} \right) (0.020\ 0 - x) &= (6.28 \times 10^{-5})(0.020\ 0 - x) \\ &= (1.25 \times 10^{-6}) - (6.28 \times 10^{-5})x \end{aligned}$$

Collecting terms gives a quadratic equation (in which the highest power is x^2):

$$x^2 + (6.28 \times 10^{-5})x - (1.25 \times 10^{-6}) = 0 \quad (8-15)$$

Equation 8-15 has two solutions (called *roots*) described in Box 8-1. One root is positive and the other is negative. Because a concentration cannot be negative, we reject the negative solution:

$$x = 1.09 \times 10^{-3}\ M \text{ (negative root rejected)}$$

From the value of x , we can find concentrations and pH:

$$[H^+] = [A^-] = x = 1.09 \times 10^{-3}\ M$$

$$[HA] = F - x = 0.020\ 0 - (1.09 \times 10^{-3}) = 0.018\ 9\ M$$

$$pH = -\log x = 2.96$$

Was the approximation $[H^+] \approx [A^-]$ justified? The concentration of $[H^+]$ is $1.09 \times 10^{-3}\ M$, which means $[OH^-] = K_w/[H^+] = 9.20 \times 10^{-12}\ M$.

$$[H^+] \text{ from HA dissociation} = [A^-] \text{ from HA dissociation} = 1.09 \times 10^{-3}\ M$$

$$[H^+] \text{ from } H_2O \text{ dissociation} = [OH^-] \text{ from } H_2O \text{ dissociation} = 9.20 \times 10^{-12}\ M$$

You should **check your answer** by plugging it back into Equation 8-13 and seeing whether the equation is satisfied.

For uniformity, we are going to express pH to the 0.01 decimal place, even though significant figures in this problem justify another digit.

In a weak-acid solution, H^+ is derived almost entirely from HA, not from H_2O .

Box 8-1 Quadratic Equations

A quadratic equation of the general form $ax^2 + bx + c = 0$ has two solutions:

$$x = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \quad x = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

The solutions to Equation 8-15

$$(1)[H^+]^2 + \underbrace{(6.28 \times 10^{-5})[H^+]}_{a=1} - \underbrace{(1.25_6 \times 10^{-6})}_{c=-1.25_6 \times 10^{-6}} = 0$$

$$b = 6.28 \times 10^{-5}$$

are

$$[H^+] = \frac{-(6.28 \times 10^{-5}) + \sqrt{(6.28 \times 10^{-5})^2 - 4(1)(-1.25_6 \times 10^{-6})}}{2(1)} = 1.09 \times 10^{-3} \text{ M}$$

and

$$[H^+] = \frac{-(6.28 \times 10^{-5}) - \sqrt{(6.28 \times 10^{-5})^2 - 4(1)(-1.25_6 \times 10^{-6})}}{2(1)} = -1.09 \times 10^{-3} \text{ M}$$

Because the concentration of $[H^+]$ cannot be negative, we reject the negative solution and choose $1.09 \times 10^{-3} \text{ M}$ as the correct answer.

When you solve a quadratic equation, retain all the digits in your calculator during the computation, or serious round-off errors can occur in some cases. Alternatively, create a spreadsheet to solve quadratic equations (Problem 8-36) and use it often.

Sometimes, the equation $x^2/(F - x) = K$ has an easy solution. If $x \ll F$, then x can be neglected in comparison with F and the denominator can be simplified to just F . In this case, the solution is $x \approx \sqrt{KF}$. If $K < 10^{-4} F$, then the error in the approximation $x \approx \sqrt{KF}$ is <0.5%.

The assumption that H^+ is derived mainly from HA is excellent because $1.09 \times 10^{-3} \text{ M} \gg 9.20 \times 10^{-12} \text{ M}$.

Fraction of Dissociation

What fraction of HA is dissociated? If the total concentration of acid ($= [HA] + [A^-]$) is 0.020 M and the concentration of A^- is $1.09 \times 10^{-3} \text{ M}$, then the *fraction of dissociation* is

Fraction of dissociation of an acid:

$$\frac{[A^-]}{[A^-] + [HA]} = \frac{1.09 \times 10^{-3}}{0.020 \text{ M}} = 0.054 \quad (8-16)$$

The acid is indeed weak. It is only 5.4% dissociated.

Figure 8-4 compares the fraction of dissociation of two weak acids as a function of formal concentration. As the solution becomes more dilute, the fraction of dissociation increases. The stronger acid has a greater fraction of dissociation at any formal concentration. Demonstration 8-2 illustrates the chemistry of acid rain.

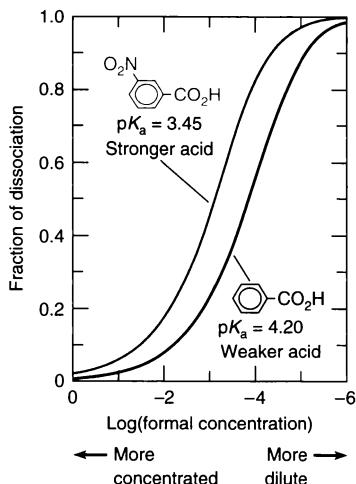
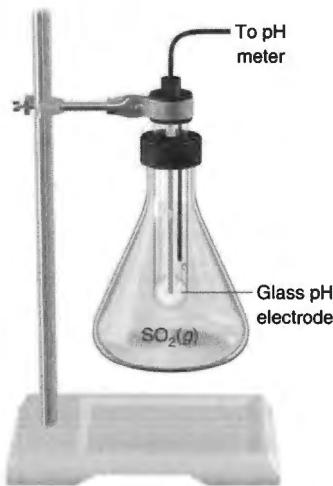


Figure 8-4 The fraction of dissociation of a weak acid increases as it is diluted. The stronger of the two acids is more dissociated at any given concentration. ($pK_a = 3.45$ is stronger than $pK_a = 4.20$.)



Demonstration 8-2 Acid Rain Chemistry²

As described at the opening of this chapter, rainfall acidity arises from oxidation products of nitrogen oxides (NO and NO_2 , called NO_x) and sulfur dioxide (SO_2). This demonstration illustrates SO_2 chemistry with a glass pH electrode suspended in the air space of a flask.



Apparatus for demonstrating acid rain chemistry.

Prepare three stoppered 500-mL flasks containing SO_2 , H_2O_2 , or NH_3 .

1. Prepare SO_2 by winding a conical coil on the end of a copper wire and filling the coil with 20 mg of sulfur

powder. Ignite the sulfur with a match and place the coil inside a loosely stoppered flask until burning is complete. Then insert the stopper tightly. Alternatively, place 10 mL of 0.5 M NaHSO_3 in the flask and add 10 drops (~0.5 mL) of 3 M H_2SO_4 to generate $\text{SO}_2(g)$.

2. Add 1 mL of 30 wt% H_2O_2 to 9 mL of water in a second stoppered flask.
3. Add 0.2 mL of 28 wt% NH_3 to 10 mL of water in a third stoppered flask.

Fit a rubber collar to a glass pH electrode so that the electrode can be inserted into the airspace of a 500-mL Erlenmeyer flask. Calibrate the electrode in buffers at pH 4, 7, and 10. Instead of rinsing the electrode with water, rinse with 0.2 wt% NaCl solution so that the liquid film adhering to the electrode contains enough electrolyte for stable pH readings when the electrode is not immersed in liquid. After washing the electrode, touch a tissue to the bottom to remove liquid hanging from the glass bulb. A thin film of liquid remains on the glass. Store the electrode in humid air in a flask containing water so that the liquid film on the electrode does not evaporate.

Monitor the glass electrode suspended in the humid flask with a pH meter, preferably interfaced to a computer to record pH versus time. After 1 min, place the electrode in the airspace of the SO_2 flask for 3 s. Observe a sudden drop in pH. Remove the electrode so that SO_2 can diffuse from the liquid film back into the air. The pH changes are shown in the graph.

The Essence of a Weak-Acid Problem

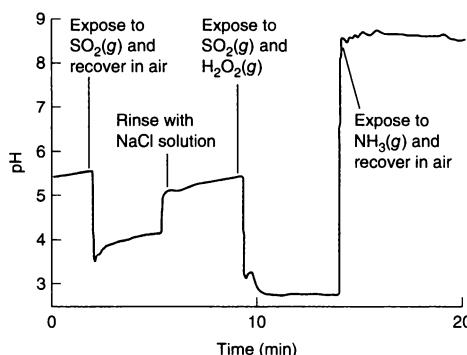
When faced with a weak acid, you should immediately realize that $[\text{H}^+] \approx [\text{A}^-] = x$ and proceed to set up and solve the equation

The way to do it.

Equation for weak acids:

$$\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = \frac{x^2}{F - x} = K_a \quad (8-17)$$

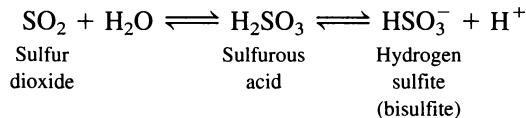
where F is the formal concentration of HA. The approximation $[\text{H}^+] \approx [\text{A}^-] = x$ would be poor only if the acid were very dilute or very weak, neither of which constitutes a practical problem.



Response of glass electrode exposed to different gases. [From F. S. Lopes, L. H. G. Coelho, and I. G. R. Gutz, *J. Chem. Ed.* **2010**, 87, 157.]

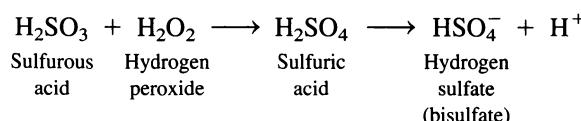
After a few minutes, rinse the electrode with 0.2 wt% NaCl until a pH of 5.4 ± 0.2 is restored. Insert the electrode back into the SO_2 flask for 3 s and then quickly transfer it to the gas phase of the H_2O_2 flask for 10 s. Observe an even steeper drop in pH that is not restored when the electrode is removed and held in the air. Finally, expose the electrode to the air space of the NH_3 flask for 3 s and observe the rise in pH that remains when the electrode is removed and held in air.

Here is an explanation of what you observe. In the first experiment, $\text{SO}_2(g)$ dissolves in the thin film of water to form the weak acid sulfurous acid:

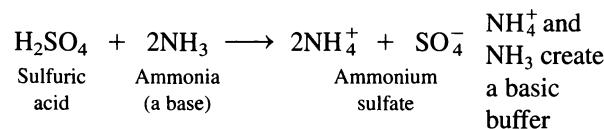


The pH drops below 4 and recovers to above 4 when the electrode is held in the air and SO_2 diffuses from the liquid film into the air.

When exposed to SO_2 followed by hydrogen peroxide, H_2SO_3 is irreversibly oxidized to the strong acid sulfuric acid, thereby taking the pH below 3. (SO_2 is also oxidized to H_2SO_4 in the atmosphere.) Sulfuric acid is not volatile, so it does not diffuse back into the air. The pH remains low when the electrode is held in the air.



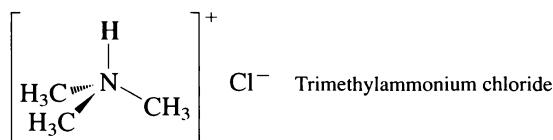
When the electrode is exposed to ammonia (an atmospheric pollutant from fertilizer and other sources), the pH jumps to near 9 by the buffering action of NH_4^+ plus excess NH_3 in the liquid. We will study buffers in the next chapter. NH_3 is very soluble in water and does not readily diffuse back into the air when the electrode is held in the air.



As an extension of this demonstration, the electrode can be exposed to a flask containing $\text{CO}_2(g)$ to demonstrate ocean acidification by CO_2 (Box 11-1).

Example Finding the pH of a Weak Acid

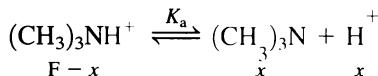
Find the pH of 0.100 M trimethylammonium chloride.



SOLUTION We must first realize that salts of this type are *completely dissociated* to give $(\text{CH}_3)_3\text{NH}^+$ and Cl^- . We then recognize that trimethylammonium ion is a weak acid, being conjugate to trimethylamine, $(\text{CH}_3)_3\text{N}$, a weak base. The ion Cl^- has no basic or acidic properties and should be ignored. In Appendix B, we find trimethylammonium ion listed under the name trimethylamine but drawn as the trimethylammonium ion. The value of pK_a is 9.799, so

$$K_a = 10^{-pK_a} = 10^{-9.799} = 1.59 \times 10^{-10}$$

It's all downhill from here:



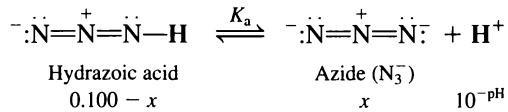
$$\frac{x^2}{0.100 - x} = 1.59 \times 10^{-10}$$

$$x = 3.99 \times 10^{-6} \text{ M} \Rightarrow \text{pH} = -\log(3.99 \times 10^{-6}) = 5.40$$

 **Test Yourself** What is the pH of 0.010 M dimethylammonium nitrate? (Answer: 6.39)

 **Example** Finding pK_a of a Weak Acid

A 0.100 M solution of hydrazoic acid has pH = 2.83. Find pK_a for this acid.



SOLUTION We know that $[\text{H}^+] = 10^{-\text{pH}} = 10^{-2.83} = 1.48 \times 10^{-3} \text{ M}$. Because $[\text{N}_3^-] = [\text{H}^+]$ in this solution, $[\text{N}_3^-] = 1.48 \times 10^{-3} \text{ M}$ and $[\text{HN}_3] = 0.100 - 1.48 \times 10^{-3} = 0.0985 \text{ M}$. From these concentrations, we calculate K_a and pK_a :

$$K_a = \frac{[\text{N}_3^-][\text{H}^+]}{[\text{HN}_3]} = \frac{(1.48 \times 10^{-3})^2}{0.0985} = 2.22 \times 10^{-5}$$
$$\Rightarrow pK_a = -\log(2.22 \times 10^{-5}) = 4.65$$

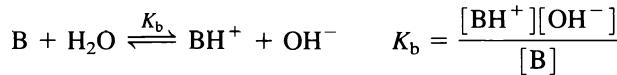
 **Test Yourself** A 0.063 M solution of hydroxybenzene has pH = 5.60. Find pK_a for this acid. (Answer: 10.00)

 **Ask Yourself**

- 8-F. (a) What is the pH and fraction of dissociation of a 0.100 M solution of the weak acid HA with $K_a = 1.00 \times 10^{-5}$?
(b) A 0.045 0 M solution of HA has a pH of 2.78. Find pK_a for HA.
(c) A 0.045 0 M solution of HA is 0.60% dissociated. Find pK_a for HA.

8-7 Weak-Base Equilibrium

The treatment of weak bases is almost the same as that of weak acids.



Nearly all OH^- comes from the reaction of $\text{B} + \text{H}_2\text{O}$, and little comes from dissociation of H_2O . Setting $[\text{OH}^-] = x$, we must also set $[\text{BH}^+] = x$, because one BH^+ is produced for each OH^- . Setting $F = [\text{B}] + [\text{BH}^+]$, we can write

$$[\text{B}] = F - [\text{BH}^+] = F - x$$

Plugging these values into the K_b equilibrium expression, we get

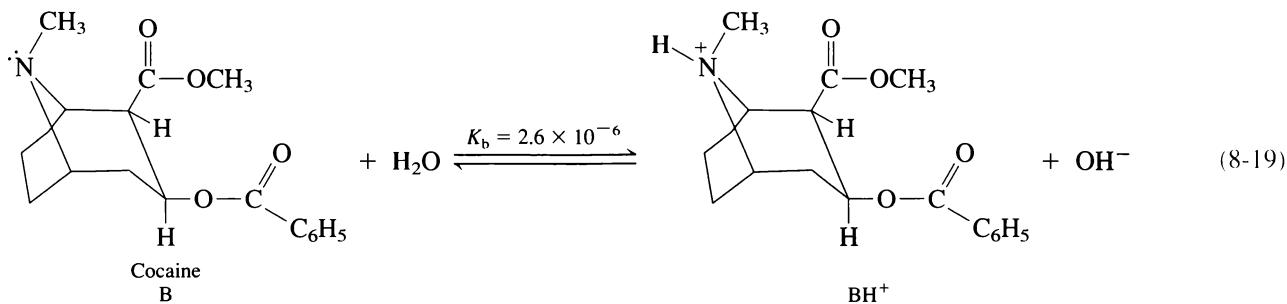


A weak-base problem has the same algebra as a weak-acid problem, except $K = K_b$ and $x = [\text{OH}^-]$.

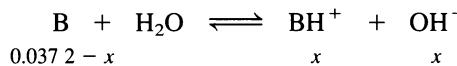
which looks a lot like a weak-acid problem, except that now $x = [\text{OH}^-]$.

Example Finding the pH of a Weak Base

Find the pH of a 0.0372 M solution of the commonly encountered weak base, cocaine:



SOLUTION Designating cocaine as B, we formulate the problem as follows:



$$\frac{x^2}{0.0372 - x} = 2.6 \times 10^{-6} \Rightarrow x = 3.10 \times 10^{-4} \text{ M}$$

Because $x = [\text{OH}^-]$, we can write

$$\begin{aligned} [\text{H}^+] &= K_w/[\text{OH}^-] = (1.0 \times 10^{-14})/(3.10 \times 10^{-4}) = 3.22 \times 10^{-11} \text{ M} \\ \text{pH} &= -\log[\text{H}^+] = 10.49 \end{aligned}$$

This is a reasonable pH for a weak base.

Question What concentration of OH^- is produced by H_2O dissociation in this solution? Were we justified in neglecting water dissociation as a source of OH^- ?

Test Yourself Find the pH of a 0.010 M solution of morphine, another weak base with $K_b = 1.6 \times 10^{-6}$. (**Answer:** 10.10)

What fraction of cocaine reacted with water?

$$\text{Fraction of association of a base: } \frac{[\text{BH}^+]}{[\text{BH}^+] + [\text{B}]} = \frac{3.1_0 \times 10^{-4}}{0.0372} = 0.0083 \quad (8-20)$$

because $[\text{BH}^+] = [\text{OH}^-] = 3.1 \times 10^{-4} \text{ M}$. Only 0.83% of the base has reacted.

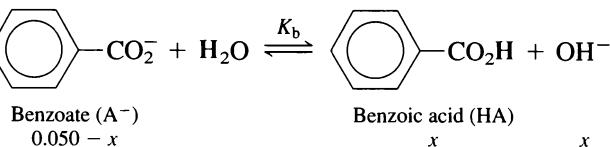
Conjugate Acids and Bases—Revisited

HA and A^- are a conjugate acid-base pair. So are BH^+ and B .

The conjugate base of a weak acid is a weak base, and the conjugate acid of a weak base is a weak acid. The exceedingly important relation between the equilibrium constants for a conjugate acid-base pair is

$$K_a \cdot K_b = K_w$$

Reaction 8-12 featured benzoic acid, designated HA . Now consider 0.050 M sodium benzoate, Na^+A^- , which contains the conjugate base of benzoic acid. When this salt dissolves in water, it dissociates to Na^+ and A^- . Na^+ does not react with water, but A^- is a weak base:



$$K_b = \frac{K_w}{K_a \text{ (for benzoic acid)}} = \frac{1.0 \times 10^{-14}}{6.28 \times 10^{-5}} = 1.5_9 \times 10^{-10}$$

To find the pH of this solution, we write

$$\frac{[\text{HA}][\text{OH}^-]}{[\text{A}^-]} = \frac{x^2}{0.050 - x} = 1.5_9 \times 10^{-10} \Rightarrow x = [\text{OH}^-] = 2.8 \times 10^{-6} \text{ M}$$

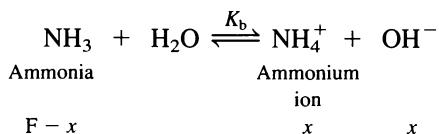
$$[\text{H}^+] = K_w/[\text{OH}^-] = 3.5 \times 10^{-9} \text{ M} \Rightarrow \text{pH} = 8.45$$

This is a reasonable pH for a solution of a weak base.

Example A Weak-Base Problem

Find the pH of 0.10 M ammonia.

SOLUTION When ammonia dissolves in water, its reaction is



In Appendix B, we find the ammonium ion, NH_4^+ , listed next to ammonia. K_a for ammonium ion is 5.69×10^{-10} . Therefore K_b for NH_3 is

$$K_b = \frac{K_w}{K_a} = \frac{1.0 \times 10^{-14}}{5.69 \times 10^{-10}} = 1.7_6 \times 10^{-5}$$

To find the pH of 0.10 M NH₃, we set up and solve the equation

$$\frac{[\text{NH}_4^+][\text{OH}^-]}{[\text{NH}_3]} = \frac{x^2}{0.10 - x} = K_b = 1.76 \times 10^{-5}$$

$$x = [\text{OH}^-] = 1.32 \times 10^{-3} \text{ M}$$

$$[\text{H}^+] = \frac{K_w}{[\text{OH}^-]} = 7.59 \times 10^{-12} \text{ M} \Rightarrow \text{pH} = -\log[\text{H}^+] = 11.12$$

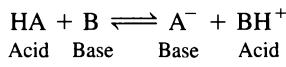
 **Test Yourself** Find the pH of 5.0 mM diethylamine. (Answer: 11.25)

Ask Yourself

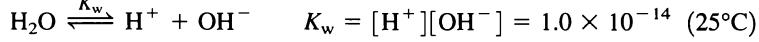
- 8-G. (a) What is the pH and fraction of association of a 0.100 M solution of a weak base with $K_b = 1.00 \times 10^{-5}$?
 (b) A 0.10 M solution of a base has pH = 9.28. Find K_b .
 (c) A 0.10 M solution of a base is 2.0% associated. Find K_b .

Key Equations

Conjugate acids and bases



Autoprotolysis of water



Finding [OH⁻] from [H⁺]

$$[\text{OH}^-] = K_w / [\text{H}^+]$$

Definition of pH

$$\text{pH} = -\log[\text{H}^+] \quad (\text{This relation is only approximate—but it is the one we use in this book.})$$

Acid dissociation constant

$$\text{HA} \xrightleftharpoons{K_a} \text{H}^+ + \text{A}^- \quad K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

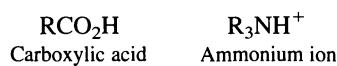
Base hydrolysis constant

$$\text{B} + \text{H}_2\text{O} \xrightleftharpoons{K_b} \text{BH}^+ + \text{OH}^- \quad K_b = \frac{[\text{BH}^+][\text{OH}^-]}{[\text{B}]}$$

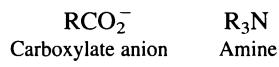
Relation between K_a and K_b for conjugate acid-base pair

$$K_a \cdot K_b = K_w$$

Common weak acids



Common weak bases



Definitions of pK

$$\text{p}K_a = -\log K_a \quad \text{p}K_b = -\log K_b$$

Weak-acid equilibrium

$$\text{HA} \xrightleftharpoons{F-x} \text{H}^+ + \text{A}^- \quad K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = \frac{x^2}{F-x}$$

(F = formal concentration of weak acid or weak base)

Weak-base equilibrium

$$\text{B} + \text{H}_2\text{O} \xrightleftharpoons{F-x} \text{BH}^+ + \text{OH}^- \quad K_b = \frac{[\text{BH}^+][\text{OH}^-]}{[\text{B}]} = \frac{x^2}{F-x}$$

Fraction of dissociation of HA

$$\text{fraction of dissociation} = \frac{[\text{A}^-]}{[\text{A}^-] + [\text{HA}]}$$

Fraction of association of B

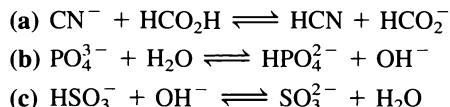
$$\text{fraction of association} = \frac{[\text{BH}^+]}{[\text{BH}^+] + [\text{B}]}$$

Important Terms

acid	base	conjugate acid-base pair	salt
acid dissociation constant	base hydrolysis constant	hydronium ion	strong acid
acidic solution	basic solution	neutralization	strong base
amine	carboxylate anion	pH	weak acid
ammonium ion	carboxylic acid	pK	weak base
autoprotonysis			

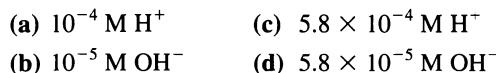
Problems

8-1. Identify the conjugate acid-base pairs in the following reactions:



8-2. A solution is *acidic* if _____. A solution is *basic* if _____.

8-3. Find the pH of a solution containing



8-4. The concentration of H^+ in your blood is $0.035 \mu\text{M}$.

- (a) What is the pH of blood?
 (b) Find the concentration of OH^- in blood.

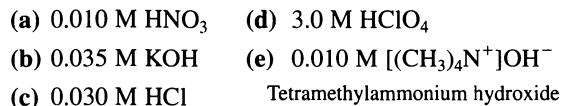
8-5. Sulfuric acid is the principal acidic component of acid rain. The mean pH of rainfall in southern Norway in 2000 was 4.6. What concentration of H_2SO_4 will produce this pH by the reaction $\text{H}_2\text{SO}_4 \rightleftharpoons \text{H}^+ + \text{HSO}_4^-$?

8-6. An acidic solution containing 0.010 M La^{3+} is treated with NaOH until $\text{La}(\text{OH})_3$ precipitates. Use the solubility product for $\text{La}(\text{OH})_3$ to find the concentration of OH^- when La^{3+} first precipitates. At what pH does this occur?

8-7. Make a list of the common strong acids and strong bases. Memorize this list.

8-8. Write the structures and names for two classes of weak acids and two classes of weak bases.

8-9. Calculate $[\text{H}^+]$ and pH for the following solutions:

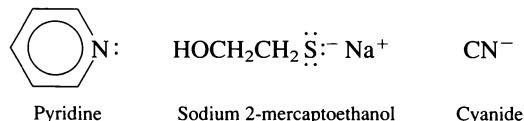


8-10. (a) Write the K_a reaction for trichloroacetic acid, $\text{Cl}_3\text{CCO}_2\text{H}$ ($K_a = 0.3$), for anilinium ion, and for Cu^{2+} ($K_a = 3 \times 10^{-8}$)



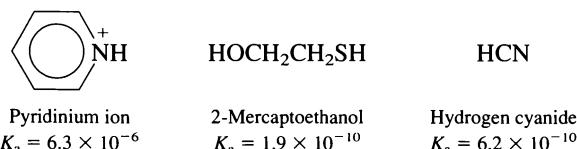
(b) Which of the three is the strongest acid?

8-11. (a) Write the K_b reaction for pyridine, for sodium 2-mercaptoethanol, and for cyanide.

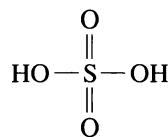


H^+ binds to S in sodium 2-mercaptoethanol and to C in cyanide.

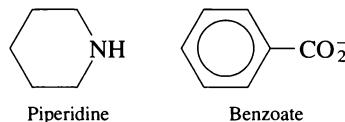
(b) From K_a for the conjugate acids, which base in part (a) is strongest?



8-12. Write the autoprotolysis reaction of H_2SO_4 , whose structure is



8-13. Write the K_b reactions for piperidine and benzoate.



8-14. Hypochlorous acid has the structure $\text{H}-\text{O}-\text{Cl}$. Write the base hydrolysis reaction of hypochlorite, OCl^- . Given that K_a for HOCl is 3.0×10^{-8} , find K_b for hypochlorite.

8-15. Calculate the pH of $3.0 \times 10^{-5} \text{ M Mg(OH)}_2$, which completely dissociates to Mg^{2+} and OH^- .

8-16. Find the concentration of H^+ in a solution whose pH is 11.65.

8-17. Find the pH and fraction of dissociation of a 0.010 M solution of the weak acid HA with $K_a = 1.00 \times 10^{-4}$.

8-18. Find the pH and fraction of dissociation in a 0.150 M solution of hydroxybenzene (also called phenol).

8-19. Calculate the pH of 0.085 M pyridinium bromide, $\text{C}_5\text{H}_5\text{NH}^+\text{Br}^-$. Find the concentrations of pyridine ($\text{C}_5\text{H}_5\text{N}$), pyridinium ion ($\text{C}_5\text{H}_5\text{NH}^+$), and Br^- in the solution.

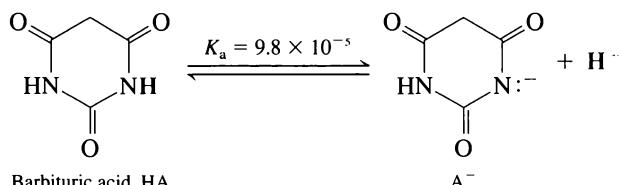
8-20. Calculate the pH of 0.10 M $\text{Zn}(\text{NO}_3)_2$, which dissociates to give 0.10 M Zn^{2+} ($\text{p}K_a = 9.0$) and 0.20 M NO_3^- .

8-21. A 0.100 M solution of the weak acid HA has a pH of 2.36. Calculate $\text{p}K_a$ for HA.

8-22. A 0.022 2 M solution of HA is 0.15% dissociated. Calculate $\text{p}K_a$ for this acid.

8-23. Find the pH and concentrations of $(\text{CH}_3)_3\text{N}$ and $(\text{CH}_3)_3\text{NH}^+$ in a 0.060 M solution of trimethylammonium chloride.

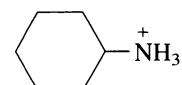
8-24. Calculate the pH and fraction of dissociation of (a) $10^{-2.00}$ M and (b) $10^{-10.00}$ M barbituric acid.



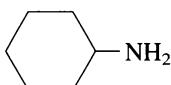
Barbituric acid, HA

8-25. $\text{BH}^+\text{ClO}_4^-$ is a salt formed from the base B ($K_b = 1.00 \times 10^{-4}$) and perchloric acid. It dissociates into BH^+ , a weak acid, and ClO_4^- , which is neither an acid nor a base. Find the pH of 0.100 M $\text{BH}^+\text{ClO}_4^-$.

8-26. Find K_a for cyclohexylammonium ion and K_b for cyclohexylamine.

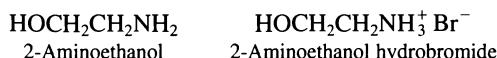


Cyclohexylammonium ion



Cyclohexylamine

8-27. Write the chemical reaction whose equilibrium constant is (a) K_b for 2-aminoethanol and (b) K_a for 2-aminoethanol hydrobromide.



8-28. Find the pH and concentrations of $(\text{CH}_3)_3\text{N}$ and $(\text{CH}_3)_3\text{NH}^+$ in a 0.060 M solution of trimethylamine.

8-29. Calculate the pH and fraction of association of 1.00×10^{-1} , 1.00×10^{-2} , and 1.00×10^{-12} M sodium acetate.

8-30. Find the pH of 0.050 M NaCN.

8-31. Find the pH and fraction of association of 0.026 M NaOCl.

8-32. If a 0.030 M solution of a base has pH = 10.50, find K_b for the base.

8-33. In a 0.030 M solution of a base, 0.27% of B underwent hydrolysis to make BH^+ . Find K_b for the base.

8-34. The smell (and taste) of fish arises from amine compounds. Ionic compounds, such as ammonium salts, have lower vapor pressure than nonionic compounds. Suggest a reason why adding lemon juice (which is acidic) reduces the “fishy” smell.

8-35. Cr^{3+} has $\text{p}K_a = 3.80$. Find the pH of 0.010 M $\text{Cr}(\text{ClO}_4)_3$. What fraction of chromium is in the form $\text{Cr}(\text{H}_2\text{O})_{w-1}(\text{OH})^{2+}$?

8-36. Create a spreadsheet to solve the equation $x^2/(F-x) = K$ by using the roots of a quadratic equation. The input will be F and K. The output is the positive value of x. Use your spreadsheet to check your answer to (a) of Ask Yourself Problem 8-F.

8-37. Excel Goal Seek. Solve the equation $x^2/(F-x) = K$ by using Goal Seek described in Problem 6-24. Guess a value of x in cell A4 and evaluate $x^2/(F-x)$ in cell B4. Use Goal Seek to vary the value of x until $x^2/(F-x)$ is equal to K. Use your spreadsheet to check your answer to (a) of Ask Yourself Problem 8-F.

	A	B
1	Using Excel Goal Seek	
2		
3	x	$x^2/(F-x)$
4	0.01	1.11E-03
5	F =	
6	0.1	

How Would You Do It?

8-38. *Weighted average pH in precipitation.* The following data were reported for rainfall at the Philadelphia airport in 1990:

Season	Precipitation (cm)	Weighted average pH
Winter	17.3	4.40
Spring	30.5	4.68
Summer	17.8	4.68
Fall	14.7	5.10

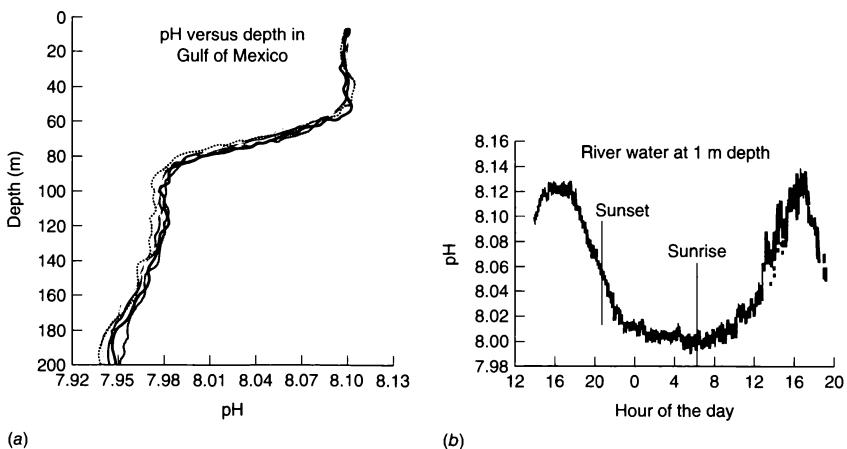
Suggest a procedure to calculate the average pH for the entire year, which would be the pH observed if all the rain for the whole year were pooled in one container.

Notes and References

1. For related demonstrations, see S.-J. Kang and E.-H. Ryu, “Carbon Dioxide Fountain,” *J. Chem. Ed.* **2007**, 84, 1671; M. D. Alexander, “The Ammonia Smoke Fountain,” *J. Chem. Ed.* **1999**, 76, 210.

2. F. S. Lopes, L. H. G. Coelho, and I. G. R. Gutz, *J. Chem. Ed.* **2010**, 87, 157. This article goes on to demonstrate the reaction of H_2SO_3 with formaldehyde vapor. For “A Demonstration of Acid Rain and Lake Acidification: Wet Deposition of Sulfur Dioxide,” see L. M. Goss, *J. Chem. Ed.* **2003**, 80, 39.

Measuring pH of Natural Waters with Acid-Base Indicators

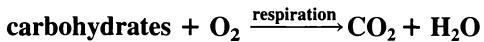
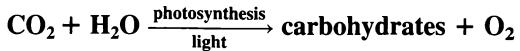


(a) pH in Gulf of Mexico measured with the indicator thymol blue shows reproducibility of four measurements. (b) Variation of pH at 1 m depth in the course of a day in a river in Florida, measured with the indicator phenol red. [From X. Liu, Z. A. Wang, R. H. Byrne, E. A. Kaltenbacher, and R. E. Bernstein, *Environ. Sci. Technol.* **2006**, *40*, 5036.]

Acid-base indicators, which we study in this chapter, are compounds whose color changes over a certain pH range. The color change that enables us to observe the end point of a titration can be used to measure pH with a spectrophotometer. The spectrophotometer measures the ratio of the acidic and basic forms of the indicator, from which we find pH with Equation 9-4.

Glass pH electrodes are convenient in the lab but are not well suited to long-term environmental measurements because electrode potentials drift. We compensate for drift in the lab by frequent calibration in standard buffers. To monitor pH in natural waters, a spectrophotometer measures the *ratio* of absorbance of light at wavelengths corresponding to the two colored forms of an indicator. This ratio provides stable measurements without calibration. The precision (repeatability) of shipboard spectrophotometric pH measurements is $\sim 0.000\text{4}$ pH units.

pH measurements in panel *a* show a distinct upper layer of the ocean (called the *mixed layer*) extending to a depth of $\sim 50\text{--}80$ m. The upper layer, which is mixed by wind and water currents, is less salty and has higher pH than water below. Sunlight penetrating this layer enables photosynthesis by phytoplankton to occur. Photosynthesis consumes CO₂ (a weak acid) from the water, thereby raising the pH. In river water (panel *b*), photosynthesis by microorganisms raises the pH by consumption of CO₂ during daylight hours. At night, respiration restores CO₂ to the water and lowers the pH (Demonstration 9-2).



Buffers

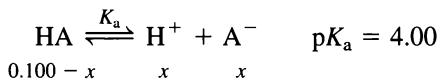
A buffered solution resists changes in pH when small amounts of acids or bases are added or when it is diluted. The **buffer** consists of a mixture of a weak acid and its conjugate base. The two forms of an *acid-base indicator*, which we introduce at the end of this chapter, have the same chemistry as a buffer.

Biochemists are particularly interested in buffers because the functioning of biological systems depends critically on pH. For example, Figure 9-1 shows how the rate of a particular enzyme-catalyzed reaction varies with pH. Enzymes are proteins that *catalyze* (increase the rate of) selected chemical reactions. Organisms control the pH of subcellular compartments so that enzyme-catalyzed reactions can proceed at the proper rate.

9-1 | What You Mix Is What You Get

If you mix A moles of a weak acid with B moles of its conjugate base, the moles of acid remain close to A and the moles of base remain close to B. Little reaction occurs to change either concentration.

To understand why this should be so, look at the K_a and K_b reactions in terms of Le Châtelier's principle. Consider an acid with $pK_a = 4.00$ and its conjugate base with $pK_b = 10.00$. We will calculate the fraction of acid that dissociates in a 0.100 M solution of HA.

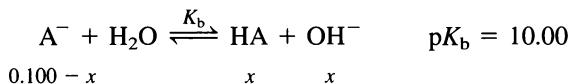


$$\frac{x^2}{F - x} = K_a = 1.0 \times 10^{-4} \Rightarrow x = 3.1 \times 10^{-3} \text{ M}$$

$$\text{fraction of dissociation} = \frac{[\text{A}^-]}{[\text{A}^-] + [\text{HA}]} = \frac{x}{F} = 0.031$$

The acid is only 3.1% dissociated under these conditions.

In a solution containing 0.100 mol of A^- dissolved in 1.00 L of water, the extent of reaction of A^- with water is even smaller:



$$\frac{x^2}{F - x} = K_b = 1.0 \times 10^{-10} \Rightarrow x = 3.2 \times 10^{-6} \text{ M}$$

$$\text{fraction of association} = \frac{[\text{HA}]}{[\text{A}^-] + [\text{HA}]} = \frac{x}{F} = 3.2 \times 10^{-5}$$

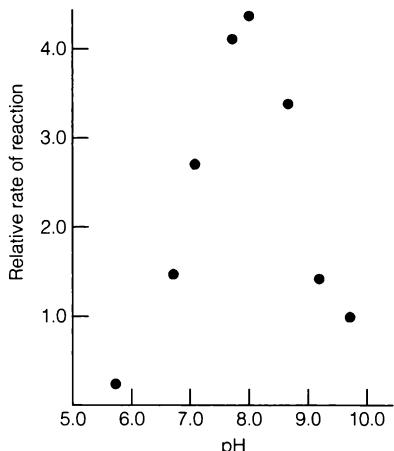
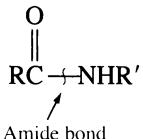


Figure 9-1 pH dependence of the rate of cleavage of an amide bond by the enzyme chymotrypsin.



The rate near pH 8 is twice as great as the rate near pH 7 or pH 9. Chymotrypsin helps digest proteins in your intestine. [From M. L. Bender, G. E. Clement, F. J. Kézdy, and H. A. Heck, *J. Am. Chem. Soc.* **1964**, 86, 3680.]

F is the formal concentration of HA, which is 0.100 M in this example.

When you mix a weak acid and its conjugate base, what you mix is what you get. This approximation breaks down if the solution is too dilute or the acid or base is too weak. We will not consider these cases.

HA dissociates very little, and Le Châtelier's principle tells us that adding extra A⁻ to the solution will make the HA dissociate even less. Similarly, A⁻ does not react very much with water, and adding extra HA makes A⁻ react even less. If 0.050 mol of A⁻ plus 0.036 mol of HA are added to water, there will be close to 0.050 mol of A⁻ and close to 0.036 mol of HA in the solution at equilibrium.

9-2 The Henderson-Hasselbalch Equation

The central equation for buffers is the **Henderson-Hasselbalch equation**, which is merely a rearranged form of the K_a equilibrium expression:

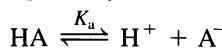
$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$\log K_a = \log\left(\frac{[H^+][A^-]}{[HA]}\right) = \log[H^+] + \log\left(\frac{[A^-]}{[HA]}\right)$$

Now rearrange to isolate $-\log[H^+]$:

$$\underbrace{-\log[H^+]}_{\text{pH}} = \underbrace{-\log K_a}_{\text{p}K_a} + \log\left(\frac{[A^-]}{[HA]}\right)$$

Henderson-Hasselbalch equation for an acid:



$$\text{pH} = \text{p}K_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

Base in numerator
Acid in denominator

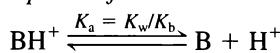
(9-1)

L. J. Henderson was a physician who wrote $[H^+] = K_a[\text{acid}]/[\text{salt}]$ in a physiology article in 1908, a year before the word “buffer” and the concept of pH were invented by the biochemist Sørensen. Henderson’s contribution was the approximation of setting [acid] equal to the concentration of HA placed in solution and [salt] equal to the concentration of A⁻ placed in solution. In 1916, K. A. Hasselbalch wrote what we call the Henderson-Hasselbalch equation in a biochemical journal.¹

The Henderson-Hasselbalch equation tells us the pH of a solution, provided we know the ratio of concentrations of conjugate acid and base, as well as p K_a for the acid.

For the weak base B and its conjugate acid, the analogous equation is

Henderson-Hasselbalch equation for a base:



$$\text{pH} = \text{p}K_a + \log\left(\frac{[B]}{[BH^+]}\right)$$

Base in numerator
p K_a applies to this acid

(9-2)

where p K_a is the acid dissociation constant of the weak acid BH⁺. The important features of Equations 9-1 and 9-2 are that (1) the base (A⁻ or B) appears in the numerator and (2) p K_a applies to the acid in the denominator.

When $[A^-] = [HA]$, $\text{pH} = \text{p}K_a$

When the concentrations of A⁻ and HA are equal in Equation 9-1, the log term is 0 because $\log(1) = 0$. Therefore, when $[A^-] = [HA]$, $\text{pH} = \text{p}K_a$.

$$\text{pH} = \text{p}K_a + \log\left(\frac{[A^-]}{[HA]}\right) = \text{p}K_a + \log(1) = \text{p}K_a$$

Whenever $\text{pH} = \text{p}K_a$, $[\text{A}^-]$ must equal $[\text{HA}]$. This relation is true because *all equilibria must be satisfied simultaneously in any solution at equilibrium*. If there are 10 different acids and bases in the solution, the 10 forms of Equation 9-1 must all give the same pH, because **there can be only one concentration of H^+ in a solution**.

When $[\text{A}^-]/[\text{HA}]$ Changes by a Factor of 10, the pH Changes by One Unit

The Henderson-Hasselbalch equation tells us that a factor-of-10 change in the ratio $[\text{A}^-]/[\text{HA}]$ changes the pH by one unit (Table 9-1). As $[\text{A}^-]$ increases, pH goes up. As $[\text{HA}]$ increases, the pH goes down. For any conjugate acid-base pair, you can say, for example, that, if $\text{pH} = \text{p}K_a - 1$, there must be 10 times as much HA as A^- . HA is ten-elevenths of the mixture and A^- is one-eleventh.

Table 9-1 Change of pH with change of $[\text{A}^-]/[\text{HA}]$

$[\text{A}^-]/[\text{HA}]$	pH
100 : 1	$\text{p}K_a + 2$
10 : 1	$\text{p}K_a + 1$
1 : 1	$\text{p}K_a$
1 : 10	$\text{p}K_a - 1$
1 : 100	$\text{p}K_a - 2$

If $\text{pH} = \text{p}K_a$, $[\text{HA}] = [\text{A}^-]$.

If $\text{pH} < \text{p}K_a$, $[\text{HA}] > [\text{A}^-]$.

If $\text{pH} > \text{p}K_a$, $[\text{HA}] < [\text{A}^-]$.

Example Using the Henderson-Hasselbalch Equation

Sodium hypochlorite (NaOCl , the active ingredient of bleach) was dissolved in a solution buffered to pH 6.20. Find the ratio $[\text{OCl}^-]/[\text{HOCl}]$ in this solution.

SOLUTION OCl^- is the conjugate base of hypochlorous acid, HOCl . In Appendix B, we find that $\text{p}K_a = 7.53$ for HOCl . Knowing the pH, we calculate the ratio $[\text{OCl}^-]/[\text{HOCl}]$ from the Henderson-Hasselbalch equation:

$$\text{HOCl} \rightleftharpoons \text{H}^+ + \text{OCl}^- \quad \text{pH} = \text{p}K_a + \log\left(\frac{[\text{OCl}^-]}{[\text{HOCl}]}\right)$$

$$6.20 = 7.53 + \log\left(\frac{[\text{OCl}^-]}{[\text{HOCl}]}\right)$$

$$-1.33 = \log\left(\frac{[\text{OCl}^-]}{[\text{HOCl}]}\right)$$

To solve this equation, raise 10 to the power shown on each side:

$$10^{-1.33} = 10^{\log([\text{OCl}^-]/[\text{HOCl}])} = \frac{[\text{OCl}^-]}{[\text{HOCl}]}$$

$$0.047 = \frac{[\text{OCl}^-]}{[\text{HOCl}]}$$

To find $10^{-1.33}$ with my calculator, I use the 10^x function, with $x = -1.33$. If you have the *antilog* function instead of 10^x on your calculator, you should compute $\text{antilog}(-1.33)$.

Finding the ratio $[\text{OCl}^-]/[\text{HOCl}]$ requires only pH and $\text{p}K_a$. We do not care what else is in the solution, how much NaOCl was added, or what the volume of the solution is.

 **Test Yourself** Find the ratio $[\text{OCl}^-]/[\text{HOCl}]$ if $\text{pH} = 7.20$. (Answer: 0.47, which makes sense—when pH changes by 1 unit, the ratio changes by 1 power of 10.)

When we say that the solution is buffered to pH 6.20, we mean that unspecified acids and bases were used to set the pH to 6.20. If buffering is sufficient, then adding a little more acid or base does not change the pH appreciably.

It doesn't matter how the pH got to 6.20. If we know that $\text{pH} = 6.20$, the Henderson-Hasselbalch equation tells us the ratio $[\text{OCl}^-]/[\text{HOCl}]$.

If $a = b$, then $10^a = 10^b$.

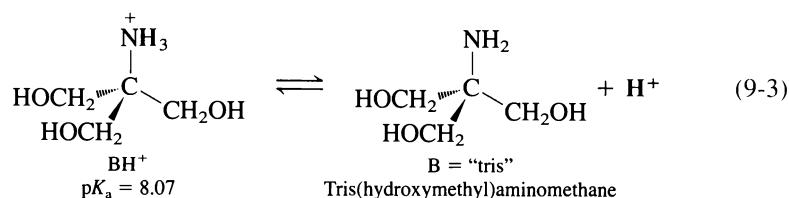
$$10^{\log a} = a$$

Ask Yourself

- 9-A. (a) What is the pH of a buffer prepared by dissolving 0.100 mol of the weak acid HA ($K_a = 1.0 \times 10^{-5}$) plus 0.050 mol of its conjugate base Na^+A^- in 1.00 L?
 (b) Write the Henderson-Hasselbalch equation for a solution of formic acid, HCO_2H . What is the quotient $[\text{HCO}_2^-]/[\text{HCO}_2\text{H}]$ at pH 3.00, 3.744, and 4.00?

9-3 A Buffer in Action

For illustration, we choose a widely used buffer called “tris.”



In Appendix B, we find $\text{p}K_a$ for BH^+ , the conjugate acid of tris, to be 8.07. An example of a salt containing BH^+ is tris hydrochloride, which is BH^+Cl^- . When BH^+Cl^- is dissolved in water, it dissociates completely to BH^+ and Cl^- . To find the pH of a known mixture of B and BH^+ , simply plug their concentrations into the Henderson-Hasselbalch equation.

Example A Buffer Solution

Find the pH of a 1.00-L aqueous solution containing 12.43 g of tris (FM 121.14) plus 4.67 g of tris hydrochloride (FM 157.60).

SOLUTION The concentrations of B and BH^+ added to the solution are

$$[\text{B}] = \frac{12.43 \text{ g/L}}{121.14 \text{ g/mol}} = 0.1026 \text{ M} \quad [\text{BH}^+] = \frac{4.67 \text{ g/L}}{157.60 \text{ g/mol}} = 0.0296 \text{ M}$$

Assuming that what we mixed stays in the same form, we insert the concentrations into the Henderson-Hasselbalch equation to find the pH:

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{B}]}{[\text{BH}^+]}\right) = 8.07 + \log\left(\frac{0.1026}{0.0296}\right) = 8.61$$

 **Test Yourself** Find the pH if we had mixed 4.67 g of tris with 12.43 g of tris hydrochloride. (Answer: 7.76; makes sense—more acid and less base give a lower pH.)

Don't panic over significant figures in pH. For consistency, we are almost always going to express pH to the 0.01 place.

Notice that *the volume of solution is irrelevant to finding the pH*, because volume cancels in the numerator and denominator of the log term:

$$\begin{aligned}\text{pH} &= \text{p}K_a + \log\left(\frac{\text{moles of B/L of solution}}{\text{moles of } \text{BH}^+/\text{L of solution}}\right) \\ &= \text{p}K_a + \log\left(\frac{\text{moles of B}}{\text{moles of } \text{BH}^+}\right)\end{aligned}$$

The pH of a buffer is nearly independent of dilution.

If strong acid is added to a buffered solution, some of the buffer base will be converted into the conjugate acid and the quotient $[\text{B}]/[\text{BH}^+]$ changes. If a strong base is added to a buffered solution, some BH^+ is converted into B. Knowing how much strong acid or base is added, we can compute the new quotient $[\text{B}]/[\text{BH}^+]$ and the new pH.

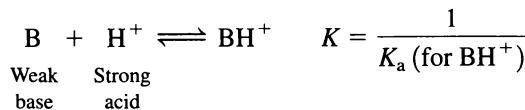
Example Effect of Adding Acid to a Buffer

If we add 12.0 mL of 1.00 M HCl to the solution in the preceding example, what will the new pH be?

SOLUTION The key to this problem is to realize that, **when a strong acid is added to a weak base, they react completely to give BH^+** (Box 9-1). We add 12.0 mL

Box 9-1 Strong Plus Weak Reacts Completely

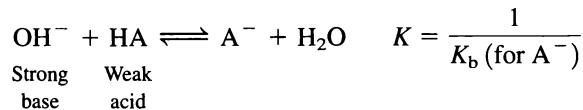
A strong acid reacts with a weak base “completely” because the equilibrium constant is large:



If B is tris, then the equilibrium constant for reaction with HCl is

$$K = \frac{1}{K_a} = \frac{1}{10^{-8.07}} = 1.2 \times 10^8 \leftarrow \text{A big number}$$

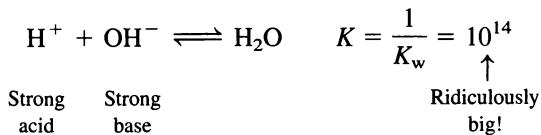
A strong base reacts “completely” with a weak acid because the equilibrium constant is, again, very large:



If HA is acetic acid, then the equilibrium constant for reaction with NaOH is

$$K = \frac{1}{K_b} = \frac{K_a \text{ (for HA)}}{K_w} = 1.7 \times 10^9 \leftarrow \text{Another big number}$$

The reaction of a strong acid with a strong base is even more complete than a strong-plus-weak reaction:



If you mix a strong acid, a strong base, a weak acid, and a weak base, the strong acid and base will neutralize each other until one is used up. The remaining strong acid or base will then react with the weak base or weak acid.



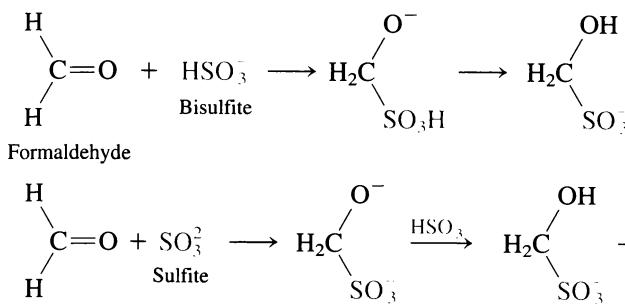
Demonstration 9-1 How Buffers Work

A buffer resists changes in pH because the added acid or base is consumed by the buffer. As the buffer is used up, it becomes less resistant to changes in pH.

In this demonstration,² a mixture containing a 10 : 1 mole ratio of HSO_3^- : SO_3^{2-} is prepared. Because pK_a for HSO_3^- is 7.2, the pH should be approximately

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{SO}_3^{2-}]}{[\text{HSO}_3^-]}\right) = 7.2 + \log\left(\frac{1}{10}\right) = 6.2$$

When formaldehyde is added, the net reaction is the consumption of HSO_3^- , but not of SO_3^{2-} .



(Sequence A consumes bisulfite. In sequence B, the net reaction is destruction of HSO_3^- , with no change in the SO_3^{2-} concentration.)

We can prepare a table showing how the pH should change as the HSO_3^- reacts:

Percentage of reaction completed	$[\text{SO}_3^{2-}] : [\text{HSO}_3^-]$	Calculated pH
0	1 : 10	6.2
90	1 : 1	7.2
99	1 : 0.1	8.2
99.9	1 : 0.01	9.2
99.99	1 : 0.001	10.2

Through 90% of the reaction, pH rises by just one unit. In the next 9%, pH rises by another unit. At the end of the reaction, pH changes abruptly.

In the formaldehyde clock reaction, $\text{H}_2\text{C}=\text{O}$ is added to a solution containing HSO_3^- , SO_3^{2-} , and phenolphthalein indicator. Phenolphthalein is colorless below pH 8.5 and pink above this pH. We observe that the solution remains colorless for more than a minute

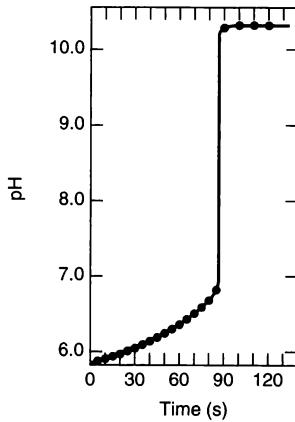
after adding formaldehyde. Suddenly the pH shoots up and the liquid turns pink.

The hydrogen sulfite ion, HSO_3^- , is obtained for this demonstration from sodium metabisulfite, $\text{Na}_2\text{S}_2\text{O}_5$, plus water: $\text{S}_2\text{O}_5^{2-} + \text{H}_2\text{O} \rightleftharpoons 2\text{HSO}_3^-$

Procedure: Prepare phenolphthalein solution by dissolving 50 mg of solid indicator in 50 mL of ethanol and diluting with 50 mL of water. The following solutions should be fresh: Dilute 9 mL of 37 wt% formaldehyde to 100 mL. (CAUTION Formaldehyde is a carcinogen.) Dissolve 1.4 g of $\text{Na}_2\text{S}_2\text{O}_5$ and 0.18 g of Na_2SO_3 in 400 mL of water and add 1 mL of phenolphthalein indicator solution. To initiate the clock reaction, add 23 mL of formaldehyde solution to the well-stirred buffer solution. Reaction time is affected by temperature, concentrations, and volume.

For a less toxic variant of this dem-

onstration use glyoxal ($\text{HC}=\text{CH}_2$) in place of formaldehyde.³ Prepare phenol red indicator by dissolving 0.010 g in 2.82 mL of 0.01 M NaOH and adding 22 mL H_2O . A day in advance, dilute 2.9 g of 40 wt% glyoxal to 25 mL with H_2O . Dissolve 0.90 g $\text{Na}_2\text{S}_2\text{O}_5$, 0.15 g Na_2SO_3 , and 0.18 g $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (to protect sulfite from metal-catalyzed air oxidation) in 50 mL H_2O . For the demonstration, add 0.5 mL phenol red indicator to 400 mL H_2O plus 5.0 mL sulfite solution. Add 2.5 mL glyoxal solution to the well-stirred sulfite solution to start the clock reaction.



Measured pH versus time in the formaldehyde clock reaction.

of 1.00 M HCl, which contain $(0.012\text{ L})(1.00\text{ mol/L}) = 0.012\text{ mol}$ of H^+ . The H^+ consumes 0.012 0 mol of B to create 0.012 0 mol of BH^+ :

	B tris	+	H^+ From HCl	→	BH^+
Initial moles	0.102 6		0.012 0		0.029 6
Final moles	0.102 6 – 0.012 0		—		0.029 6 + 0.012 0
	$\underbrace{0.090\text{ 6}}$				$\underbrace{0.041\text{ 6}}$

$$\text{pH} = \text{p}K_a + \log\left(\frac{\text{moles of B}}{\text{moles of } \text{BH}^+}\right)$$

$$= 8.07 + \log\left(\frac{0.090\text{ 6}}{0.041\text{ 6}}\right) = 8.41$$

Question Does the pH change in the right direction when HCl is added?

Again, the volume of solution is irrelevant.

 **Test Yourself** What would be the pH if we had added 12.0 mL of 1.00 M NaOH instead of HCl? (**Answer:** 8.88, which makes sense—pH goes up when we add base.)

The example shows that *the pH of a buffer does not change very much when a limited amount of a strong acid or base is added*. Addition of 12.0 mL of 1.00 M HCl changed the pH from 8.61 to 8.41. Addition of 12.0 mL of 1.00 M HCl to 1.00 L of pure water would have lowered the pH from 7.00 to 1.93.

But *why* does a buffer resist changes in pH? **It does so because the strong acid or base is consumed by B or BH^+ .** If you add HCl to tris, B is converted into BH^+ . If you add NaOH, BH^+ is converted into B. As long as you don't use up B or BH^+ by adding too much HCl or NaOH, the log term of the Henderson-Hasselbalch equation does not change very much and the pH does not change very much. A factor-of-10 change in $[\text{B}]/[\text{BH}^+]$ changes the pH by only one unit. Demonstration 9-1 illustrates what happens when the buffer *is* used up. The buffer has its maximum capacity to resist changes of pH when $\text{pH} = \text{p}K_a$. We return to this point later.

A buffer resists changes in pH . . .

. . . because the buffer consumes the added acid or base.

Ask Yourself

- 9-B. (a) What is the pH of a solution prepared by dissolving 10.0 g of tris plus 10.0 g of tris hydrochloride in 0.250 L water?
 (b) What will the pH be if 10.5 mL of 0.500 M HClO_4 are added to (a)?
 (c) What will the pH be if 10.5 mL of 0.500 M NaOH are added to (a)?

9-4 Preparing Buffers

Buffers are usually prepared by starting with a measured amount of either a weak acid (HA) or a weak base (B). Then OH^- is added to HA to make a mixture of HA and A^- (a buffer) or H^+ is added to B to make a mixture of B and BH^+ (a buffer).

Example Calculating How to Prepare a Buffer Solution

How many milliliters of 0.500 M NaOH should be added to 10.0 g of tris hydrochloride (BH^+ , Equation 9-3) to give a pH of 7.60 in a final volume of 250 mL?

SOLUTION The number of moles of tris hydrochloride in 10.0 g is $(10.0 \text{ g}) / (157.60 \text{ g/mol}) = 0.0635$. We can make a table to help solve the problem:

<i>Reaction with OH^-:</i>	BH^+	+	OH^-	\longrightarrow	B
Initial moles	0.0635		x		—
Final moles	$0.0635 - x$			—	x

The Henderson-Hasselbalch equation allows us to find x , because we know pH and pK_a :

$$\begin{aligned}\text{pH} &= pK_a + \log\left(\frac{\text{mol B}}{\text{mol } \text{BH}^+}\right) \\ 7.60 &= 8.07 + \log\left(\frac{x}{0.0635 - x}\right) \\ -0.47 &= \log\left(\frac{x}{0.0635 - x}\right)\end{aligned}$$

To solve for x , raise 10 to the power of the terms on both sides, remembering that $10^{\log z} = z$:

$$\begin{aligned}10^{-0.47} &= 10^{\log[x/(0.0635 - x)]} \\ 0.339 &= \frac{x}{0.0635 - x} \Rightarrow x = 0.0161 \text{ mol}\end{aligned}$$

This many moles of NaOH is contained in

$$\frac{0.0161 \text{ mol}}{0.500 \text{ mol/L}} = 0.0322 \text{ L} = 32.2 \text{ mL}$$

Our calculation tells us to mix 32.2 mL of 0.500 M NaOH with 10.0 g of tris hydrochloride to get a pH of 7.60.

 **Test Yourself** How much 0.500 M NaOH should be added to 12.0 g of tris hydrochloride to reach pH 7.77 in a final volume of 317 mL? (Answer: 50.8 mL; volume is irrelevant)

Preparing a Buffer in Real Life

When you mix calculated quantities of acid and base to make a buffer, the pH *does not* come out exactly as expected. The main reason for the discrepancy is that pH is governed by the *activities* of the conjugate acid-base pair, not by the concentrations. (Activity is discussed in Section 12-2.) If you really want to prepare tris buffer at pH 7.60, you should use a pH electrode to get exactly what you need.

Suppose you wish to prepare 1.00 L of buffer containing 0.100 M tris at pH 7.60. When we say 0.100 M tris, we mean that the total concentration of tris plus

trisH^+ will be 0.100 M. You have available solid tris hydrochloride and \sim 1 M NaOH. Here's how to do it:

1. Weigh out 0.100 mol tris hydrochloride and dissolve it in a beaker containing about 800 mL water and a stirring bar.
2. Place a pH electrode in the solution and monitor the pH.
3. Add NaOH solution until the pH is exactly 7.60. Allow time for the pH reading to stabilize after each addition of reagent.
4. Transfer the solution to a volumetric flask and wash the beaker and stirring bar a few times. Add the washings to the volumetric flask.
5. Dilute to the mark and mix.

The reason for using 800 mL of water in the first step is so that the volume will be close to the final volume during pH adjustment. Otherwise, the pH will change slightly when the sample is diluted to its final volume because the *ionic strength* changes.

Before making a buffer, calculate how much strong acid or base will be required. It helps to know if you are going to need 10 mL or 10 drops of reagent before you dispense it.



Ask Yourself

- 9-C. (a) How many milliliters of 1.20 M HCl should be added to 10.0 g of tris (B, Equation 9-3) to give a pH of 7.60 in a final volume of 250 mL?
(b) How would you prepare 100.0 mL of 0.200 M acetate buffer, pH 5.00, starting with pure liquid acetic acid and solutions of \sim 3 M HCl and \sim 3 M NaOH?

Ionic strength is a measure of the total concentration of ions in a solution. Changing ionic strength changes activities of ionic species such as H^+ and A^- . Diluting a buffer with water changes the ionic strength and therefore changes the pH slightly.

9-5 Buffer Capacity

Buffer capacity measures how well a solution resists changes in pH when acid or base is added. The greater the buffer capacity, the smaller the pH change. We will find that *buffer capacity is maximum when $\text{pH} = \text{p}K_a$ for the buffer*.

Figure 9-2 shows the calculated response of a buffer to small additions of H^+ or OH^- . The buffer is a mixture of HA (with acid dissociation constant $K_a = 10^{-5}$)

The greater the buffer capacity, the less the pH changes when H^+ or OH^- is added. Buffer capacity is maximum when $\text{pH} = \text{p}K_a$.

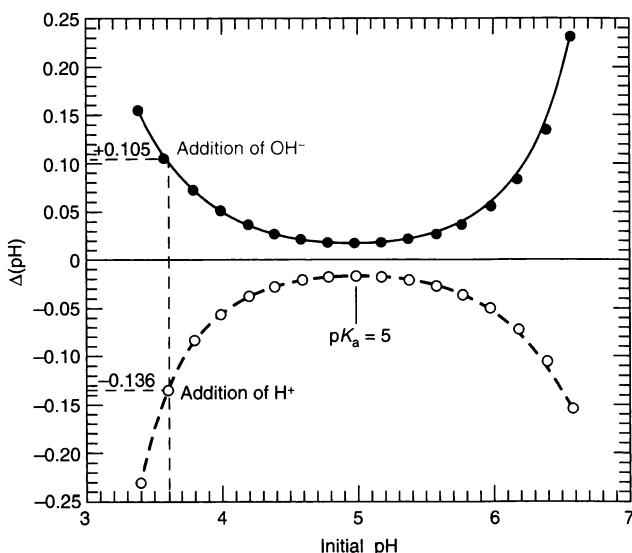


Figure 9-2 Buffer capacity: Effect of adding 0.01 mol of H^+ or OH^- to a buffer containing HA and A^- (total quantity of HA + A^- = 1 mol). The minimum change in pH occurs when the initial pH of the buffer equals $\text{p}K_a$ for HA. That is, buffer capacity is maximum when $\text{pH} = \text{p}K_a$.

plus A^- . The total moles of $HA + A^-$ were set equal to 1. Relative quantities of HA and A^- were varied to give initial pH values from 3.4 to 6.6. Then 0.01 mol of either H^+ or OH^- was added to the solution and the new pH was calculated. Figure 9-2 shows the change in pH, that is, $\Delta(pH)$, as a function of the initial pH of the buffer.

For example, mixing 0.038 3 mol of A^- and 0.961 7 mol of HA gives an initial pH of 3.600:

$$pH = pK_a + \log\left(\frac{\text{mol } A^-}{\text{mol HA}}\right) = 5.000 + \log\left(\frac{0.038\ 3}{0.961\ 7}\right) = 3.600$$

When 0.010 0 mol of OH^- is added to this mixture, the concentrations and pH change in a manner that you are now smart enough to calculate:

<i>Reaction with OH^-:</i>	HA	+	OH^-	→	A^-	+	H_2O
Initial moles	0.961 7		0.010 0		0.038 3		
Final moles	0.961 7 - 0.010 0		—		0.038 3 + 0.010 0		
	$\overbrace{0.951\ 7}$				$\overbrace{0.048\ 3}$		

$$pH = pK_a + \log\left(\frac{\text{mol } A^-}{\text{mol HA}}\right) = 5.000 + \log\left(\frac{0.048\ 3}{0.951\ 7}\right) = 3.705$$

The change in pH is $\Delta(pH) = 3.705 - 3.600 = +0.105$. This is the value plotted in Figure 9-2 on the upper curve for an initial pH of 3.600. If the same starting mixture had been treated with 0.010 0 mol of H^+ , the pH would change by -0.136 , which is shown on the lower curve.

In Figure 9-2, the magnitude of the pH change is smallest when the initial pH is equal to pK_a for the buffer. That is, *the buffer capacity is greatest when $pH = pK_a$* .

In choosing a buffer, you should seek one whose pK_a is as close as possible to the desired pH. The useful pH range of a buffer is approximately $pK_a \pm 1$ pH unit. Outside this range, there is not enough of either the weak acid or the weak base to react with added base or acid. Buffer capacity increases with increasing buffer concentration. To maintain a stable pH, you must use enough buffer to react with the acid or base expected to be encountered.

Table 9-2 lists pK_a for common buffers. Some buffers have more than one acidic proton, so more than one pK is listed. We consider polyprotic acids and bases in Chapter 11.

Buffer pH Depends on Temperature and Ionic Strength

Table 9-2 shows that pK_a for tris decreases by 0.028 per degree near 25°C. A solution of tris made up to pH 8.07 at 25°C will have pH ≈ 8.7 at 4°C and pH ≈ 7.7 at 37°C. When a 0.5 M stock solution of phosphate buffer at pH 6.6 is diluted to 0.05 M, the pH rises to 6.9 because the ionic strength and activities of the buffering species, $H_2PO_4^-$ and HPO_4^{2-} , change.

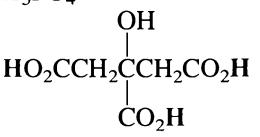
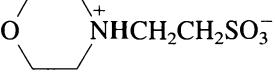
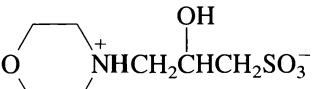
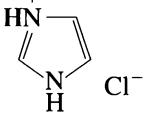
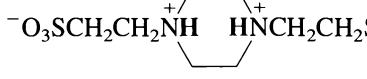
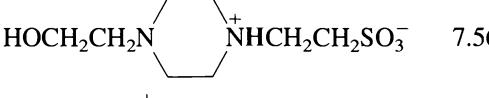
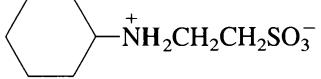
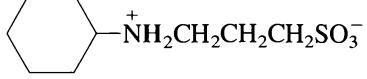
Summary

A buffer is a mixture of a weak acid and its conjugate base. The buffer is most useful when $pH \approx pK_a$. Buffer pH is nearly independent of concentration. A buffer resists changes in pH because it reacts with added acid or base. If too much acid or base is added, the buffer will be consumed and will no longer resist changes in pH.

Choose a buffer whose pK_a is close to the desired pH.

$$\begin{aligned} pK_a(\text{at } 37^\circ\text{C}) &= pK_a(\text{at } 25^\circ\text{C}) + \left(\frac{\Delta pK_a}{\Delta T}\right)(\Delta T) \\ &= 8.07 + (-0.028^\circ\text{C}^{-1})(12^\circ\text{C}) \\ &= 7.73 \end{aligned}$$

Table 9-2 Structures and pK_a values for common buffers

Name	Structure ^a	pK_a ^{b,c}	$\Delta(pK_a)/\Delta T^c$ (K^{-1})	Formula mass
Phosphoric acid	H_3PO_4	2.15 (pK_1)	0.005	98.00
Citric acid		3.13 (pK_1)	-0.002	192.12
Citric acid	$H_2(\text{citrate})^{2-}$	4.76 (pK_2)	-0.001	192.12
Acetic acid	CH_3CO_2H	4.76	0.000	60.05
2-(<i>N</i> -Morpholino)ethane-sulfonic acid (MES)		6.27	-0.009	195.24
Citric acid	$H(\text{citrate})^{2-}$	6.40 (pK_3)	0.002	192.12
3-(<i>N</i> -Morpholino)-2-hydroxypropanesulfonic acid (MOPSO)		6.90	-0.015	225.26
Imidazole hydrochloride		6.99	-0.022	104.54
Piperazine- <i>N,N'</i> -bis(2-ethanesulfonic acid) (PIPES)		7.14	-0.007	302.37
Phosphoric acid	$H_2PO_4^-$	7.20 (pK_2)	-0.002	98.00
<i>N</i> -2-Hydroxyethylpiperazine- <i>N'</i> -2-ethanesulfonic acid (HEPES)		7.56	-0.012	238.30
Tris(hydroxymethyl)aminomethane hydrochloride (tris hydrochloride)	$(HOCH_2)_3CNH_3^+ Cl^-$	8.07	-0.028	157.60
Glycylglycine	$H_3^+NCH_2\overset{\text{O}}{\parallel}CNHCH_2CO_2^-$	8.26	-0.026	132.12
Ammonia	NH_3	9.24	-0.031	17.03
Boric acid	$B(OH)_3$	9.24 (pK_1)	-0.008	61.83
Cyclohexylaminoethanesulfonic acid (CHES)		9.39	-0.023	207.29
3-(Cyclohexylamino)propane-sulfonic acid (CAPS)		10.50	-0.028	221.32
Phosphoric acid	HPO_4^{2-}	12.375 (pK_3)	-0.009	98.00
Boric acid	$OB(OH)_2^-$	12.74 (pK_2)	61.83	

a. The protonated form of each molecule is shown. Acidic hydrogen atoms are shown in bold type.

b. pK_a is generally for 25°C and zero ionic strength.

c. R. N. Goldberg, N. Kishore, and R. M. Lennen, *J. Phys. Chem. Ref. Data* **2002**, 31, 231; A. E. Martell and R. J. Motekaitis, *NIST Database 46* (Gaithersburg, MD: National Institute of Standards and Technology, 2001).

Ask Yourself

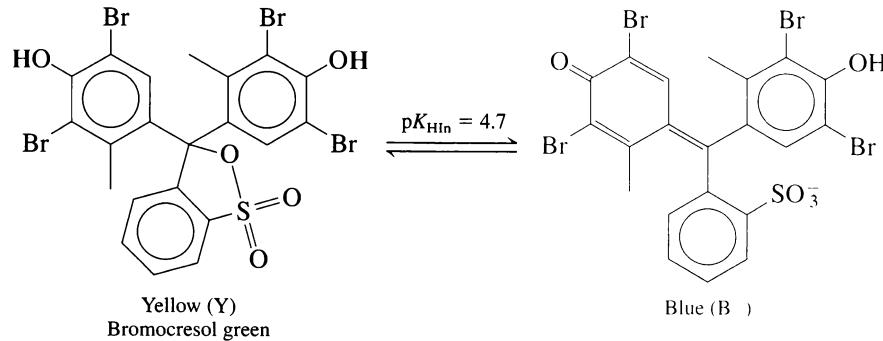
- 9-D. (a) Look up pK_a for each of the following acids and decide which one would be best for preparing a buffer of pH 3.10: (i) hydroxybenzene, (ii) propanoic acid, (iii) cyanoacetic acid, (iv) sulfuric acid.
- (b) Why does buffer capacity increase as the concentration of buffer increases?
- (c) From their K_b values, which of the following bases would be best for preparing a buffer of pH 9.00: (i) NH_3 (ammonia, $K_b = 1.8 \times 10^{-5}$), (ii) $\text{C}_6\text{H}_5\text{NH}_2$ (aniline, $K_b = 4.0 \times 10^{-10}$), (iii) H_2NNH_2 (hydrazine, $K_b = 9.5 \times 10^{-7}$), (iv) $\text{C}_5\text{H}_5\text{N}$ (pyridine, $K_b = 1.6 \times 10^{-9}$)?

9-6 How Acid-Base Indicators Work

An *acid-base indicator* is an acid or a base whose various protonated forms have different colors.

An acid-base **indicator** is itself an acid or base whose various protonated species have different colors. The indicator is added at such a low concentration that it has negligible effect on acid-base equilibria of the major components of the solution. In the next chapter, we will learn how to choose an indicator to find the end point for a titration. For now, let's use the Henderson-Hasselbalch equation to understand the pH range over which color changes are observed.

Consider bromocresol green as an example. We will call pK_a of the indicator pK_{HIn} , to allow us to distinguish it from pK_a of an acid being titrated.



pK_{HIn} for bromocresol green is 4.7. Below pH 4.7, the predominant species is yellow (Y); above pH 4.7, the predominant species is blue (B⁻).

The equilibrium between Y and B⁻ can be written

$$\text{Y} \rightleftharpoons \text{B}^- + \text{H}^+ \quad K_{\text{HIn}} = \frac{[\text{B}^-][\text{H}^+]}{[\text{Y}]}$$

for which the Henderson-Hasselbalch equation is

$$\text{pH} = pK_{\text{HIn}} + \log \left(\frac{[\text{B}^-]}{[\text{Y}]} \right) \quad (9-4)$$

pH	[B ⁻] : [Y]	Color
3.7	1 : 10	Yellow
4.7	1 : 1	Green
5.7	10 : 1	Blue

At $\text{pH} = pK_a = 4.7$, there is a 1:1 mixture of yellow and blue species, which appears green. As a crude rule of thumb, we predict that the solution will appear yellow when $[\text{Y}]/[\text{B}^-] \geq 10/1$ and blue when $[\text{B}]/[\text{Y}] \geq 10/1$. (The symbol \geq means “is approximately equal to or greater than.”) From Equation 9-4, we predict that the solution will be yellow when $\text{pH} \leq pK_{\text{HIn}} - 1 (= 3.7)$ and blue when $\text{pH} \geq pK_{\text{HIn}} + 1 (= 5.7)$. By comparison, Table 9-3 lists bromocresol green as yellow below pH 3.8 and blue above pH 5.4. Between pH 3.8 and 5.4, various shades of green are seen. Demonstration 9-2 illustrates indicator color changes and Box 9-2 describes an everyday application of indicators.

Table 9-3 Common indicators

Indicator	Transition range (pH)	Acid color	Base color	Indicator	Transition range (pH)	Acid color	Base color
Methyl violet	0.0–1.6	Yellow	Violet	Litmus	5.0–8.0	Red	Blue
Cresol red	0.2–1.8	Red	Yellow	Bromothymol blue	6.0–7.6	Yellow	Blue
Thymol blue	1.2–2.8	Red	Yellow	Phenol red	6.4–8.0	Yellow	Red
Cresol purple	1.2–2.8	Red	Yellow	Neutral red	6.8–8.0	Red	Yellow
Erythrosine, disodium	2.2–3.6	Orange	Red	Cresol red	7.2–8.8	Yellow	Red
Methyl orange	3.1–4.4	Red	Yellow	α -Naphtholphthalein	7.3–8.7	Pink	Green
Congo red	3.0–5.0	Violet	Red	Cresol purple	7.6–9.2	Yellow	Purple
Ethyl orange	3.4–4.8	Red	Yellow	Thymol blue	8.0–9.6	Yellow	Blue
Bromocresol green	3.8–5.4	Yellow	Blue	Phenolphthalein	8.0–9.6	Colorless	Pink
Methyl red	4.8–6.0	Red	Yellow	Thymolphthalein	8.3–10.5	Colorless	Blue
Chlorophenol red	4.8–6.4	Yellow	Red	Alizarin yellow	10.1–12.0	Yellow	Orange-red
Bromocresol purple	5.2–6.8	Yellow	Purple	Nitramine	10.8–13.0	Colorless	Orange-brown
p-Nitrophenol	5.6–7.6	Colorless	Yellow	Tropaeolin O	11.1–12.7	Yellow	Orange



Demonstration 9-2 Indicators and Carbonic Acid⁴

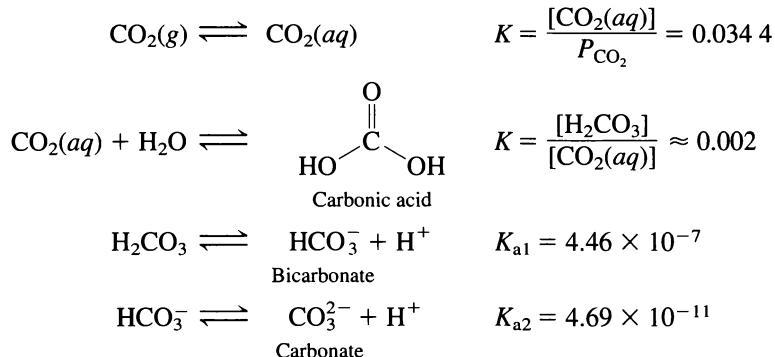
This one is just plain fun. Fill two 1-L graduated cylinders with 900 mL of water plus 10 mL of 1 M NH₃ and place a magnetic stirring bar in each. Then put 2 mL of phenolphthalein indicator solution in one and 2 mL of bromothymol blue indicator solution in the other. Both indicators will have the color of their basic species.

Drop a few chunks of Dry Ice (solid CO₂) into each cylinder. As CO₂ bubbles through each cylinder, the solutions become acidic. First, pink phenolphthalein color

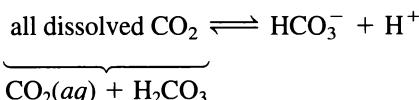
disappears. Then bromothymol blue changes from blue to its pale green intermediate color. The pH does not go low enough to turn bromothymol blue into its yellow color.

Add about 20 mL of 6 M HCl to *the bottom* of each cylinder through Tygon tubing attached to a funnel. Then stir for a few seconds on a magnetic stirrer. Explain what happens. The sequence of events is shown in Color Plate 4.

CO₂ in water makes carbonic acid, which has two acidic protons:



The value $K_{a1} = 4.46 \times 10^{-7}$ applies to the equation



$$K_{a1} = \frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{CO}_2(aq) + \text{H}_2\text{CO}_3]} = 4.46 \times 10^{-7}$$

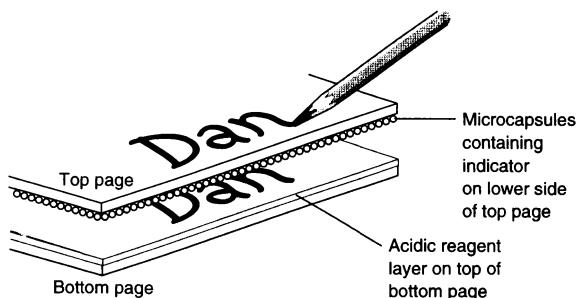
Only about 0.2% of dissolved CO₂ is in the form H₂CO₃. If the true value of [H₂CO₃] were used instead of [H₂CO₃ + CO₂(aq)], the equilibrium constant would be $\sim 2 \times 10^{-4}$.

Box 9-2 The Secret of Carbonless Copy Paper⁵

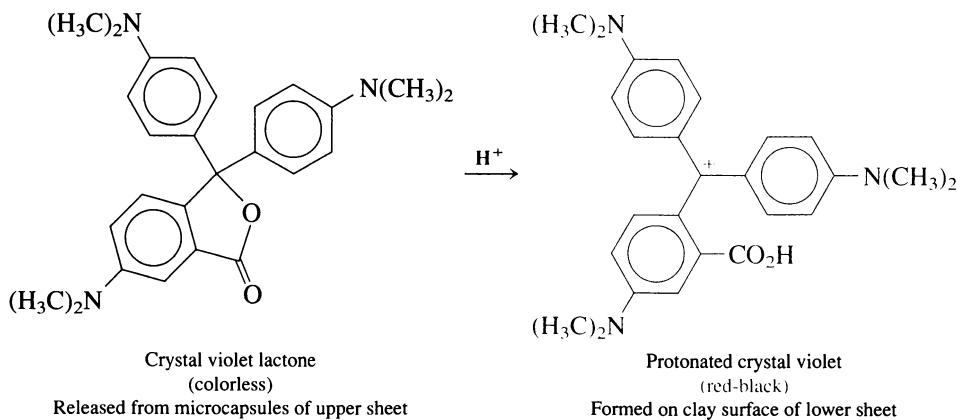
Back in the days when dinosaurs roamed the Earth and I was a boy, people inserted a messy piece of carbon paper between two pages so that a copy of the upper page would appear on the lower page. Then carbonless copy paper was invented to perform the same task without the extra sheet of carbon paper. Alas, now even carbonless copy paper is gone.

Carbonless copy paper has an acid-base indicator inside polymeric microcapsules stuck to the underside of the upper sheet of paper. When you write on the upper sheet, the pressure of your pen breaks the microcapsules on the bottom side to release indicator.

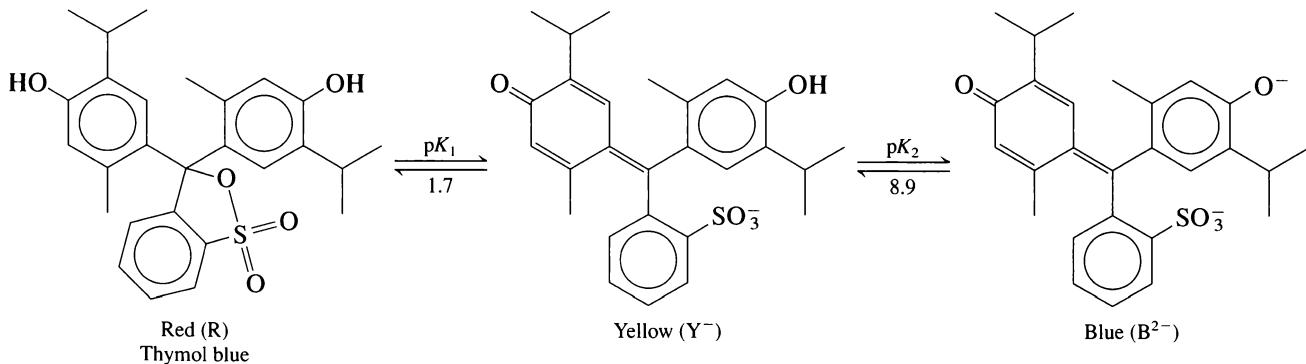
The indicator becomes adsorbed on the upper surface of the lower page, which is coated with microscopic particles of an acidic material such as the clay



bentonite. This clay contains negatively charged aluminosilicate layers with hydronium ion (H_3O^+) between the layers to balance the charge. Adsorbed indicator reacts with H_3O^+ to give a colored product that appears as a copy of what you wrote.



Several indicators are listed twice in Table 9-3, with two different sets of colors. For example, thymol blue loses one proton with a pK of 1.7 and a second proton with a pK of 8.9:



Below pH 1.7, the predominant species is red (R); between pH 1.7 and pH 8.9, the predominant species is yellow (Y^-); and above pH 8.9, the predominant species is blue (B^{2-}). The sequence of color changes for thymol blue is shown in Color Plate 5.

The equilibrium between R and Y^- can be written



$$\text{pH} = \text{p}K_1 + \log\left(\frac{[\text{Y}^-]}{[\text{R}]}\right) \quad (9-5)$$

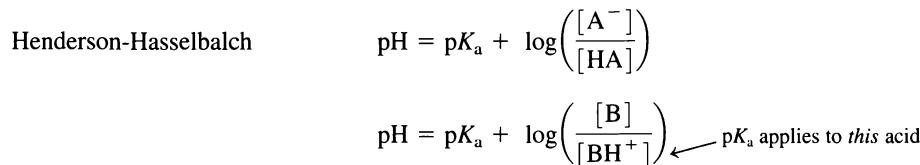
At pH = 1.7 (= pK_1), there is a 1:1 mixture of yellow and red species, which appears orange. We expect the solution to be red when $[R]/[Y^-] \geq 10/1$ and yellow when $[Y^-]/[R] \geq 10/1$. From the Henderson-Hasselbalch equation 9-5, we expect to see red when $\text{pH} \leq pK_1 - 1$ (= 0.7) and yellow when $\text{pH} \geq pK_1 + 1$ (= 2.7). Table 9-3 states that thymol blue is red below pH 1.2 and yellow above pH 2.8. There is another transition, from yellow to blue, between pH 8.0 and pH 9.6. In this range, various shades of green are seen.

pH	[Y ⁻]:[R]	Color
0.7	1 : 10	Red
1.7	1 : 1	Orange
2.7	10 : 1	Yellow

?) Ask Yourself

- 9-E. (a)** Why do indicator color changes occur near $pK_{\text{HIn}} \pm 1$?
(b) What color do you expect for cresol purple (Table 9-3) at pH 1.0, 2.0, and 3.0?

Key Equations



Important Terms

buffer

Henderson-Hasselbalch equation

indicator

Problems

- 9-1.** Explain what happens when acid is added to a buffer and the pH does not change very much.

9-2. Why is the pH of a buffer nearly independent of concentration?

9-3. A solution contains 63 different conjugate acid-base pairs. Among them is acrylic acid and acrylate ion, with the ratio $[\text{acrylate}]/[\text{acrylic acid}] = 0.75$. What is the pH of the solution?

$\text{H}_2\text{C}=\text{CHCO}_2\text{H} \rightleftharpoons \text{H}_2\text{C}=\text{CHCO}_2^- + \text{H}^+$ $pK_a = 4.25$

Acrylic acid	Acrylate
--------------	----------

9-4. Table 9-1 shows the relation of pH to the quotient $[\text{A}^-]/[\text{HA}]$.

(a) Use the Henderson-Hasselbalch equation to show that $\text{pH} = pK_a + 2$ when $[\text{A}^-]/[\text{HA}] = 100$.

(b) Find the quotient $[A^-]/[HA]$ when $pH = pK_a - 3$.

(c) Find the pH when $[A^-]/[HA] = 10^{-4}$.

9-5. Explain why the indicator cresol red changes color when the pH is lowered from 10 to 6. What colors will be observed at pH 10, 8, and 6? Why does the color transition require ~ 2 pH units for completion?

9-6. Given that pK_b for iodate ion (IO_3^-) is 13.83, find the quotient $[HIO_3]/[IO_3^-]$ in a solution of sodium iodate at

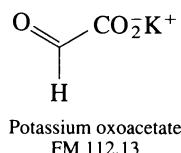
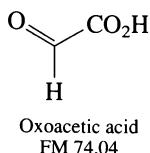
(a) pH 7.00; (b) pH 1.00.

9-7. Given that pK_b for nitrite ion (NO_2^-) is 10.85, find the quotient $[HNO_2]/[NO_2^-]$ in a solution of sodium nitrite at

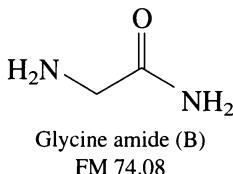
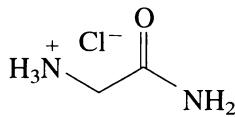
(a) pH 2.00; (b) pH 10.00.

9-8. Write the Henderson-Hasselbalch equation for a solution of methylamine. Calculate the quotient $[CH_3NH_2]/[CH_3NH_3^+]$ at (a) pH 4.00; (b) pH 10.632; (c) pH 12.00.

9-9. Find the pH of a solution prepared from 2.53 g of oxoacetic acid, 5.13 g of potassium oxoacetate, and 103 g of water.



9-10. (a) Find the pH of a solution prepared by dissolving 1.00 g of glycine amide hydrochloride plus 1.00 g of glycine amide in 0.100 L.



(b) How many grams of glycine amide should be added to 1.00 g of glycine amide hydrochloride to give 100 mL of solution with pH 8.00?

(c) What would be the pH if the solution in (a) is mixed with 5.00 mL of 0.100 M HCl?

(d) What would be the pH if the solution in (c) is mixed with 10.00 mL of 0.100 M NaOH?

9-11. (a) Write the chemical reactions whose equilibrium constants are K_b and K_a for imidazole and imidazole hydrochloride, respectively.

(b) Calculate the pH of a 100-mL solution containing 1.00 g of imidazole (FM 68.08) and 1.00 g of imidazole hydrochloride (FM 104.54).

(c) Calculate the pH of the solution if 2.30 mL of 1.07 M $HClO_4$ are added to the solution.

(d) How many milliliters of 1.07 M $HClO_4$ should be added to 1.00 g of imidazole to give a pH of 6.993?

9-12. (a) Calculate the pH of a solution prepared by mixing 0.080 0 mol of chloroacetic acid plus 0.040 0 mol of sodium chloroacetate in 1.00 L of water.

(b) Using first your head and then the Henderson-Hasselbalch equation, find the pH of a solution prepared by dissolving all of the following compounds in a total volume of 1.00 L: 0.180 mol $ClCH_2CO_2H$, 0.020 mol $ClCH_2CO_2Na$, 0.080 mol HNO_3 , and 0.080 mol $Ca(OH)_2$. Assume that $Ca(OH)_2$ dissociates completely.

9-13. How many milliliters of 0.246 M HNO_3 should be added to 213 mL of 0.006 66 M 2,2'-bipyridine to give a pH of 4.19?

9-14. How many milliliters of 0.626 M KOH should be added to a solution containing 5.00 g of HEPES (Table 9-2) to give a pH of 7.40?

9-15. How many milliliters of 0.113 M HBr should be added to 52.2 mL of 0.013 4 M morpholine to give a pH of 8.00?

9-16. For a fixed buffer concentration, such as 0.05 M, which buffer from Table 9-2 will provide the highest buffer capacity at pH (a) 4.00, (b) 7.00, (c) 10.00? (d) What other buffers in Table 9-2 would be useful at pH 10.00?

9-17. Which buffer system will have the greatest buffer capacity at pH 9.0: (i) dimethylamine/dimethylammonium ion, (ii) ammonia/ammonium ion, (iii) hydroxylamine/hydroxylammonium ion, (iv) 4-nitrophenol/4-nitrophenolate ion?

9-18. (a) Would you need NaOH or HCl to bring the pH of 0.050 0 M HEPES (Table 9-2) to 7.45?

(b) Describe how to prepare 0.250 L of 0.050 0 M HEPES, pH 7.45.

9-19. (a) Describe how to prepare 0.500 L of 0.100 M imidazole buffer, pH 7.50, starting with imidazole hydrochloride. Would you use NaOH or HCl to bring the pH to 7.50?

(b) Starting with imidazole, would you need NaOH or HCl to bring the pH to 7.50?

9-20. (a) Calculate how many milliliters of 0.100 M HCl should be added to how many grams of sodium acetate dihydrate ($NaOAc \cdot 2H_2O$, FM 118.06) to prepare 250.0 mL of 0.100 M buffer, pH 5.00.

(b) If you mixed what you calculated, the pH would not be 5.00. Describe how you would actually prepare this buffer in the lab.

9-21. Cresol red has two transition ranges listed in Table 9-3. What color would you expect it to be at the following pH values?

(a) 0 (b) 1 (c) 6 (d) 9

9-22. Consider the buffer in Figure 9-2 with an initial pH of 6.200 and pK_a of 5.000. It is made from p mol of A^- plus q mol of HA such that $p + q = 1$ mol.

(a) Setting mol HA = q and mol A^- = $1 - q$, use the Henderson-Hasselbalch equation to find p and q .

(b) Using three decimal places for pH, calculate the pH change when 0.010 0 mol of H^+ is added to the buffer. Did you get the same value shown in Figure 9-2?

9-23. Ammonia buffer is made up to pH 9.50 at 25°C and then warmed to 37°C. The pH of the solution changes because pK_a changes. Using $\Delta(pK_a)/\Delta T$ from Table 9-2, predict the pH that would be observed at 37°C.

9-24.  *Henderson-Hasselbalch spreadsheet.* Prepare a spreadsheet with the constant $pK_a = 4$ in column A. Type the heading $[\text{A}^-]/[\text{HA}]$ for column B and enter values ranging from 0.001 to 1 000, with a selection of values in between. In column C, compute pH from the Henderson-Hasselbalch equation, by using pK_a from column A and $[\text{A}^-]/[\text{HA}]$ from

column B. In column D, compute $\log([\text{A}^-]/[\text{HA}])$. Use values from columns C and D to prepare a graph of pH versus $\log([\text{A}^-]/[\text{HA}])$. Explain the shape of the resulting graph.

How Would You Do It?

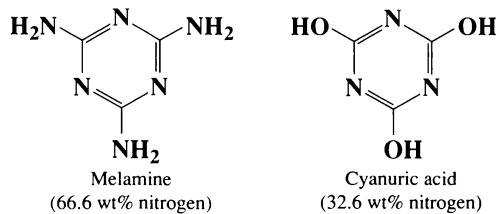
9-25. Consider the spectrophotometric pH measurement of seawater with the indicator thymol blue illustrated at the opening of this chapter. The blue form of the indicator In^{2-} on page 202 has maximum absorbance at a wavelength of 596 nm (nanometers). The yellow form HIn^- has maximum absorbance at 435 nm. Measurements of absorbance at the two wavelengths allows us to find the ratio $[\text{In}^{2-}]/[\text{HIn}^-]$. How would you use this measurement to find the pH?

Notes and References

1. H. N. Po and N. M. Senozan, *J. Chem. Ed.* **2001**, 78, 1499; R. de Levie, *J. Chem. Ed.* **2003**, 80, 146.
2. R. L. Barrett, *J. Chem. Ed.* **1955**, 32, 78.
3. J. B. Early, A. R. Negron, J. Stephens, R. Stauffer, and S. D. Furrow, *J. Chem. Ed.* **2007**, 84, 1965.
4. You can find more indicator demonstrations in J. T. Riley, *J. Chem. Ed.* **1977**, 54, 29. The chemistry of carbonic acid is discussed by M. Kern, *J. Chem. Ed.* **1960**, 37, 14.
5. M. A. White, *J. Chem. Ed.* **1998**, 75, 1119.

Kjeldahl Nitrogen Analysis Behind the Headlines

In 2007, dogs and cats in North America suddenly began to die from kidney failure. The illness was traced to animal food from China. Melamine, used to make plastics, had been deliberately added to food “in a bid to meet the contractual demand for the amount of protein in the products.” Cyanuric acid, used to disinfect swimming pools, was also found in the food. Melamine alone does not cause kidney failure, but the combination of melamine and cyanuric acid makes a crystalline product that does cause kidney failure.



What do these compounds have to do with protein? Nothing—except that they are high in nitrogen. Protein, which contains ~16 wt% nitrogen, is the main source of nitrogen in food. Kjeldahl nitrogen analysis—an acid-base titration method introduced in this chapter—is a surrogate measurement for protein in food. For example, if food contains 10 wt% protein, it will contain ~16% of 10% = 1.6 wt% N. If you find 1.6 wt% N in food, you could conclude that the food contains ~10 wt% protein. Melamine contains 66.6 wt% N—four times more than protein. Adding 1 wt% melamine to food makes it appear that the food contains an additional 4 wt% protein.

Incredibly, in the summer of 2008, approximately 300 000 Chinese babies became sick and at least six died of kidney failure. Chinese companies had diluted milk with water and added melamine to make the protein content appear normal. Two people were executed in 2009 for their role in producing tainted milk. In 2010, Chinese authorities found 40 tons of powdered milk containing melamine.

In response to these events, a spectrophotometric method was developed to distinguish protein from melamine.¹ Also, mass spectrometric² and chromatographic methods were developed to measure melamine and cyanuric acid in foods.

Protein source	Weight percent nitrogen
Meat	16.0
Blood plasma	15.3
Milk	15.6
Flour	17.5
Egg	14.9

From D. J. Holme and H. Peck, *Analytical Biochemistry*, 3rd ed. (New York: Addison Wesley Longman, 1998), p. 388.

Acid-Base Titrations

In a *titration*, we measure the quantity of a known reagent required to react with an unknown sample. From this quantity, we deduce the concentration of analyte in the unknown. Titrations of acids and bases are among the most widespread procedures in chemical analysis. Later in this chapter, we will see how acid-base titrations are used to measure nitrogen in foods, a measure of protein content.

10-1 Titration of Strong Base with Strong Acid

For each type of titration in this chapter, *our goal is to construct a graph showing how the pH changes as titrant is added*. If you can do this, then you understand what is happening during the titration, and you will be able to interpret an experimental titration curve.

The first step is to write the balanced chemical reaction between titrant and analyte. Then use that reaction to calculate the composition and pH after each addition of titrant. Let's consider the titration of 50.00 mL of 0.020 00 M KOH with 0.100 0 M HBr. The chemical reaction between titrant and analyte is merely



Because the equilibrium constant for this reaction is 10^{14} , it is fair to say that it “goes to completion.” Prior to the equivalence point, *any amount of H^+ added will consume a stoichiometric amount of OH^-* .

A useful starting point is to calculate the volume of HBr (V_e) needed to reach the equivalence point:

$$\text{mol HBr at equivalence point} = \text{mol OH}^- \text{ being titrated}$$

$$(\text{equivalence volume})(\text{HBr molarity}) = (\text{initial volume of OH}^-)(\text{OH}^- \text{ molarity})$$

$$\underbrace{(\text{V}_e \text{ (mL)})(0.100 \text{ M})}_{\text{mmol of HBr at equivalence point}} = \underbrace{(50.00 \text{ mL})(0.020 \text{ M})}_{\text{mmol of OH}^- \text{ being titrated}} \Rightarrow \text{V}_e = 10.00 \text{ mL}$$

When 10.00 mL of HBr have been added, the titration is complete. Prior to V_e , excess, unreacted OH^- is present. After V_e , there is excess H^+ in the solution.

First write the reaction between titrant and analyte.

The titration reaction.

Equivalence point: when moles of added titrant are exactly sufficient for stoichiometric reaction with analyte

$$\text{mL} \times \frac{\text{mol}}{\text{L}} = \text{mmol}$$

You can do all calculations with mol and L instead of mmol and mL, if you wish. I find mmol and mL to be more convenient.

In the titration of a strong base with a strong acid, three regions of the titration curve require different kinds of calculations:

1. Before the equivalence point, the pH is determined by excess OH^- in the solution.
2. At the equivalence point, added H^+ is just sufficient to react with all the OH^- to make H_2O . The pH is determined by the dissociation of water.
3. After the equivalence point, pH is determined by excess H^+ in the solution.

We will do one sample calculation for each region.

Region 1: Before the Equivalence Point

Before the equivalence point, there is excess OH^- .

Before we add any HBr titrant from the buret, the flask of analyte contains 50.00 mL of 0.020 00 M KOH, which amounts to $(50.00 \text{ mL})(0.020 00 \text{ M}) = 1.000 \text{ mmol}$ of OH^- . Remember that $\text{mL} \times (\text{mol/L}) = \text{mmol}$.

If we add 3.00 mL of HBr, we add $(3.00 \text{ mL})(0.100 0 \text{ M}) = 0.300 \text{ mmol}$ of H^+ , which consumes 0.300 mmol of OH^- .

$$\text{OH}^- \text{ remaining} = \underbrace{1.000 \text{ mmol}}_{\text{Initial OH}^-} - \underbrace{0.300 \text{ mmol}}_{\text{OH}^- \text{ consumed by HBr}} = 0.700 \text{ mmol}$$

The total volume in the flask is now $50.00 \text{ mL} + 3.00 \text{ mL} = 53.00 \text{ mL}$. Therefore the concentration of OH^- in the flask is

$$\frac{\text{mmol}}{\text{mL}} = \frac{\text{mol}}{\text{L}} = \text{M}$$

$$[\text{OH}^-] = \frac{0.700 \text{ mmol}}{53.00 \text{ mL}} = 0.013 2 \text{ M}$$

From the concentration of OH^- , it is easy to find the pH:

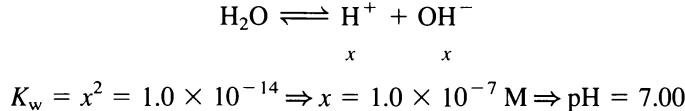
$$[\text{H}^+] = \frac{K_w}{[\text{OH}^-]} = \frac{1.0 \times 10^{-14}}{0.013 2} = 7.5_8 \times 10^{-13} \text{ M}$$
$$\Rightarrow \text{pH} = -\log(7.5_8 \times 10^{-13}) = 12.12$$

Challenge Calculate $[\text{OH}^-]$ and pH when 6.00 mL of HBr have been added. Check your answers against Table 10-1.

If you had to, you could reproduce the calculations prior to the equivalence point in Table 10-1 in the same way as the 3.00-mL point. (A spreadsheet would be helpful.) The volume of acid added is designated V_a , and pH is expressed to the 0.01 decimal place, regardless of what is justified by significant figures. We do this for the sake of consistency and also because 0.01 is near the limit of accuracy in pH measurements.

Region 2: At the Equivalence Point

At the equivalence point, enough H^+ has been added to react with all the OH^- . We could prepare the same solution by dissolving KBr in water. The equivalence point pH of a strong acid-strong base titration is determined by the dissociation of water:



The pH at the equivalence point in the titration of any strong base (or acid) with strong acid (or base) will be 7.00 at 25°C.

Table 10-1 Calculation of the titration curve for 50.00 mL of 0.020 00 M KOH treated with 0.100 0 M HBr

	mL HBr added (V_a)	Concentration of unreacted OH^- (M)	Concentration of excess H^+ (M)	pH
Region 1 (excess OH^-)	0.00	0.020 0		12.30
	1.00	0.017 6		12.24
	2.00	0.015 4		12.18
	3.00	0.013 2		12.12
	4.00	0.011 1		12.04
	5.00	0.009 09		11.95
	6.00	0.007 14		11.85
	7.00	0.005 26		11.72
	8.00	0.003 45		11.53
	9.00	0.001 69		11.22
	9.50	0.000 840		10.92
	9.90	0.000 167		10.22
Region 2	9.99	0.000 016 6		9.22
	10.00	—	—	7.00
Region 3 (excess H^+)	10.01		0.000 016 7	4.78
	10.10		0.000 166	3.78
	10.50		0.000 826	3.08
	11.00		0.001 64	2.79
	12.00		0.003 23	2.49
	13.00		0.004 76	2.32
	14.00		0.006 25	2.20
	15.00		0.007 69	2.11
	16.00		0.009 09	2.04

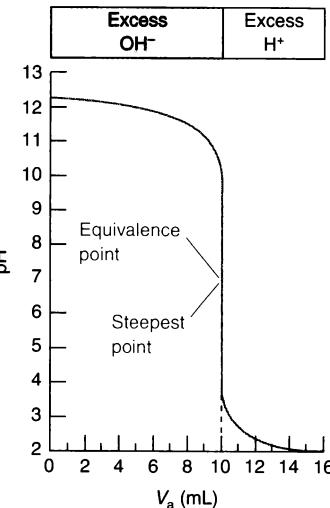


Figure 10-1 Calculated titration curve showing how pH changes as 0.100 0 M HBr is added to 50.00 mL of 0.020 00 M KOH. At the equivalence point, the curve is steepest. The first derivative reaches a maximum and the second derivative is 0. We discuss derivatives later in this chapter.

As we will soon discover, the pH is **not** 7.00 at the equivalence point in the titration of weak acids or bases. The pH is 7.00 only if the titrant and analyte are both strong.

At the equivalence point, $\text{pH} = 7.00$, but **only** in a strong-acid–strong-base reaction.

Region 3: After the Equivalence Point

Beyond the equivalence point, excess HBr is present. For example, at the point where 10.50 mL of HBr have been added, there is an excess of $10.50 - 10.00 = 0.50$ mL. The excess H^+ amounts to

$$\text{excess } \text{H}^+ = (0.50 \text{ mL})(0.100 0 \text{ M}) = 0.050 \text{ mmol}$$

Using the total volume of solution ($50.00 + 10.50 = 60.50$ mL), we find the pH:

$$[\text{H}^+] = \frac{0.050 \text{ mmol}}{60.50 \text{ mL}} = 8.26 \times 10^{-4} \text{ M} \Rightarrow \text{pH} = -\log(8.26 \times 10^{-4}) = 3.08$$

After the equivalence point, there is excess H^+ .

The Titration Curve

The titration curve in Figure 10-1 is a graph of pH versus V_a , the volume of acid added. The sudden change in pH near the equivalence point is characteristic of all analytically useful titrations. The curve is steepest at the equivalence point, which means that the slope is greatest there. The pH at the equivalence point is 7.00 **only**

in a strong-acid-strong-base titration. If one or both of the reactants are weak, the equivalence-point pH is *not* 7.00.

Example Titration of Strong Acid with Strong Base

Find the pH when 12.74 mL of 0.087 42 M NaOH have been added to 25.00 mL of 0.066 66 M HClO₄.

SOLUTION The titration reaction is H⁺ + OH⁻ → H₂O. The equivalence point is

$$\underbrace{(V_e \text{ (mL)})(0.087 \text{ 42 M})}_{\text{mmol of NaOH at equivalence point}} = \underbrace{(25.00 \text{ mL})(0.066 \text{ 66 M})}_{\text{mmol of HClO}_4 \text{ being titrated}} \Rightarrow V_e = 19.06 \text{ mL}$$

At V_b (volume of base) = 12.74 mL, there is excess acid in the solution:

$$\text{H}^+ \text{ remaining} = \underbrace{(25.00 \text{ mL})(0.066 \text{ 66 M})}_{\text{Initial mmol of HClO}_4} - \underbrace{(12.74 \text{ mL})(0.087 \text{ 42 M})}_{\text{Added mmol of NaOH}} = 0.553 \text{ mmol}$$

$$[\text{H}^+] = \frac{0.553 \text{ mmol}}{(25.00 + 12.74) \text{ mL}} = 0.0147 \text{ M}$$

$$\text{pH} = -\log(0.0147) = 1.83$$

 **Test Yourself** Find the pH when 20.00 mL of 0.087 42 M NaOH have been added to 25.00 mL of 0.066 66 M HClO₄. (Answer: 11.26)

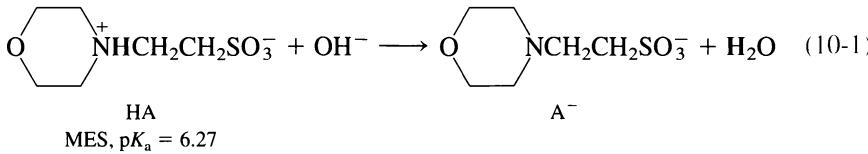
 **Ask Yourself**

10-A. What is the equivalence volume in the titration of 50.00 mL of 0.010 0 M NaOH with 0.100 M HCl? Calculate the pH at the following points: V_a = 0.00, 1.00, 2.00, 3.00, 4.00, 4.50, 4.90, 4.99, 5.00, 5.01, 5.10, 5.50, 6.00, 8.00, and 10.00 mL. Make a graph of pH versus V_a.

10-2 Titration of Weak Acid with Strong Base

The titration of a weak acid with a strong base puts all our knowledge of acid-base chemistry to work. The example we treat is the titration of 50.00 mL of 0.020 00 M MES with 0.100 0 M NaOH. MES is an abbreviation for 2-(*N*-morpholino)ethane-sulfonic acid, a weak acid with pK_a = 6.27. It is widely used in biochemistry as a buffer for the pH 6 region.

The *titration reaction* is



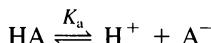
Reaction 10-1 is the reverse of the K_b reaction for the base A^- . The equilibrium constant is $1/K_b = 1/(K_w/K_{HA}) = 5.4 \times 10^7$. The equilibrium constant is so large that we can say that the reaction goes “to completion” after each addition of OH^- . As we saw in Box 9-1, *strong + weak react completely*.

It is helpful first to calculate the volume of base needed to reach the equivalence point. Because 1 mol of OH^- reacts with 1 mol of MES, we can say

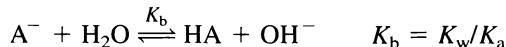
$$\underbrace{(V_e \text{ (mL)})(0.100 \text{ M})}_{\text{mmol of base}} = \underbrace{(50.00 \text{ mL})(0.020 \text{ M})}_{\text{mmol of HA}} \Rightarrow V_e = 10.00 \text{ mL}$$

The titration calculations for this problem are of four types:

- Before any base is added, the solution contains just HA in water. This is a weak-acid problem in which the pH is determined by the equilibrium



- From the first addition of NaOH until immediately before the equivalence point, there is a mixture of unreacted HA plus the A^- produced by Reaction 10-1. *Aha! A buffer!* We can use the Henderson-Hasselbalch equation to find the pH.
- At the equivalence point, “all” HA has been converted into A^- , the conjugate base. Therefore the pH will be higher than 7. The problem is the same as if the solution had been made by dissolving A^- in water. We have a weak-base problem in which pH is determined by the reaction



- Beyond the equivalence point, excess NaOH is being added to a solution of A^- , creating a mixture of strong and weak base. We calculate the pH as if we had simply added excess NaOH to water. We ignore the small effect from A^- .

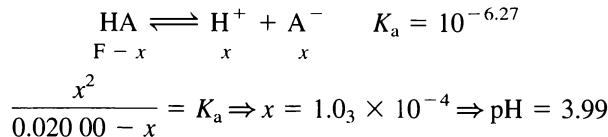
strong + weak →
complete reaction

The four regions of the titration curve are important enough to be shown inside the back cover of this book.

Region 1: Before Base Is Added

Before adding any base, we have a solution of 0.020 00 M HA with $\text{p}K_a = 6.27$. This is simply a weak-acid problem.

The initial solution contains just the *weak acid* HA.



F is the formal concentration of HA, which is 0.020 00 M.

Region 2: Before the Equivalence Point

Once we begin to add OH^- , a mixture of HA and A^- is created by the titration reaction 10-1. This mixture is a buffer whose pH can be calculated with the Henderson-Hasselbalch equation (9-1) once we know the quotient $[\text{A}^-]/[\text{HA}]$.

Before the equivalence point, there is a mixture of HA and A^- , which is a *buffer*. *Aha! A buffer!*

Henderson-Hasselbalch equation:

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right) \quad (9-1)$$

Consider the point where 3.00 mL of OH^- have been added:

<i>Titration reaction:</i>	HA	+	OH^-	\longrightarrow	A^-	+	H_2O
Initial mmol	1.000		0.300		—		
Final mmol	0.700		—		0.300		

↑ ↑
Aha! A buffer! ($\text{HA} + \text{A}^-$)

Once we know the *quotient* $[\text{A}^-]/[\text{HA}]$ in any solution, we know its pH:

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right) = 6.27 + \log\left(\frac{0.300}{0.700}\right) = 5.90$$

The point at which the volume of titrant is $\frac{1}{2}V_e$ is a special one in any titration.

<i>Titration reaction:</i>	HA	+	OH^-	\longrightarrow	A^-	+	H_2O
Initial mmol	1.000		0.500		—		
Final mmol	0.500		—		0.500		

$$\text{pH} = \text{p}K_a + \log\left(\frac{0.500}{0.500}\right) = \text{p}K_a$$

because $\log(1) = 0$. When the volume of titrant is $\frac{1}{2}V_e$, $\text{pH} = \text{p}K_a$ for the acid HA. From the experimental titration curve, you can find $\text{p}K_a$ by reading the pH when $V_b = \frac{1}{2}V_e$, where V_b is the volume of added base.

Advice. As soon as you recognize a mixture of HA and A^- in any solution, *you have a buffer!* Stop right there. You can find the pH from the quotient $[\text{A}^-]/[\text{HA}]$ with the Henderson-Hasselbalch equation.

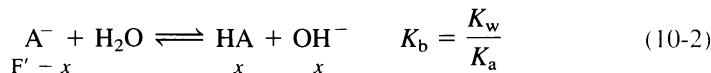
Region 3: At the Equivalence Point

At the equivalence point, HA has been converted into A^- , a *weak base*.

<i>Titration reaction:</i>	HA	+	OH^-	\longrightarrow	A^-	+	H_2O
Initial mmol	1.000		1.000		—		
Final mmol	—		—		1.000		

The resulting solution contains “just” A^- . We could have prepared the same solution by dissolving the salt Na^+A^- in water. Na^+A^- is a *weak base*, so the pH must be >7 .

To compute the pH of a weak base, write the reaction of the base with water:



The only tricky point is that the formal concentration of A^- is no longer 0.020 00 M, which was the initial concentration of HA. The initial 1.000 mmol of HA in 50.00 mL has been diluted with 10.00 mL of titrant:

$$[\text{A}^-] = \frac{1.000 \text{ mmol}}{(50.00 + 10.00) \text{ mL}} = 0.016\ 67 \text{ M} \equiv F'$$

Designating the formal concentration of A^- as F' , we can find the pH from Reaction 10-2:

$$\frac{x^2}{F' - x} = K_b = \frac{K_w}{K_a} = 1.86 \times 10^{-8} \Rightarrow x = 1.76 \times 10^{-5} \text{ M}$$

$$\text{pH} = -\log[H^+] = -\log\left(\frac{K_w}{x}\right) = 9.25$$

The pH at the equivalence point in this titration is 9.25. **It is not 7.00.** The equivalence-point pH will *always* be above 7 for the titration of a weak acid with a strong base, because the acid is converted into its conjugate base at the equivalence point.

pH > 7 at the equivalence point in the titration of a weak acid with a strong base because the product of the reaction is a weak base.

Region 4: After the Equivalence Point

Now we are adding NaOH to a solution of A^- . The base NaOH is so much stronger than the base A^- that it is fair to say that the pH is determined by the excess OH^- .

Let's calculate the pH when $V_b = 10.10 \text{ mL}$, which is just 0.10 mL past V_e . The quantity of excess OH^- is $(0.10 \text{ mL})(0.100 \text{ M}) = 0.010 \text{ mmol}$, and the total volume of solution is $50.00 + 10.10 \text{ mL} = 60.10 \text{ mL}$.

$$[\text{OH}^-] = \frac{0.010 \text{ mmol}}{50.00 + 10.10 \text{ mL}} = 1.66 \times 10^{-4} \text{ M}$$

$$\text{pH} = -\log\left(\frac{K_w}{[\text{OH}^-]}\right) = 10.22$$

The Titration Curve

A summary of the calculations for the titration of MES with NaOH appears in Table 10-2. The titration curve in Figure 10-2 has two easily identified points. One is the equivalence point, which is the steepest part of the curve. The other landmark is the point where $V_b = \frac{1}{2}V_e$ and $\text{pH} = \text{p}K_a$. This latter point has the minimum slope, which means that the pH changes least for a given addition of NaOH. This is another way of saying that *buffer capacity* is maximum when $\text{pH} = \text{p}K_a$ and $[\text{HA}] = [\text{A}^-]$. When choosing a weak acid to make a buffer, select one whose $\text{p}K_a$ is close to the desired buffer pH to obtain a high buffer capacity.

Here we assume that the pH is governed by the excess OH^- .

Challenge At $V_b = 10.10 \text{ mL}$, show that excess NaOH = 0.17 mM. Show that $F_{A^-} = 17 \text{ mM}$. Show that, in the presence of 0.17 mM NaOH, 17 mM A^- produces only 1.9 $\mu\text{M OH}^-$. That is, ignoring A^- in comparison to excess NaOH is justified.

Landmarks in a titration:
At $V_b = V_e$, curve is steepest.
At $V_b = \frac{1}{2}V_e$, $\text{pH} = \text{p}K_a$ and the slope is minimal.

Buffer capacity measures the ability of the solution to resist changes in pH.

Ask Yourself

10-B. Write the reaction between formic acid (Appendix B) and KOH. What is the equivalence volume (V_e) in the titration of 50.0 mL of 0.050 0 M formic acid with 0.050 0 M KOH? Calculate the pH at the points $V_b = 0.0, 10.0, 20.0, 25.0, 30.0, 40.0, 45.0, 48.0, 49.0, 49.5, 50.0, 50.5, 51.0, 52.0, 55.0$, and 60.0 mL . Draw a graph of pH versus V_b . Without doing any calculations, what should the pH be at $V_b = \frac{1}{2}V_e$? Does your calculated result agree with the prediction?

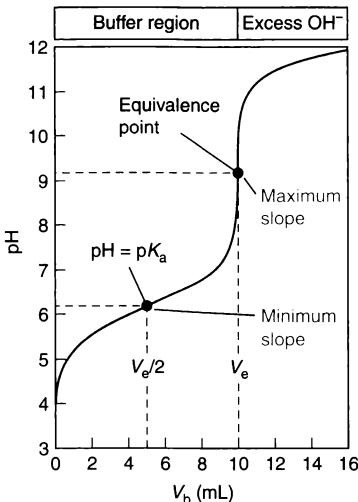


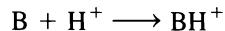
Figure 10-2 Calculated titration curve for the reaction of 50.00 mL of 0.020 00 M MES with 0.100 0 M NaOH. Landmarks occur at half of the equivalence volume ($\text{pH} = \text{p}K_{\text{a}}$) and at the equivalence point, which is the steepest part of the curve.

Table 10-2 Calculation of the titration curve for 50.00 mL of 0.020 00 M MES treated with 0.100 0 M NaOH

	mL base added (V_b)	pH
Region 1 (weak acid)	0.00	3.99
	0.50	4.99
	1.00	5.32
	2.00	5.67
	3.00	5.90
	4.00	6.09
	5.00	6.27
	6.00	6.45
	7.00	6.64
	8.00	6.87
Region 2 (buffer)	9.00	7.22
	9.50	7.55
	9.90	8.27
	10.00	9.25
	10.10	10.22
	10.50	10.91
	11.00	11.21
	12.00	11.50
	13.00	11.67
	14.00	11.79
Region 3 (weak base)	15.00	11.88
	16.00	11.95
Region 4 (excess OH^-)		

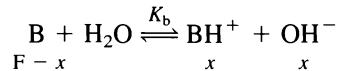
10-3 Titration of Weak Base with Strong Acid

The titration of a weak base with a strong acid is just the reverse of the titration of a weak acid with a strong base. The *titration reaction* is



Because the reactants are weak + strong, the reaction goes essentially to completion after each addition of acid. There are four distinct regions of the titration curve:

1. Before acid is added, the solution contains just the weak base, B, in water. The pH is determined by the K_b reaction:



2. Between the initial point and the equivalence point, there is a mixture of B and BH^+ —Aha! A buffer! The pH is computed by using

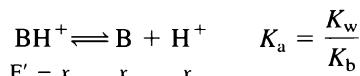
$$\text{pH} = \text{p}K_{\text{a}} (\text{for } \text{BH}^+) + \log \left(\frac{[\text{B}]}{[\text{BH}^+]} \right)$$

At the special point where $V_a = \frac{1}{2}V_e$, $\text{pH} = \text{p}K_{\text{a}} (\text{for } \text{BH}^+)$.

When $V_a = 0$, we have a *weak-base problem*.

When $0 < V_a < V_e$, we have a *buffer*.

3. At the equivalence point, B has been converted into BH^+ , a weak acid. The pH is calculated by considering the acid dissociation reaction of BH^+ :



When $V_a = V_e$, the solution contains the weak acid BH^+ .

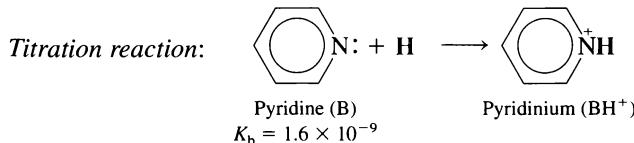
The formal concentration of BH^+ , F' , is not the same as the original formal concentration of B, because there has been some dilution. Because the solution contains BH^+ at the equivalence point, it is acidic. *The pH at the equivalence point must be below 7.*

4. After the equivalence point, there is excess strong acid in the solution. We treat this problem by considering only the concentration of excess H^+ and ignoring the contribution of weak acid, BH^+ .

For $V_a > V_e$, there is excess strong acid.

Example Titration of Pyridine with HCl

Consider the titration of 25.00 mL of 0.083 64 M pyridine with 0.106 7 M HCl, for which the equivalence point is $V_e = 19.60$ mL.



$$\underbrace{(V_e \text{ (mL)})(0.106 7 \text{ M})}_{\text{mmol of HCl}} = \underbrace{(25.00 \text{ mL})(0.083 64 \text{ M})}_{\text{mmol of pyridine}} \Rightarrow V_e = 19.60 \text{ mL}$$

- (a) Find the pH when $V_a = 4.63$ mL, which is before V_e , and (b) find the pH at V_e .

SOLUTION (a) At 4.63 mL, part of the pyridine has been neutralized, so there is a mixture of pyridine and pyridinium ion—*Aha! A buffer!* The initial millimoles of pyridine are $(25.00 \text{ mL})(0.083 64 \text{ M}) = 2.091 \text{ mmol}$. The added H^+ is $(4.63 \text{ mL}) \times (0.106 7 \text{ M}) = 0.494 \text{ mmol}$. Therefore we can write

<i>Titration reaction:</i>	B	+	H^+	\longrightarrow	BH^+
Initial mmol	2.091		0.494		—
Final mmol	1.597		—		0.494

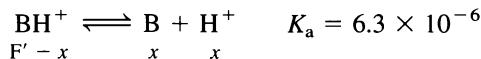
Aha! A buffer! (B + BH⁺)

$$\text{pH} = \underbrace{\text{p}K_{\text{BH}^+}}_{-\log(K_w/K_b)} + \log\left(\frac{[\text{B}]}{[\text{BH}^+]}\right) = 5.20 + \log\left(\frac{1.597}{0.494}\right) = 5.71$$

$\text{p}K_{\text{BH}^+}$ is the acid $\text{p}K_a$ for the acid BH^+ .

- (b) At the equivalence point (19.60 mL), enough acid has been added to convert all of the pyridine (B) into BH^+ . The pH is governed by dissociation of the weak acid, BH^+ , whose acid dissociation constant is $K_a = K_w/K_b = 6.3 \times 10^{-6}$. The formal concentration of BH^+ is equal to the initial millimoles of pyridine divided by the

milliliters of solution at the equivalence point: $F' = (2.091 \text{ mmol})/(25.00 + 19.60 \text{ mL}) = 0.04688 \text{ M}$.



$$\frac{x^2}{F' - x} = \frac{x^2}{0.04688 - x} = K_a = 6.3 \times 10^{-6} \Rightarrow x = [\text{H}^+] = 5.40 \times 10^{-4} \text{ M}$$

$$\text{pH} = -\log[\text{H}^+] = 3.27$$

The pH at the equivalence point is acidic because the weak base has been converted into a weak acid.



Test Yourself Find the pH when 19.00 mL of HCl have been added.
(Answer: 3.70)

Ask Yourself

- 10-C. (a) Why is the equivalence point pH necessarily below 7 when a weak base is titrated with strong acid?
(b) What is the equivalence volume in the titration of 100.0 mL of 0.100 M cocaine (Reaction 8-19, $K_b = 2.6 \times 10^{-6}$) with 0.200 M HNO_3 ? Calculate the pH at $V_a = 0.0, 10.0, 20.0, 25.0, 30.0, 40.0, 49.0, 49.9, 50.0, 50.1, 51.0$, and 60.0 mL. Draw a graph of pH versus V_a .

10-4 Finding the End Point

The *equivalence point* in a titration is defined by the stoichiometry of the reaction. The *end point* is the abrupt change in a physical property (such as pH) that we measure to locate the equivalence point. Indicators and pH measurements are commonly used to find the end point in an acid-base titration.

Using Indicators to Find the End Point

In Section 9-6, we learned that an indicator is an acid or base whose various protonated species have different colors. For the weak-acid indicator HIn , the solution takes on the color of HIn when $\text{pH} \lesssim \text{p}K_{\text{HIn}} - 1$ and has the color of In^- when $\text{pH} \gtrsim \text{p}K_{\text{HIn}} + 1$. In the interval $\text{p}K_{\text{HIn}} - 1 \lesssim \text{pH} \lesssim \text{p}K_{\text{HIn}} + 1$, a mixture of both colors is observed.

A titration curve for which $\text{pH} = 5.54$ at the equivalence point is shown in Figure 10-3. The pH drops steeply from 7 to 4 over a small volume interval. An indicator with a color change in this pH interval would provide a fair approximation to the equivalence point. The closer the color change is to pH 5.54, the more accurate will be the end point. The difference between the observed end point (color change) and the true equivalence point is called the **indicator error**.

If you dump half a bottle of indicator into your reaction, you will introduce a different indicator error. Because indicators are acids or bases, they consume analyte

Choose an indicator whose color change comes as close as possible to the theoretical pH of the equivalence point.

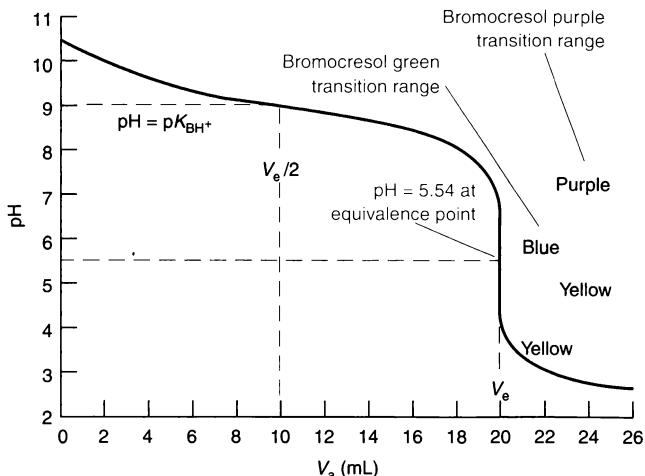


Figure 10-3 Calculated titration curve for the reaction of 100 mL of 0.010 0 M base ($\text{p}K_b = 5.00$) with 0.050 0 M HCl. As in the titration of HA with OH^- , $\text{pH} = \text{p}K_{\text{BH}^+}$ when $V_a = \frac{1}{2}V_e$.

or titrant. Therefore the moles of indicator in a reaction should be negligible relative to the moles of analyte. Never use more than a few drops of dilute indicator.

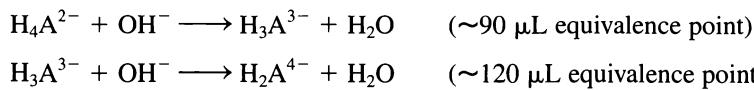
Many indicators in Table 9-3 would be useful for the titration in Figure 10-3. For example, if bromocresol purple were used, we would use the purple-to-yellow color change as the end point. The last trace of purple should disappear near pH 5.2, which is quite close to the true equivalence point in Figure 10-3. If bromocresol green were used as the indicator, a color change from blue to green (= yellow + blue) would mark the end point.

In general, we seek an indicator whose transition range overlaps the steepest part of the titration curve as closely as possible. The steepness of the titration curve near the equivalence point in Figure 10-3 ensures that the indicator error caused by the noncoincidence of the color change and equivalence point will not be large. For example, if the indicator color changed at pH 6.4 instead of 5.54, the error in V_e would be only 0.25%. You could compute the titration error by finding what volume of titrant gives a pH of 6.4.

Using a pH Electrode to Find the End Point

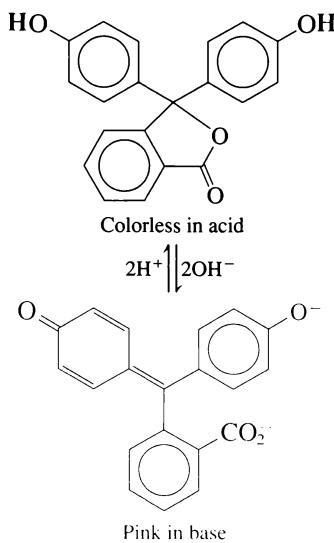
Figure 10-4 shows the observed titration curve for the weak acid, H_6A , with NaOH. Because H_6A is difficult to purify, just 1.430 mg was dissolved in 1.000 mL of aqueous solution and titrated with microliter (μL) quantities of 0.065 92 M NaOH delivered with a Hamilton syringe.

When H_6A is titrated, we might expect to see an abrupt change in pH at all six equivalence points. The curve in Figure 10-4 shows two clear breaks, near 90 and 120 μL , which correspond to titration of the *third* and *fourth* protons of H_6A .



The first two and last two equivalence points give unrecognizable end points, because they occur at pH values that are too low or too high.

One of the most common indicators is phenolphthalein, which changes from colorless in acid to pink in base:



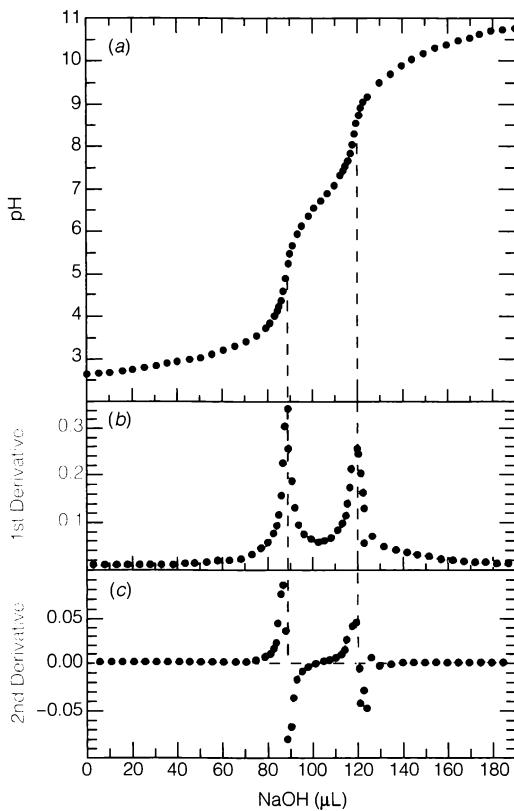


Figure 10-4 (a) Experimental points in the titration of 1.430 mg of xylene orange, a hexaprotonic acid, dissolved in 1.000 mL of 0.10 M NaNO_3 . The titrant was 0.065 92 M NaOH. (b) The first derivative, $\Delta\text{pH}/\Delta V$, of the titration curve. (c) The second derivative, $\Delta(\Delta\text{pH}/\Delta V)/\Delta V$, which is the derivative of the curve in panel b. Derivatives for the first end point are calculated in Figure 10-5. End points are taken as maxima in the derivative curve and zero crossings of the second derivative.

The end point has maximum slope.

The end point is where the slope of the titration curve is greatest. The slope is the change in pH (ΔpH) divided by the change in volume (ΔV) between the points:

$$\text{Slope of titration curve:} \quad \text{slope} = \frac{\Delta\text{pH}}{\Delta V} \quad (10-3)$$

The slope (which is also called the *first derivative*) displayed in the middle of Figure 10-4 is calculated in Figure 10-5. The first two columns of this spreadsheet give experimental volumes and pH measurements. (The pH meter was precise to three digits, even though accuracy ends in the second decimal place.) To compute the first derivative, each pair of volumes is averaged and the quantity $\Delta\text{pH}/\Delta V$ is calculated.

The last two columns of Figure 10-5 and the graph in Figure 10-4c give the slope of the slope (called the *second derivative*), computed as follows:

$$\text{Second derivative:} \quad \frac{\Delta(\text{slope})}{\Delta V} = \frac{\Delta(\Delta\text{pH}/\Delta V)}{\Delta V} \quad (10-4)$$

The slope of the slope (the second derivative) is 0 at the end point.

The end point is the volume at which the second derivative is 0. A graph on the scale of Figure 10-6 allows us to make a good estimate of the end-point volume.

	A	B	C	D	E	F
1	Derivatives of a Titration Curve					
2	Data		1st derivative		2nd derivative	
3	µL NaOH	pH	µL	ΔpH/ΔµL		Δ(ΔpH/ΔµL)
4	85.0	4.245	85.5	0.155	86.0	0.0710
5						
6	86.0	4.400	86.5	0.226	87.0	0.0810
7						
8	87.0	4.626			88.0	0.0330
9			87.5	0.307		
10	88.0	4.933			89.0	-0.0830
11			88.5	0.340		
12	89.0	5.273			90.0	-0.0680
13			89.0	0.257		
14	90.0	5.530			91.25	-0.0390
15			90.5	0.189		
16	91.0	5.719				
17			92.0	0.131		
18	93.0	5.980				
19	Representative formulas:					
20	C5 = (A6+A4)/2		E6 = (C7+C5)/2			
21	D5 = (B6 - B4)/(A6 - A4)		F6 = (D7 - D5)/(C7 - C5)			

Figure 10-5 Spreadsheet for computing first and second derivatives near 90 µL in Figure 10-4.

Example Computing Derivatives of a Titration Curve

Let's see how the first and second derivatives in Figure 10-5 are calculated.

SOLUTION The volume in cell C5, 85.5, is the average of the first two volumes (85.0 and 86.0) in column A. The slope (first derivative) $\Delta p\text{H}/\Delta V$ in cell D5 is calculated from the first two pH values and the first two volumes:

$$\frac{\Delta p\text{H}}{\Delta V} = \frac{4.400 - 4.245}{86.0 - 85.0} = 0.15_5$$

Retain extra, insignificant digits for these calculations.

The coordinates ($x = 85.5$, $y = 0.15_5$) are one point in Figure 10-4b.

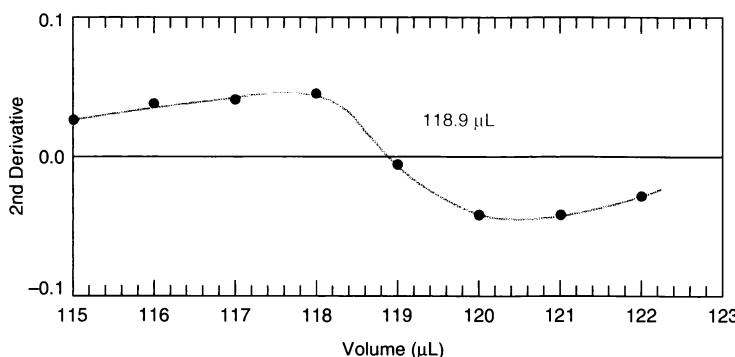


Figure 10-6 Enlargement of the second end point in the second derivative curve of Figure 10-4c.

The second derivative is computed from the first derivative. The volume in cell E6 is 86.0, which is the average of 85.5 and 86.5. The second derivative in cell F6 is

$$\frac{\Delta(\Delta \text{pH}/\Delta V)}{\Delta V} = \frac{0.22_6 - 0.15_5}{86.5 - 85.5} = 0.071_0$$

The coordinates ($x = 86.0$, $y = 0.071_0$) are one point in Figure 10-4c. These calculations are tedious by hand, but not bad in a spreadsheet.

 **Test Yourself** Verify the first and second derivatives in cells D17 and F16 of Figure 10-5. (Answer: 0.130 5 and -0.038 67. Round-off errors arise because the spreadsheet uses more digits than are displayed in Figure 10-5.)

Figure 10-7 shows an *autotitrator*, which performs titrations automatically and sends results directly to a spreadsheet. Titrant from the bottle is dispensed in small increments by a syringe pump, and pH is measured by the electrode in the beaker. After each addition, the instrument waits for the pH to stabilize before adding the next increment.

Ask Yourself

10-D. (a) Select indicators from Table 9-3 that would be useful for the titrations in Figures 10-1 and 10-2 and for the $\text{pK}_a = 8$ curve in Figure 10-11. Select a different indicator for each titration and state what color change you would use as the end point.

(b)  Data near the second end point in Figure 10-4 are given in the table. Find the first and second derivatives with a spreadsheet like Figure 10-5. Plot both derivatives versus V_b and locate the end point from each plot.

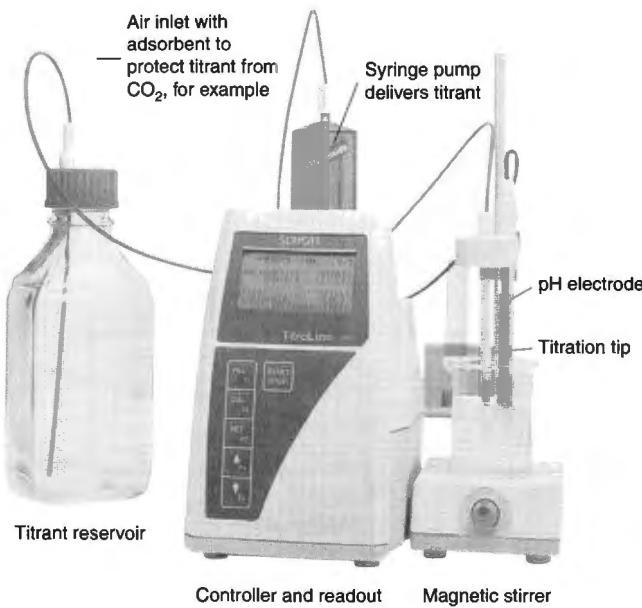


Figure 10-7 Autotitrator delivers titrant from the bottle at the left to the beaker of analyte on the stirring motor at the right. The electrode immersed in the beaker monitors pH or concentrations of specific ions. Volume and pH readings can go directly to a spreadsheet. [Schott Instruments, Mainz, Germany, and Cole-Parmer Instruments, Vernon Hills, IL.]

V_b (μL)	pH						
107.0	6.921	114.0	7.457	117.0	7.878	120.0	8.591
110.0	7.117	115.0	7.569	118.0	8.090	121.0	8.794
113.0	7.359	116.0	7.705	119.0	8.343	122.0	8.952

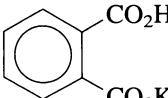
10-5 Practical Notes

Acids and bases in Table 10-3 can be purchased in forms pure enough to be *primary standards*. NaOH and KOH are not primary standards because the reagent-grade materials contain carbonate (from reaction with atmospheric CO_2) and adsorbed water. Solutions of NaOH and KOH must be standardized against a primary standard. Potassium hydrogen phthalate is convenient for this purpose. Solutions of NaOH for titrations are prepared by diluting a stock solution of 50 wt% aqueous NaOH. Sodium carbonate is relatively insoluble in this stock solution and settles to the bottom.

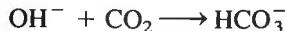
Primary standards must be pure, stable, easily dried, and not hygroscopic. **Hygroscopic** compounds adsorb water while you are weighing them. NaOH and KOH are not primary standards.

Question Why are densities of primary standards listed in Table 10-3?

Table 10-3 Primary standards

Compound	Density (g/mL) for buoyancy correction	Notes
ACIDS		
 Potassium hydrogen phthalate FM 204.22	1.64	The pure solid is dried at 105°C and used to standardize base. A phenolphthalein end point is satisfactory.
		$\text{C}_6\text{H}_4(\text{CO}_2\text{H})(\text{CO}_2\text{K}) + \text{OH}^- \rightarrow \text{C}_6\text{H}_4(\text{CO}_2^-)(\text{CO}_2^-) + \text{H}_2\text{O}$
KH(IO_3) ₂ Potassium hydrogen iodate FM 389.91	—	This is a strong acid, so any indicator with an end point between ~5 and ~9 is adequate.
BASES		
$\text{H}_2\text{NC}(\text{CH}_2\text{OH})_3$ Tris(hydroxymethyl)aminomethane (also called tris or tham) FM 121.14	1.33	The pure solid is dried at 100°–103°C and titrated with strong acid. The end point is in the range pH 4.5–5.
Na_2CO_3 Sodium carbonate FM 105.99	2.53	$\text{H}_2\text{NC}(\text{CH}_2\text{OH})_3 + \text{H}^+ \longrightarrow \text{H}_3\dot{\text{N}}(\text{CH}_2\text{OH})_3$ Primary-standard-grade Na_2CO_3 is titrated with acid to an end point of pH 4–5. Just before the end point, the solution is boiled to expel CO_2 .
$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ Borax FM 381.37	1.73	The recrystallized material is dried in a chamber containing an aqueous solution saturated with NaCl and sucrose. This procedure gives the decahydrate in pure form. The standard is titrated with acid to a methyl red end point.
		$\text{B}_4\text{O}_7^{2-} \cdot 10\text{H}_2\text{O} + 2\text{H}^+ \longrightarrow 4\text{B}(\text{OH})_3 + 5\text{H}_2\text{O}$

Alkaline solutions must be protected from the atmosphere because they absorb CO₂:



CO₂ changes the concentration of base over a period of time and reduces the sharpness of the end point in the titration of weak acids. Strong base attacks glass and should not be kept in a glass bottle or in a buret longer than necessary. If base is kept in a tightly capped polyethylene bottle, it can be used for weeks with little change.

Ask Yourself

- 10-E. (a) Give the name and formula of a primary standard used to standardize (i) HCl and (ii) NaOH.
(b) Referring to Table 10-3, determine how many grams of potassium hydrogen phthalate should be used to standardize ~0.05 M NaOH if you wish to use ~30 mL of base for the titration.

10-6 Kjeldahl Nitrogen Analysis

Developed in 1883, the **Kjeldahl nitrogen analysis** remains one of the most widely used methods for determining nitrogen in organic substances such as protein, cereal, and flour. The solid is *digested* (decomposed and dissolved) in boiling sulfuric acid to convert nitrogen into ammonium ion, NH₄⁺:

Each atom of nitrogen in the unknown is converted into one NH₄⁺ ion.



Mercury, copper, and selenium compounds catalyze the digestion. To speed the reaction, the boiling point of concentrated (98 wt%) sulfuric acid (338°C) is raised by adding K₂SO₄. Digestion is carried out in a long-neck *Kjeldahl flask* (Figure 10-8) that prevents loss of sample by spattering. (An alternative to the Kjeldahl flask is a microwave bomb containing H₂SO₄ and H₂O₂, like that in Figure 2-18.)

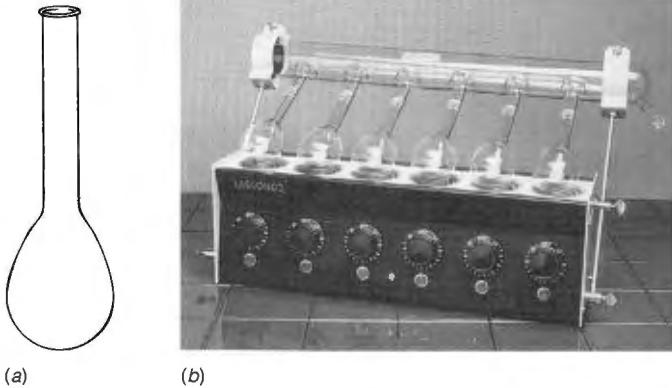


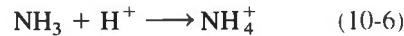
Figure 10-8 (a) Kjeldahl digestion flask has a long neck to minimize loss by spattering. (b) Six-port manifold for multiple samples provides for exhaust of fumes. [Fisher Scientific, Pittsburgh, PA.]

After digestion is complete, the solution containing NH_4^+ is made basic, and the liberated NH_3 is distilled (with a large excess of steam) into a receiver containing a known amount of HCl (Figure 10-9). Excess, unreacted HCl is titrated with standard NaOH to determine how much HCl was consumed by NH_3 .

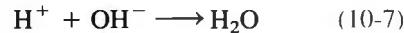
Neutralization of NH_4^+ :



Distillation of NH_3 into standard HCl:



Titration of unreacted HCl with NaOH:



An alternative to the acid-base titration is to neutralize the acid and raise the pH with a buffer, followed by addition of reagents that form a colored product with NH_3 .³ The absorbance of the colored product gives the concentration of NH_3 from the digestion.

Example Kjeldahl Analysis

A typical protein contains 16.2 wt% nitrogen. A 0.500-mL aliquot of protein solution was digested, and the liberated NH_3 was distilled into 10.00 mL of 0.021 40 M HCl. Unreacted HCl required 3.26 mL of 0.019 8 M NaOH for complete titration. Find the concentration of protein (mg protein/mL) in the original sample.

SOLUTION The original amount of HCl in the receiver was $(10.00 \text{ mL}) \times (0.021 40 \text{ M}) = 0.214 \text{ g}$ mmol. The NaOH required for titration of unreacted HCl in Reaction 10-7 was $(3.26 \text{ mL})(0.019 8 \text{ M}) = 0.064 \text{ g}$ mmol. The difference, $0.214 \text{ g} - 0.064 \text{ g} = 0.149 \text{ g}$ mmol, must equal the quantity of NH_3 produced in Reaction 10-5 and distilled into the HCl.

Because 1 mmol of nitrogen in the protein gives rise to 1 mmol of NH_3 , there must have been 0.149 5 mmol of nitrogen in the protein, corresponding to

$$(0.149 \text{ g}) \left(14.0067 \frac{\text{mg N}}{\text{mmol}} \right) = 2.093 \text{ mg N}$$

If the protein contains 16.2 wt% N, there must be

$$\frac{2.093 \text{ mg N}}{0.162 \text{ mg N/mg protein}} = 12.9 \text{ mg protein}$$

$$\frac{12.9 \text{ mg protein}}{0.500 \text{ mL}} = 25.8 \frac{\text{mg protein}}{\text{mL}}$$

 **Test Yourself** Find the protein concentration if 4.00 mL, instead of 3.26 mL, of NaOH were required. (Answer: 23.3 mg/mL)

Ask Yourself

10-F. The Kjeldahl procedure was used to analyze 256 μL of a solution containing 37.9 mg protein/mL. The liberated NH_3 was collected in 5.00 mL of 0.033 6 M HCl, and the remaining acid required 6.34 mL of 0.010 0 M NaOH for complete titration.

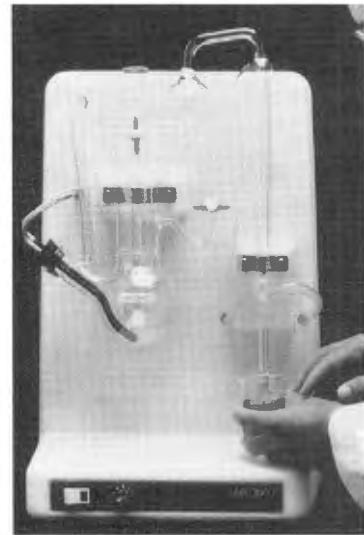


Figure 10-9 Kjeldahl distillation unit employs electric immersion heater in flask at left to carry out distillation in 5 min. Beaker at right collects liberated NH_3 in standard HCl. Round bulb at the top center is a baffle to prevent liquid from bumping over into the receiver.
[Fisher Scientific, Pittsburgh, PA.]

- (a) How many moles of NH_3 were liberated?
 - (b) How many milligrams of nitrogen are contained in the NH_3 in (a)?
 - (c) How many milligrams of protein were analyzed?
 - (d) What is the weight percent of nitrogen in the protein?
-

10-7 Putting Your Spreadsheet to Work

In Sections 10-1 to 10-3, we calculated titration curves because they helped us understand the chemistry behind a titration curve. Now we will see how a spreadsheet decreases the agony and mistakes of titration calculations. First we must derive equations relating pH to volume of titrant for use in the spreadsheet.

Charge Balance

The **charge balance** states that, in any solution, the sum of positive charges must equal the sum of negative charges, because the solution must have zero net charge. For a solution of the weak acid HA plus NaOH, the charge balance is

If the solution contained HA and $\text{Ca}(\text{OH})_2$, the charge balance would be
 $[\text{H}^+] + 2[\text{Ca}^{2+}] = [\text{A}^-] + [\text{OH}^-]$
because one mole of Ca^{2+} provides two moles of charge. If $[\text{Ca}^{2+}] = 0.1 \text{ M}$, the positive charge it contributes is 0.2 M.

$$\text{Charge balance: } [\text{H}^+] + [\text{Na}^+] = [\text{A}^-] + [\text{OH}^-] \quad (10-8)$$

The sum of the positive charges of H^+ and Na^+ equals the sum of the negative charges of A^- and OH^- .

Titrating a Weak Acid with a Strong Base

Consider the titration of a volume V_a of acid HA (initial concentration C_a) with a volume V_b of NaOH of concentration C_b . The concentration of Na^+ is just the moles of NaOH ($C_b V_b$) divided by the total volume of solution ($V_a + V_b$):

$$[\text{Na}^+] = \frac{C_b V_b}{V_a + V_b} \quad (10-9)$$

Similarly, the formal concentration of the weak acid is

$$F = [\text{HA}] + [\text{A}^-] = \frac{C_a V_a}{V_a + V_b} \quad (10-10)$$

because we have diluted $C_a V_a$ moles of HA to a total volume of $V_a + V_b$.

Now we introduce two equations that are derived in Section 12-5:

$$\text{Fraction of weak acid in the form HA: } \alpha_{\text{HA}} = \frac{[\text{HA}]}{F} = \frac{[\text{H}^+]}{[\text{H}^+] + K_a} \quad (10-11)$$

$$\text{Fraction of weak acid in the form A}^-: \alpha_{\text{A}^-} = \frac{[\text{A}^-]}{F} = \frac{[K_a]}{[\text{H}^+] + K_a} \quad (10-12)$$

Equations 10-11 and 10-12 say that if a weak acid has a formal concentration F, the concentration of HA is $\alpha_{\text{HA}} \cdot F$ and the concentration of A^- is $\alpha_{\text{A}^-} \cdot F$. These fractions must add up to 1.

$$\alpha_{\text{HA}} + \alpha_{\text{A}^-} = 1$$

Getting back to our titration, we can write an expression for the concentration of A^- by combining Equation 10-12 with Equation 10-10:

$$[A^-] = \alpha_{A^-} \cdot F = \frac{\alpha_{A^-} \cdot C_a V_a}{V_a + V_b} \quad (10-13)$$

Substituting for $[Na^+]$ (Equation 10-9) and $[A^-]$ (Equation 10-13) in the charge balance (Equation 10-8) gives

$$[H^+] + \frac{C_b V_b}{V_a + V_b} = \frac{\alpha_{A^-} \cdot C_a V_a}{V_a + V_b} + [OH^-]$$

which can be rearranged to

Fraction of titration for weak acid by strong base:

$$\phi = \frac{C_b V_b}{C_a V_a} = \frac{\alpha_{A^-} - \frac{[H^+] - [OH^-]}{C_a}}{1 + \frac{[H^+] - [OH^-]}{C_b}} \quad (10-14)$$

At last! Equation 10-14 is really useful. It relates the volume of titrant (V_b) to the pH. The quantity ϕ ($= C_b V_b / C_a V_a$) is the fraction of the way to the equivalence point, V_e . When $\phi = 1$, the volume of base added, V_b , is equal to V_e . Equation 10-14 works backward from the way that you are accustomed to thinking, because you need to put in pH (on the right) to get out volume (on the left).

Let's set up a spreadsheet for Equation 10-14 to calculate the titration curve for 50.00 mL of the weak acid 0.020 00 M MES with 0.100 0 M NaOH, which was shown in Figure 10-2 and Table 10-2. The equivalence volume is $V_e = 10.00$ mL. The quantities in Equation 10-14 are

$$\begin{array}{ll} C_b = 0.1 & [H^+] = 10^{-pH} \\ C_a = 0.02 \text{ M} & [OH^-] = K_w/[H^+] \\ V_a = 50 \text{ mL} & \\ K_a = 5.37 \times 10^{-7} & \alpha_{A^-} = \frac{K_a}{[H^+] + K_a} \\ K_w = 10^{-14} & \end{array}$$

$$\text{pH is the input} \qquad V_b = \frac{\phi C_a V_a}{C_b} \text{ is the output}$$

The input to the spreadsheet in Figure 10-10 is pH in column B and the output is V_b in column G. From the pH, the values of $[H^+]$, $[OH^-]$, and α_{A^-} are computed in columns C, D, and E. Equation 10-14 is used in column F to find the fraction of titration, ϕ . From this value, we calculate the volume of titrant, V_b , in column G.

How do we know what pH values to put in? Trial and error allows us to find the starting pH, by putting in a pH and seeing whether V_b is positive or negative. In a few tries, it is easy to home in on the pH at which $V_b = 0$. In Figure 10-10, we see that a pH of 3.00 is too low, because ϕ and V are both negative. Input values of pH are spaced as closely as needed to allow you to generate a smooth titration curve. To save space, we show only a few points in Figure 10-10, including the midpoint

$\phi = C_b V_b / C_a V_a$ is the fraction of the way to the equivalence point:

ϕ	Volume of base
0.5	$V_b = \frac{1}{2} V_e$
1	$V_b = V_e$
2	$V_b = 2V_e$



2-(*N*-Morpholino)ethanesulfonic acid
MES, $pK_a = 6.27$

Figure 10-10 Spreadsheet uses Equation 10-14 to calculate the titration curve for 50 mL of the weak acid 0.02 M MES ($pK_a = 6.27$), treated with 0.1 M NaOH. You provide pH as input in column B, and the spreadsheet tells what volume of base in column G is required to generate that pH.

	A	B	C	D	E	F	G
Titration of Weak Acid with Strong Base							
3	$C_b =$	pH	$[H^+]$	$[OH^-]$	$\text{Alpha}(A^-)$	Φ	$V_b (\text{mL})$
4	0.1	3.00	1.00E-03	1.00E-11	0.001	-0.049	-0.490
5	$C_a =$	3.99	1.02E-04	9.77E-11	0.005	0.000	0.001
6	0.02	4.00	1.00E-04	1.00E-10	0.005	0.000	0.003
7	$V_a =$	5.00	1.00E-05	1.00E-09	0.051	0.050	0.505
8	50	6.27	5.37E-07	1.86E-08	0.500	0.500	5.000
9	$K_a =$	7.00	1.00E-07	1.00E-07	0.843	0.843	8.430
10	5.37E-07	8.00	1.00E-08	1.00E-06	0.982	0.982	9.818
11	$K_w =$	9.25	5.62E-10	1.78E-05	0.999	1.000	10.000
12	1.00E-14	10.00	1.00E-10	1.00E-04	1.000	1.006	10.058
13		11.00	1.00E-11	1.00E-03	1.000	1.061	10.606
14		12.00	1.00E-12	1.00E-02	1.000	1.667	16.667
15							
16	$C_4 = 10^{-B4}$						
17	$D4 = \$A\$12/C4$						
18	$E4 = \$A\$10/(C4+\$A\$10)$						
19	$F4 = (E4-(C4-D4)/\$A\$6)/(1+(C4-D4)/\$A\$4)$ [Equation 10-14]						
20	$G4 = F4*\$A\$6/\$A\$8/\$A\4						

To home in on an exact volume (such as V_e), set the spreadsheet to show extra digits in the cell of interest. Tables in this book have been formatted to reduce the number of digits.

($\text{pH } 6.27 \Rightarrow V_b = 5.00 \text{ mL}$) and the end point ($\text{pH } 9.25 \Rightarrow V_b = 10.00 \text{ mL}$). The spreadsheet agrees with Table 10-2 without dividing the titration into different regions that use different approximations.

The Power of a Spreadsheet

By changing K_a in cell A10 of Figure 10-10, we can calculate a family of curves for different acids. Figure 10-11 shows how the titration curve depends on the acid dissociation constant of HA. The strong-acid curve at the bottom of Figure 10-11 was computed with a large value of K_a ($K_a = 10^3$) in cell A10. Figure 10-11 shows that, as K_a decreases (pK_a increases), the pH change near the equivalence point decreases, until it becomes too shallow to detect. Similar behavior occurs as the concentrations of analyte and titrant decrease. *It is not practical to titrate an acid or a base when its strength is too weak or its concentration too dilute.*

Titrating a Weak Base with a Strong Acid

By logic similar to that used to derive Equation 10-14, we can derive an equation for the titration of weak base B with strong acid:

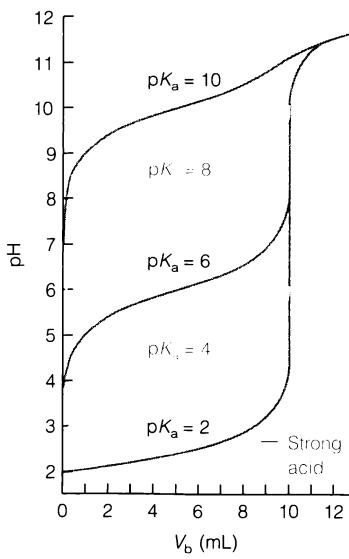


Figure 10-11 Calculated curves showing the titration of 50.0 mL of 0.020 M HA with 0.100 M NaOH. As the acid becomes weaker, the equivalence point becomes less distinct.

where C_a is the concentration of strong acid in the buret, V_a is the volume of acid added, C_b is the initial concentration of weak base being titrated, V_b is the initial volume of weak base and α_{BH^+} is the fraction of base in the form BH^+ :

$$\text{Fraction of weak base in the form } BH^+: \quad \alpha_{BH^+} = \frac{[BH^+]}{F} = \frac{[H^+]}{[H^+] + K_{BH^+}} \quad (10-16)$$

where K_{BH^+} is the acid dissociation constant of BH^+ .

Experiment 10 at the Book Companion Website www.whfreeman.com/exploringchem5e teaches you how to fit the theoretical expressions 10-14 or 10-15 to experimental titration data. Excel Solver® is used to find the best values of analyte concentration and pK_a to fit the measured data.

Ask Yourself

10-G.  (a) *Effect of pK_a in the titration of weak acid with strong base.* Use the spreadsheet in Figure 10-10 to compute and plot the family of curves in Figure 10-11. For strong acid, use $K_a = 10^3$.

(b) *Effect of concentration in the titration of weak acid with strong base.* Use your spreadsheet to prepare a family of titration curves for $pK_a = 6$, with the following combinations of concentrations: (i) $C_a = 20 \text{ mM}$, $C_b = 100 \text{ mM}$; (ii) $C_a = 2 \text{ mM}$, $C_b = 10 \text{ mM}$; and (iii) $C_a = 0.2 \text{ mM}$, $C_b = 1 \text{ mM}$.

Key Equations

Useful shortcut

$$\text{mL} \times \frac{\text{mol}}{\text{L}} = \text{mmol}$$

Equivalence volume (V_e)

$$\underbrace{C_a V_a = C_b V_e}_{\text{Titrating acid with base}} \quad \text{or} \quad \underbrace{C_a V_e = C_b V_b}_{\text{Titrating base with acid}}$$

C_a = acid concentration C_b = base concentration

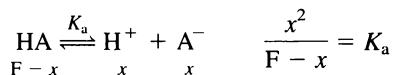
V_a = acid volume V_b = base volume

V_e = equivalence volume

Titration of weak acid

(see inside back cover)

1. Initial solution—weak acid

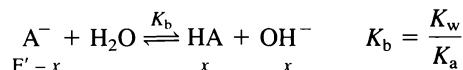


2. Before equivalence point—buffer

Titration reaction tells how much HA and A^- are present

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

3. Equivalence point—weak base— $\text{pH} > 7$



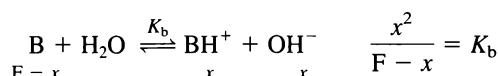
F' is diluted concentration

4. After equivalence point—excess strong base

$$\text{pH} = -\log(K_w / [\text{OH}^-]_{\text{excess}})$$

1. Initial solution—weak base

Titration of weak base
(see inside back cover)

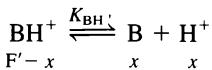


2. Before equivalence point—buffer

Titration reaction tells how much B and BH^+ are present

$$\text{pH} = \text{p}K_{\text{BH}^+} + \log\left(\frac{[\text{B}]}{[\text{BH}^+]}\right)$$

3. Equivalence point—weak acid— $\text{pH} < 7$



4. After equivalence point—excess strong acid

$$\text{pH} = -\log([\text{H}^+]_{\text{excess}})$$

Use Equations 10-14 and 10-15

Input is pH and output is volume

Use indicator with color change close to theoretical pH at equivalence point of titration

End point has greatest slope: $\Delta\text{pH}/\Delta V$ is maximum

End point has zero second derivative: $\frac{\Delta(\Delta\text{pH}/\Delta V)}{\Delta V} = 0$

Spreadsheet titration equations

Choosing indicator

Using electrodes for end point

Important Terms

charge balance

indicator error

Kjeldahl nitrogen analysis

Problems

10-1. Explain what chemistry occurs in each region of the titration of OH^- with H^+ . State how you would calculate the pH in each region.

10-2. Explain what chemistry occurs in each region of the titration of the weak acid, HA, with OH^- . State how you would calculate the pH in each region.

10-3. Explain what chemistry occurs in each region of the titration of the weak base, A^- , with strong acid, H^+ . State how you would calculate the pH in each region.

10-4. Why is the titration curve in Figure 10-3 steepest at the equivalence point?

10-5. Why do we use the maximum of the first derivative curve or the zero crossing of the second derivative curve to locate the end points in Figure 10-4?

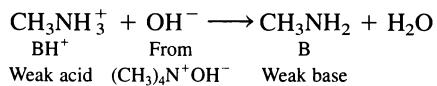
10-6. Consider the titration of 100.0 mL of 0.100 M NaOH with 1.00 M HBr. What is the equivalence volume? Find the pH at the following volumes of HBr and make a graph of pH versus V_a : $V_a = 0, 1.00, 5.00, 9.00, 9.90, 10.00, 10.10$, and 12.00 mL.

10-7. Consider the titration of 25.0 mL of 0.050 0 M HClO_4 with 0.100 M KOH. Find the equivalence volume. Find the pH at the following volumes of KOH and plot pH versus V_b : $V_b = 0, 1.00, 5.00, 10.00, 12.40, 12.50, 12.60$, and 13.00 mL.

10-8. A 50.0-mL volume of 0.050 0 M weak acid HA ($\text{p}K_a = 4.00$) was titrated with 0.500 M NaOH. Write the titration

reaction and find V_e . Find the pH at $V_b = 0, 1.00, 2.50, 4.00, 4.90, 5.00, 5.10$, and 6.00 mL and plot pH versus V_b .

10-9. When methylammonium chloride is titrated with tetramethylammonium hydroxide, the titration reaction is



Find the equivalence volume in the titration of 25.0 mL of 0.010 0 M methylammonium chloride with 0.050 0 M tetramethylammonium hydroxide. Calculate the pH at $V_b = 0, 2.50, 5.00$, and 10.00 mL. Sketch the titration curve.

10-10. Write the reaction for the titration of 100 mL of 0.100 M anilinium bromide (“aminobenzene · HBr”) with 0.100 M NaOH. Sketch the titration curve for the points $V_b = 0, 0.100V_e, 0.500V_e, 0.900V_e, V_e$, and $1.200V_e$.

10-11. What is the pH at the equivalence point when 0.100 M hydroxyacetic acid is titrated with 0.050 0 M KOH?

10-12. When 16.24 mL of 0.064 3 M KOH were added to 25.00 mL of 0.093 8 M weak acid, HA, the observed pH was 3.62. Find $\text{p}K_a$ for the acid.

10-13. When 22.63 mL of aqueous NaOH were added to 1.214 g of CHES (FM 207.29, structure in Table 9-2) dissolved in 41.37 mL of water, the pH was 9.13. Calculate the molarity of the NaOH.

10-14. (a) When 100.0 mL of weak acid HA were titrated with 0.093 81 M NaOH, 27.63 mL were required to reach the equivalence point. Find the molarity of HA.

(b) What is the formal concentration of A^- at the equivalence point?

(c) The pH at the equivalence point was 10.99. Find pK_a for HA.

(d) What was the pH when only 19.47 mL of NaOH had been added?

10-15. A 100.0-mL aliquot of 0.100 M weak base B ($pK_b = 5.00$) was titrated with 1.00 M $HClO_4$. Find V_e and calculate the pH at $V_a = 0, 1.00, 5.00, 9.00, 9.90, 10.00, 10.10$, and 12.00 mL and make a graph of pH versus V_a .

10-16. A solution of 100.0 mL of 0.040 0 M sodium propanoate (the sodium salt of propanoic acid) was titrated with 0.083 7 M HCl. Find V_e and calculate the pH at $V_a = 0, \frac{1}{4}V_e, \frac{1}{2}V_e, \frac{3}{4}V_e, V_e$, and $1.1V_e$. Sketch the titration curve.

10-17. A 50.0-mL solution of 0.031 9 M benzylamine was titrated with 0.050 0 M HCl.

(a) What is the equilibrium constant for the titration reaction?

(b) Find V_e and calculate the pH at $V_a = 0, 12.0, \frac{1}{2}V_e, 30.0, V_e$, and 35.0 mL.

10-18. Don't ever mix acid with cyanide (CN^-) because it liberates poisonous $HCN(g)$. But, just for fun, calculate the pH of a solution made by mixing 50.00 mL of 0.100 M NaCN with

(a) 4.20 mL of 0.438 M $HClO_4$.

(b) 11.82 mL of 0.438 M $HClO_4$.

(c) What is the pH at the equivalence point with 0.438 M $HClO_4$?

10-19. A 25.00-mL volume of 0.050 00 M imidazole was titrated with 0.125 0 M HNO_3 . Find V_e and calculate the pH at $V_a = 0, 1.00, 5.00, 9.00, 9.90, 10.00, 10.10$, and 12.00 mL and make a graph of pH versus V_a .

10-20. Would the indicator bromocresol green, with a transition range of pH 3.8–5.4, ever be useful in the titration of a weak acid with a strong base? Why?

10-21. Consider the titration in Figure 10-2, for which the pH at the equivalence point is calculated to be 9.25. If thymol blue is used as an indicator, what color will be observed through most of the titration prior to the equivalence point? At the equivalence point? After the equivalence point?

10-22. Why would an indicator end point not be very useful in the titration curve for $pK_a = 10.00$ in Figure 10-11?

10-23. Phenolphthalein is used as an indicator for the titration of HCl with NaOH.

(a) What color change is observed at the end point?

(b) The basic solution just after the end point slowly absorbs

CO_2 from the air and becomes more acidic by virtue of the reaction $CO_2 + OH^- \rightleftharpoons HCO_3^-$, causing the color to fade from pink to colorless. If you carry out the titration too slowly, does this reaction lead to a systematic or a random error in finding the end point?

10-24. In the titration of 0.10 M pyridinium bromide (the salt of pyridine plus HBr) by 0.10 M NaOH, the pH at $0.99V_e$ is 7.20. At V_e , pH = 8.95, and, at $1.01V_e$, pH = 10.70. Select an indicator from Table 9-3 that would be suitable for this titration and state what color change will be used.

10-25. Prepare a graph of the second derivative to find the end point from the following titration data:

mL NaOH	pH	mL NaOH	pH
10.679	7.643	10.729	5.402
10.696	7.447	10.733	4.993
10.713	7.091	10.738	4.761
10.721	6.700	10.750	4.444
10.725	6.222	10.765	4.227

10-26. Borax (Table 10-3) was used to standardize a solution of HNO_3 . Titration of 0.261 9 g of borax required 21.61 mL. What is the molarity of the HNO_3 ?

10-27. A 10.231-g sample of window cleaner containing ammonia was diluted with 39.466 g of water. Then 4.373 g of solution were titrated with 14.22 mL of 0.106 3 M HCl to reach a bromocresol green end point.

(a) What fraction of the 10.231-g sample of window cleaner is contained in the 4.373 g that were analyzed?

(b) How many grams of NH_3 (FM 17.031) were in the 4.373-g sample?

(c) Find the weight percent of NH_3 in the cleaner.

10-28. In the Kjeldahl nitrogen analysis, the final product is NH_4^+ in HCl solution. It is necessary to titrate the HCl without titrating the NH_4^+ ion.

(a) Calculate the pH of pure 0.010 M NH_4Cl .

(b) The steep part of the titration curve when HCl is titrated with NaOH runs from pH ≈ 4 to pH ≈ 10. Select an indicator that allows you to titrate HCl but not NH_4^+ .

10-29. Prepare a spreadsheet like the one in Figure 10-10, but, using Equation 10-15, reproduce the titration curve in Figure 10-3.

10-30. *Effect of pK_b in the titration of weak base with strong acid.* Use the spreadsheet from Problem 10-29 to compute and plot a family of curves analogous to Figure 10-11 for the titration of 50.0 mL of 0.020 0 M B ($pK_b = -2.00, 2.00, 4.00, 6.00, 8.00$, and 10.00) with 0.100 M HCl. ($pK_b = -2.00$ corresponds to $K_b = 10^{+2.00}$, which represents a strong base.)

How Would You Do It?

- 10-31.** The following table gives data for 100.0 mL of solution of a single unknown base titrated with 0.1114 M HCl. Provide an argument for how many protons the base is able to accept (Is it monoprotic, diprotic, etc.) and find the molarity

of the base. A high-quality pH meter provides pH to the 0.001 place, even though accuracy is limited to the 0.01 place. How could you deliver volumes up to 50 mL with a precision of 0.001 mL?

Coarse titration				Fine data near first end point				Fine data near second end point			
mL	pH	mL	pH	mL	pH	mL	pH	mL	pH	mL	pH
0.595	12.148	29.157	5.785	26.939	8.217	27.481	7.228	40.168	3.877	41.542	2.999
1.711	12.006	31.512	5.316	27.013	8.149	27.501	7.158	40.403	3.767	41.620	2.949
3.540	11.793	33.609	5.032	27.067	8.096	27.517	7.103	40.498	3.728	41.717	2.887
5.250	11.600	36.496	4.652	27.114	8.050	27.537	7.049	40.604	3.669	41.791	2.845
7.258	11.390	38.222	4.381	27.165	7.987	27.558	6.982	40.680	3.618	41.905	2.795
9.107	11.179	39.898	3.977	27.213	7.916	27.579	6.920	40.774	3.559	42.033	2.735
11.557	10.859	40.774	3.559	27.248	7.856	27.600	6.871	40.854	3.510	42.351	2.617
13.967	10.486	41.791	2.845	27.280	7.791	27.622	6.825	40.925	3.457	42.709	2.506
16.042	10.174	42.709	2.506	27.309	7.734	27.649	6.769	40.994	3.407	43.192	2.401
18.474	9.850	45.049	2.130	27.338	7.666	27.675	6.717	41.057	3.363	43.630	2.312
20.338	9.627	47.431	1.937	27.362	7.603	27.714	6.646	41.114	3.317		
22.136	9.402	49.292	1.835	27.386	7.538	27.747	6.594	41.184	3.263		
24.836	8.980			27.406	7.485	27.793	6.535	41.254	3.210		
26.216	8.608			27.427	7.418	27.846	6.470	41.329	3.150		
27.013	8.149			27.444	7.358	27.902	6.411	41.406	3.093		
27.969	6.347			27.463	7.287	27.969	6.347	41.466	3.047		

Reference Procedure:

Preparing standard acid and base

Hydrochloric acid and sodium hydroxide are the most common strong acids and bases used in the laboratory. Both reagents need to be standardized to learn their exact concentrations. Section 10-5 provides background information for the procedures described below.

Reagents

50 wt% NaOH: (3 mL/student) Dissolve 50 g of reagent-grade NaOH in 50 mL of distilled water and allow the suspension to settle overnight. Na_2CO_3 is insoluble in the solution and precipitates. Store the solution in a tightly sealed polyethylene bottle and handle it gently to avoid stirring the precipitate when liquid is withdrawn.

Phenolphthalein indicator: Dissolve 50 mg in 50 mL of ethanol and add 50 mL of distilled water.

Bromocresol green indicator: Dissolve 100 mg in 14.3 mL of 0.01 M NaOH and dilute to 250 mL with distilled water.

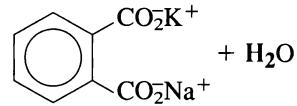
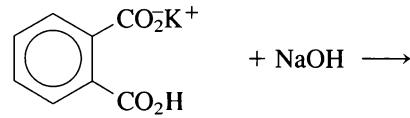
Concentrated (37 wt%) HCl: 10 mL/student

Primary standards: Potassium hydrogen phthalate (~ 2.5 g/student) and sodium carbonate (~ 1.0 g/student)

0.05 M NaCl: 50 mL/student

Standardizing NaOH

1. Dry primary-standard-grade potassium hydrogen phthalate for 1 h at 105°C and store it in a capped bottle in a desiccator.



2. Boil 1 L of distilled water for 5 min to expel CO_2 . Pour the water into a polyethylene bottle, which should be tightly capped whenever possible. Calculate the volume of 50 wt% NaOH needed to prepare 1 L of 0.1 M NaOH. (The density of 50 wt% NaOH is 1.50 g per milliliter of solution.) Use a graduated cylinder to transfer this much NaOH to the bottle of water. (CAUTION: 50 wt% NaOH eats people. Flood any spills on your skin with water.) Mix well and cool the solution to room temperature (preferably overnight).

3. Weigh four samples of solid potassium hydrogen phthalate and dissolve each in \sim 25 mL of distilled water in a 125-mL flask. Each sample should contain enough solid to react with \sim 25 mL of 0.1 M NaOH. Add 3 drops of phenolphthalein to each flask and titrate one rapidly to find the end point. The buret should have a loosely fitted cap to minimize entry of CO₂ from the air.

4. Calculate the volume of NaOH required for each of the other three samples and titrate them carefully. During each titration, periodically tilt and rotate the flask to wash all liquid from the walls into the bulk solution. Near the end, deliver less than 1 drop of titrant at a time. To do so, carefully suspend a fraction of a drop from the buret tip, touch it to the inside wall of the flask, wash it into the bulk solution by careful tilting, and swirl the solution. The end point is the first appearance of faint pink color that persists for 15 s. (The color will slowly fade as CO₂ from the air dissolves in the solution.)

5. Calculate the average molarity (\bar{x}), the standard deviation (s), and the percent relative standard deviation ($= 100 \times s/\bar{x}$). If you were careful, the relative standard deviation should be $<0.2\%$.

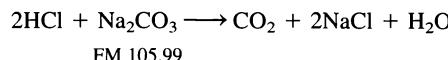
Standardizing HCl

1. Use the table inside the back cover of this book to calculate the volume of \sim 37 wt% HCl that should be added to 1 L of

distilled water to produce 0.1 M HCl and prepare this solution.

2. Dry primary-standard-grade sodium carbonate for 1 h at 105°C and cool it in a desiccator.

3. Weigh four samples, each containing enough Na₂CO₃ to react with \sim 25 mL of 0.1 M HCl and place each in a 125-mL flask. When you are ready to titrate each one, dissolve it in \sim 25 mL of distilled water. Add 3 drops of bromocresol green indicator and titrate one rapidly to a green color to find the approximate end point.



4. Carefully titrate each sample until it turns from blue to green. Then boil the solution to expel CO₂. The color should return to blue. Carefully add HCl from the buret until the solution turns green again and report the volume of acid at this point.

5. Perform one blank titration of 50 mL of 0.05 M NaCl containing 3 drops of indicator. Subtract the volume of HCl needed for the blank from that required to titrate Na₂CO₃.

6. Calculate the mean HCl molarity, standard deviation, and percent relative standard deviation.

Notes and References

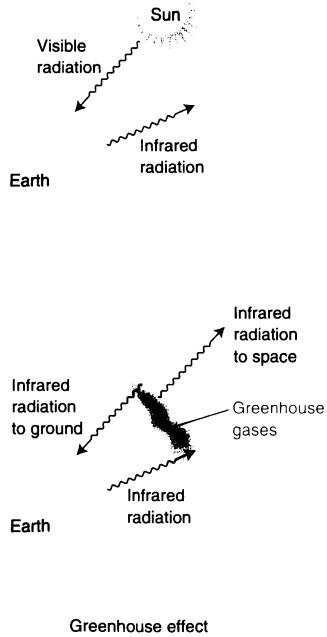
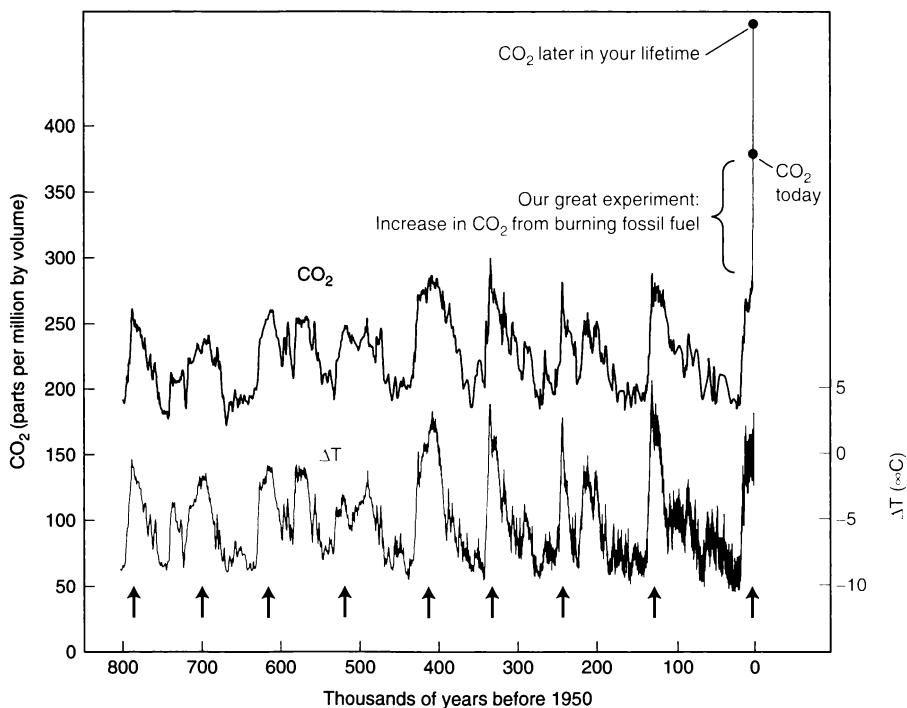
1. J. J. Urh, "Protein Testing Enters the 21st Century: Innovative Protein Analyzer Not Affected by Melamine," *Am. Lab.* October 2008, p. 18.
2. L. Zhu, G. Gamez, H. Chen, K. Chingin, and R. Zenobi, "Rapid Detection of Melamine in Untreated Milk and Wheat Gluten by Ultrasound-Assisted Extractive Electrospray

Ionization Mass Spectrometry," *Chem. Commun.* **2009**, 559; G. Huang, Z. Ouyang, and R. G. Cooks, "High-Throughput Trace Melamine Analysis in Complex Mixtures," *Chem. Commun.* **2009**, 556.

3. Colorimetric Kjeldahl NH₃ measurement: www.umass.edu/tei/mwwp/acrobat/epa351_3Norg.pdf.

Carbon Dioxide in the Air

Upper curve: Atmospheric CO₂ deduced from air trapped in Antarctic ice and from direct atmospheric measurements. Lower curve: Atmospheric temperature at the level where precipitation forms is deduced from isotopic composition of ice. [Data from J. M. Barnola, D. Raynaud, C. Lorius, and N. I. Barkov, <http://cdiac.esd.ornl.gov/ftp/trends/co2/vostok.icecore.co2>.] Figure 1-4 shows details of the last 1 000 years.



Perhaps the largest chemical experiment ever conducted is our injection of carbon dioxide into the atmosphere in sufficient quantity to alter the cycle of CO₂ concentration that has persisted for at least 800 000 years. CO₂ is produced by combustion of fossil fuels (coal, oil, natural gas), which are our predominant source of energy.

CO₂ acts as a *greenhouse gas* to affect Earth's surface temperature. Earth absorbs sunlight and then emits infrared radiation. The balance between sunlight absorbed and radiation sent back to space determines the surface temperature. A greenhouse gas absorbs infrared radiation and reradiates some of it back to the ground. By intercepting some of Earth's radiation, CO₂ keeps our planet warmer than it would otherwise be.

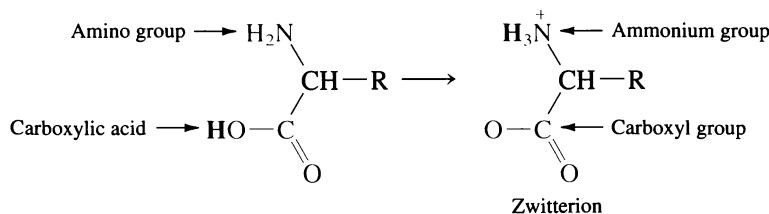
The graph shows that atmospheric temperature and CO₂ experience peaks marked by arrows roughly every 100 000 years. Temperature change is attributed principally to cyclic changes in Earth's orbit and tilt. Small increases in temperature drive dissolved CO₂ from the ocean into the atmosphere. Increased atmospheric CO₂ further increases warming by the greenhouse effect. Cooling brought on by orbital changes redissolves CO₂ in the ocean, thereby causing further cooling. Temperature and CO₂ followed each other until 200 years ago. Your generation is likely to be the first to learn the effect of our colossal experiment to alter Earth's atmosphere.

Polyprotic Acids and Bases

Carbon dioxide from air dissolves in water to make carbonic acid, which has two acidic protons. Later in this chapter, we explore potential effects of dissolving massive amounts of this acid in the ocean. Carbonic acid from CO_2 and amino acids from proteins are examples of **polyprotic acids**—those having more than one acidic proton.

11-1 Amino Acids Are Polyprotic

Amino acids from which proteins are built have an acidic carboxylic acid group, a basic amino group, and a variable substituent designated R:

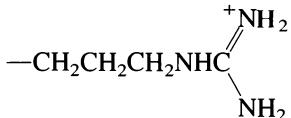
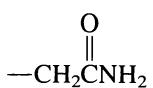
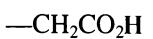
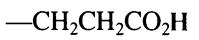
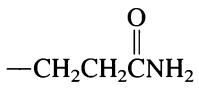
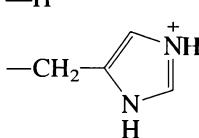
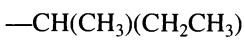
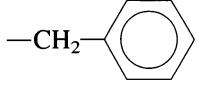
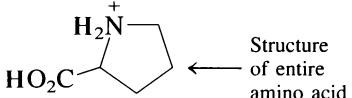
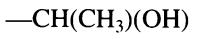
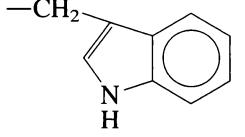
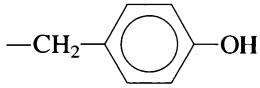


A **zwitterion** is a molecule with both positive and negative charges.

Because the amino group is more basic than the carboxyl group, the acidic proton resides on nitrogen of the amino group instead of on oxygen of the carboxyl group. The resulting structure, with positive and negative sites, is called a **zwitterion**.

At low pH, both the ammonium group and the carboxyl group are protonated. At high pH, neither is protonated. The substituent may also have acidic or basic properties. Acid dissociation constants of the 20 common amino acids are given in

Table 11-1 Acid dissociation constants of amino acids^{a, b}

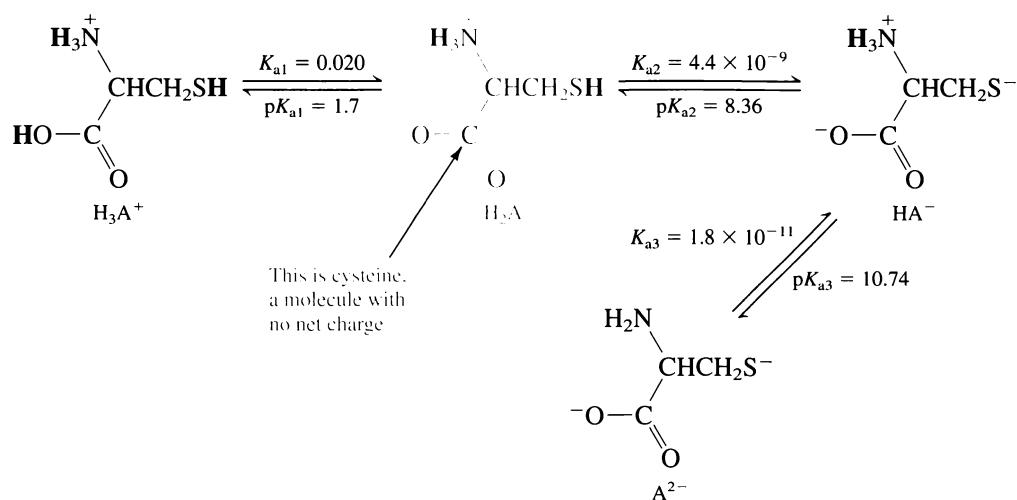
Amino acid ^c	Substituent	Carboxylic acid pK_a	Ammonium pK_a	Substituent pK_a	Molecular mass
Alanine (A)	—CH ₃	2.344	9.868		89.09
Arginine (R)		1.823	8.991	(12.1)	174.20
Asparagine (N)		2.16	8.73		132.12
Aspartic acid (D)		1.990	10.002	3.900	133.10
Cysteine (C)		(1.7)	10.74	8.36	121.16
Glutamic acid (E)		2.16	9.96	4.30	147.13
Glutamine (Q)		2.19	9.00		146.15
Glycine (G)	—H	2.350	9.778		75.07
Histidine (H)		(1.6)	9.28	5.97	155.16
Isoleucine (I)		2.318	9.758		131.17
Leucine (L)		2.328	9.744		131.17
Lysine (K)		(1.77)	9.07	10.82	146.19
Methionine (M)		2.18	9.08		149.21
Phenylalanine (F)		2.20	9.31		165.19
Proline (P)		1.952	10.640		115.13
Serine (S)		2.187	9.209		105.09
Threonine (T)		2.088	9.100		119.12
Tryptophan (W)		2.37	9.33		204.23
Tyrosine (Y)		2.41	8.67	11.01	181.19
Valine (V)		2.286	9.719		117.15

a. A. E. Martell, R. M. Smith, and R. J. Motekaitis, *NIST Critically Selected Stability Constants of Metal Complexes*, NIST Standard Reference Database 46, Gaithersburg, MD, 2001.

b. pK_a at 25°C. Values in parentheses are less certain.

c. Standard abbreviations are shown in parentheses. Acidic protons are bold. Each substituent is written in its fully protonated form.

Table 11-1, in which each substituent (R) is shown in its fully protonated form. For example, the amino acid cysteine has three acidic protons:



In general, a *diprotic* acid has two acid dissociation constants, designated K_{a1} and K_{a2} (where $K_{a1} > K_{a2}$):



The two base association constants are designated K_{b1} and K_{b2} ($K_{b1} > K_{b2}$):



K_{a1} applies to the *most acidic* proton. The subscript “a” in K_{a1} and K_{a2} is customarily omitted, so we will write K_1 and K_2 throughout most of this chapter.

Relation Between K_a and K_b

If you add the K_{a1} reaction to the K_{b2} reaction, the sum is $H_2O \rightleftharpoons H^+ + OH^-$ —the K_w reaction. From this knowledge, you can derive a most important set of relations between the acid and the base equilibrium constants:

Relation between K_a and K_b for diprotic system:

$$\begin{aligned} K_{a1} \cdot K_{b2} &= K_w \\ K_{a2} \cdot K_{b1} &= K_w \end{aligned} \quad (11-1)$$

Challenge Add the K_{a1} and K_{b2} reactions to prove that $K_{a1} \cdot K_{b2} = K_w$.

For a *triprotic* system, with three acidic protons, the corresponding relations are

Relation between K_a and K_b for triprotic system:

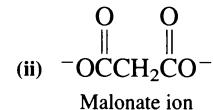
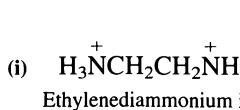
$$\begin{aligned} K_{a1} \cdot K_{b3} &= K_w \\ K_{a2} \cdot K_{b2} &= K_w \\ K_{a3} \cdot K_{b1} &= K_w \end{aligned} \quad (11-2)$$

The standard notation for successive acid dissociation constants of a polyprotic acid is K_1 , K_2 , K_3 , and so on, with the subscript “a” usually omitted. We retain or omit the subscript “a” as dictated by clarity. For successive base hydrolysis constants, we retain the subscript “b”. K_{a1} (or K_1) refers to the acidic species with the most protons, and K_{b1} refers to the basic species with no acidic protons.

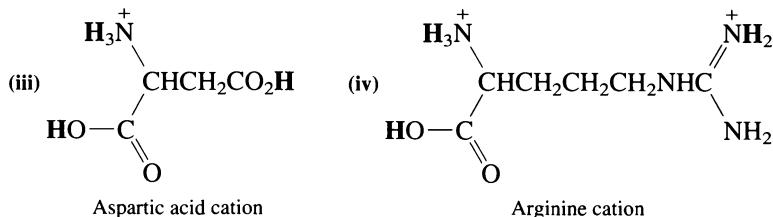
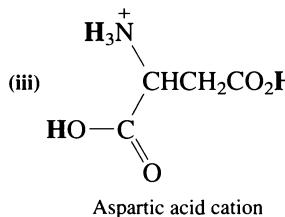


Ask Yourself

11-A. (a) Each ion below has two consecutive acid-base reactions (called stepwise acid-base reactions) when placed in water. Write the reactions and the correct symbol (for example, K_2 or K_{b1}) for the equilibrium constant for each. Use Appendix B to find the numerical value of each equilibrium constant.



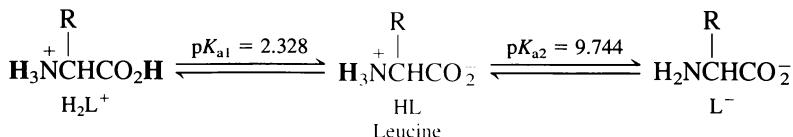
(b) Starting with the following fully protonated species, write the stepwise acid dissociation reactions of the amino acids aspartic acid and arginine. Remove protons in the correct order on the basis of pK_a values in Table 11-1. Remember that the proton with the lowest pK_a (that is, the greatest K_a) comes off first. Label the neutral molecules that we call aspartic acid and arginine.



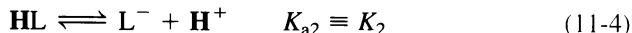
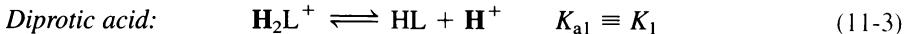
11-2 Finding the pH in Diprotic Systems

Consider the amino acid leucine, designated HL. The neutral molecule shown in color has one acidic proton on the amino group. It can accept a proton on the carboxyl group to form H_2L^+ or it can lose the proton from the ammonium group to give L^- .

The side chain in leucine is an isobutyl group: R = — $\text{CH}_2\text{CH}(\text{CH}_3)_2$.



The acid dissociation constants begin with the fully protonated form H_2L^+ :



The base hydrolysis constants begin with the fully deprotonated species L^- :

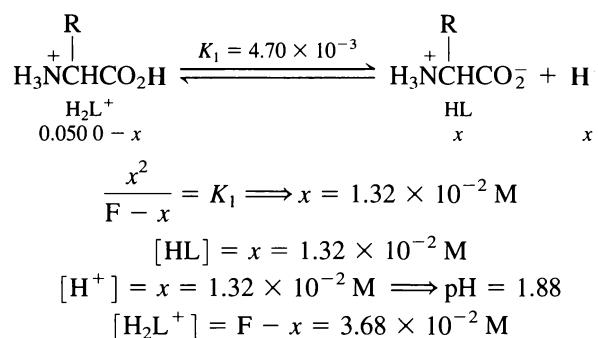


We now set out to calculate the pH and composition of individual solutions of 0.050 0 M H_2L^+ , 0.050 0 M HL, and 0.050 0 M L^- . Our methods do not depend on the charge of the acids and bases. We use the same procedure to find the pH of diprotic H_2A , where A is anything, or H_2L^+ , where HL is leucine.

The Acidic Form, H_2L^+

A salt such as leucine hydrochloride contains the protonated species H_2L^+ , which can dissociate twice, as indicated in Reactions 11-3 and 11-4. Because $K_1 = 4.70 \times 10^{-3}$, H_2L^+ is a weak acid. HL is an even weaker acid, with $K_2 = 1.80 \times 10^{-10}$. It appears that H_2L^+ will dissociate only partly, and the resulting HL will hardly dissociate at all. For this reason, we make the (superb) approximation that a solution of H_2L^+ behaves as a *monoprotic* acid, with $K_a = K_1$.

With this approximation, the calculation of the pH of 0.050 0 M H_2L^+ is simple:



Easy stuff.

H_2L^+ can be treated as monoprotic, with $K_a = K_1$.

F is the formal concentration of H_2L^+ ($= 0.050\ 0\ \text{M}$ in this example).

What is the concentration of L^- in the solution? We can find $[\text{L}^-]$ with K_2 :

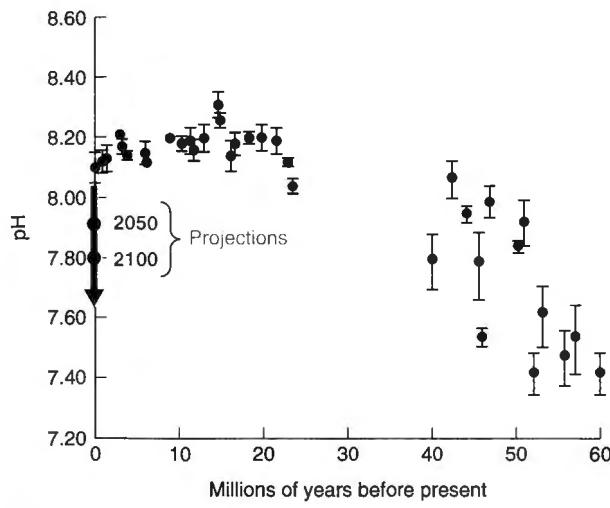
$$\begin{aligned} \text{HL} &\xrightleftharpoons{K_2} \text{L}^- + \text{H}^+ \quad K_2 = \frac{[\text{H}^+][\text{L}^-]}{[\text{HL}]} \implies [\text{L}^-] = \frac{K_2[\text{HL}]}{[\text{H}^+]} \\ [\text{L}^-] &= \frac{(1.80 \times 10^{-10})(1.32 \times 10^{-2})}{(1.32 \times 10^{-2})} = 1.80 \times 10^{-10}\ \text{M} (= K_2) \end{aligned}$$

Our approximation that the second dissociation of a diprotic acid is much less than the first dissociation is confirmed by this last result. The concentration of L^- is about eight orders of magnitude smaller than that of HL. As a source of protons, the dissociation of HL is negligible relative to the dissociation of H_2L^+ . For most diprotic acids, K_1 is sufficiently larger than K_2 for this approximation to be valid. Even if K_2 were just 10 times less than K_1 , the value of $[\text{H}^+]$ calculated by ignoring the second ionization would be in error by only 4%. The error in pH would be only 0.01 pH unit. In summary, *a solution of a diprotic acid behaves like a solution of a monoprotic acid, with $K_a = K_1$* .

Dissolved carbon dioxide is one of the most important diprotic acids in Earth's ecosystem. Box 11-1 describes imminent danger to the entire ocean food chain as a result of increasing atmospheric CO_2 dissolving in the oceans. Reaction A in Box 11-1 lowers the concentration of CO_3^{2-} in the oceans. As a result, CaCO_3 shells and skeletons of creatures at the bottom of the food chain will dissolve by Reaction B in Box 11-1. This effect is far more certain than the effects of atmospheric CO_2 on Earth's climate.

Box 11-1 Carbon Dioxide in the Ocean

The opening of this chapter shows long-term oscillations in atmospheric CO₂ concentration that we have grossly upset by burning fossil fuel. Increasing atmospheric CO₂ increases CO₂ in the ocean. Dissolved CO₂ makes carbonic acid (Demonstration 9-2), which acidifies the ocean. The surface pH of the ocean has already fallen from a preindustrial value of 8.16 to 8.04 today.¹ Without changes in our activities, the pH could be 7.8 or lower by 2100. For various marine organisms, extra dissolved CO₂ requires greater energy



(a) Equatorial Pacific Ocean surface pH deduced from $^{11}\text{B}/^{10}\text{B}$ ratio in fossil shells. [Data from M. R. Palmer and P. N. Pearson, *Science* **2003**, *300*, 480; *Nature* **2000**, *406*, 695. Projections from C. Turley et al. in *Avoiding Dangerous Climate Change*, H. J. Schellnhuber et al., eds. (Cambridge: Cambridge University Press, 2006).]

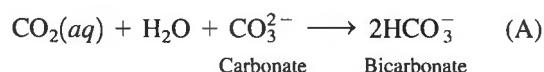


(b)

Pteropod. The shell of a live pteropod begins to dissolve after 48 h in water that is undersaturated with aragonite. [David Wrobel/Visuals Unlimited]

expenditure to maintain proper intracellular pH, reduces fertility, thwarts larval development, reduces growth rate, interferes with iron uptake, reduces muscle mass, suppresses the immune system, and kills some species.²

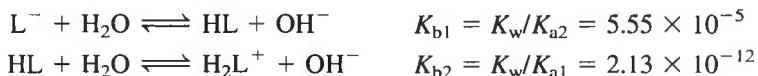
Dissolved CO₂ consumes carbonate by the reaction



The Basic Form, L

More easy stuff.

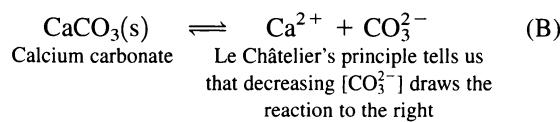
The fully basic species, L^- , would be found in a salt such as sodium leucinate, which could be prepared by treating leucine with an equimolar quantity of NaOH. Dissolving sodium leucinate in water gives a solution of L^- , the fully basic species. The K_b values for this dibasic anion are



K_{b1} tells us that L^- will not hydrolyze (react with water) very much to give HL . Furthermore, K_{b2} tells us that the resulting HL is such a weak base that hardly any further reaction to make H_2L^+ will take place.

Hydrolysis is the reaction of anything with water. Specifically, the reaction $L^- + H_2O \rightleftharpoons HL + OH^-$ is called hydrolysis.

Low carbonate concentration promotes dissolution of solid calcium carbonate:



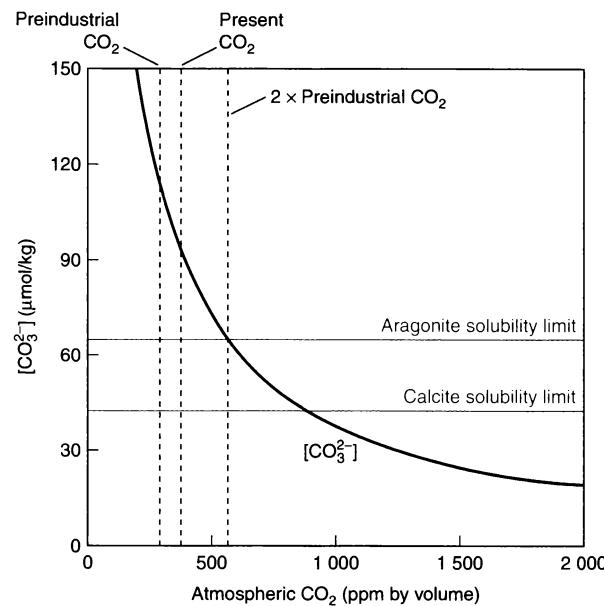
If $[\text{CO}_3^{2-}]$ in the ocean decreases enough, organisms such as plankton and coral with CaCO_3 shells or skeletons will not survive.³ Calcium carbonate has two crystalline forms called calcite and aragonite. Aragonite is more soluble than calcite. Different organisms have either calcite or aragonite in their shells and skeletons.

Pteropods are a type of plankton also known as winged snails. When pteropods collected from the subarctic Pacific Ocean are kept in water that is less than saturated with aragonite, their shells begin to dissolve within 48 h. Animals such as the pteropod lie at the base of the food chain. Their destruction would reverberate through the entire ocean.

Today, ocean surface waters contain more than enough CO_3^{2-} to sustain aragonite and calcite. As atmospheric CO_2 inexorably increases, ocean surface waters will become undersaturated with respect to aragonite—killing off organisms that depend on this mineral for their structure. Polar regions will suffer this fate first because CO_2 is more soluble in cold water than in warm water and $K_{\text{a}1}$ and $K_{\text{a}2}$ at low temperature favor HCO_3^- and $\text{CO}_2(aq)$ relative to CO_3^{2-} (Problem 11-32).

Panel c shows the predicted concentration of CO_3^{2-} in polar ocean surface water as a function of atmospheric CO_2 . The upper horizontal line is the concentration of CO_3^{2-} below which aragonite dissolves. Atmospheric CO_2 is presently near 400 ppm and $[\text{CO}_3^{2-}]$ is near

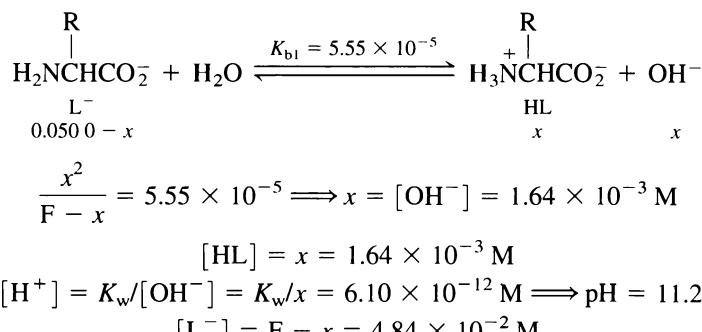
100 $\mu\text{mol/kg}$ of seawater—more than enough to precipitate aragonite or calcite. When atmospheric CO_2 reaches 600 ppm *in the present century*, $[\text{CO}_3^{2-}]$ will decrease to 60 $\mu\text{mol/kg}$ and creatures with aragonite structures will begin to disappear from polar waters. At still higher atmospheric CO_2 concentration, extinctions will move to lower latitudes and will overtake organisms with calcite structures as well as aragonite structures. *How long will we put CO_2 into the atmosphere to see if these predictions are borne out?*



(c)

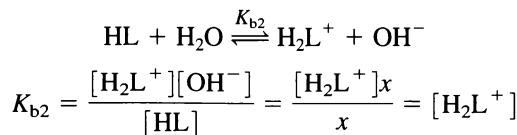
Calculated $[\text{CO}_3^{2-}]$ in polar ocean surface waters as a function of atmospheric CO_2 . When $[\text{CO}_3^{2-}]$ drops below the upper horizontal line, aragonite dissolves. [Adapted from J. C. Orr et al., *Nature* 2005, 437, 681.]

We therefore treat L^- as a monobasic species, with $K_b = K_{\text{b}1}$. The results of this (fantastic) approximation can be outlined as follows:



L^- can be treated as monobasic with $K_b = K_{\text{b}1}$.

The concentration of H_2L^+ can be found from the $K_{\text{b}2}$ equilibrium:



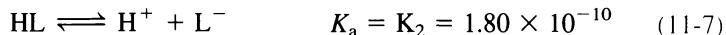
We find that $[\text{H}_2\text{L}^+] = K_{\text{b}2} = 2.13 \times 10^{-12} \text{ M}$, and the approximation that $[\text{H}_2\text{L}^+]$ is insignificant relative to $[\text{HL}]$ is well justified. In summary, if there is any reasonable separation between K_1 and K_2 (and, therefore, between $K_{\text{b}1}$ and $K_{\text{b}2}$), *the fully basic form of a diprotic acid can be treated as monobasic, with $K_b = K_{\text{b}1}$.*

The Intermediate Form, HL

A tougher problem.

A solution prepared from leucine, HL, is more complicated than one prepared from either H_2L^+ or L^- , because HL is both an acid and a base.

HL is both an acid and a base.



A molecule that can both donate and accept a proton is said to be **amphiprotic**. The acid dissociation reaction (11-7) has a larger equilibrium constant than the base hydrolysis reaction (11-8), so we expect the solution of leucine to be acidic.

However, we cannot simply ignore Reaction 11-8, even when K_a and K_b differ by several orders of magnitude. Both reactions proceed to a nearly equal extent, because H^+ produced in Reaction 11-7 reacts with OH^- from Reaction 11-8, thereby driving Reaction 11-8 to the right.

To treat this case, we write a *charge balance*, which says that the sum of positive charges in solution must equal the sum of negative charges. The procedure is applied to leucine, whose intermediate form (HL) has no net charge. However, results apply to the intermediate form of *any* diprotic acid, regardless of its charge.

Our problem concerns 0.050 0 M leucine, in which both Reactions 11-7 and 11-8 can happen. The charge balance is

The charge balance is discussed further in Section 12-3.

Charge balance for HL:

sum of positive charges =
sum of negative charges

$$\underbrace{[\text{H}_2\text{L}^+] + [\text{H}^+]}_{\text{Sum of positive charges}} = \underbrace{[\text{L}^-] + [\text{OH}^-]}_{\text{Sum of negative charges}} \quad (11-9)$$

From the acid dissociation equilibria (Equations 11-3 and 11-4), we replace $[\text{H}_2\text{L}^+]$ with $[\text{HL}][\text{H}^+]/K_1$ and $[\text{L}^-]$ with $K_2[\text{HL}]/[\text{H}^+]$. Also, we can always write $[\text{OH}^-] = K_w / [\text{H}^+]$. Putting these expressions into Equation 11-9 gives

$$\frac{[\text{HL}][\text{H}^+]}{K_1} + [\text{H}^+] = \frac{K_2[\text{HL}]}{[\text{H}^+]} + \frac{K_w}{[\text{H}^+]}$$

which can be solved for $[\text{H}^+]$. First, multiply all terms by $[\text{H}^+]$:

$$\frac{[\text{HL}][\text{H}^+]^2}{K_1} + [\text{H}^+]^2 = K_2[\text{HL}] + K_w$$

Then factor out $[H^+]^2$ and rearrange:

$$[H^+]^2 \left(\frac{[HL]}{K_1} + 1 \right) = K_2[HL] + K_w$$
$$[H^+]^2 = \frac{K_2[HL] + K_w}{\frac{[HL]}{K_1} + 1}$$

Multiplying the numerator and denominator by K_1 and taking the square root of both sides gives

$$[H^+] = \sqrt{\frac{K_1 K_2 [HL] + K_1 K_w}{K_1 + [HL]}} \quad (11-10)$$

We solved for $[H^+]$ in terms of known constants plus the single unknown, $[HL]$. Where do we proceed from here?

In our moment of despair, a chemist gallops down from the mountain mists on her snow-white unicorn to provide the missing insight: “The major species will be HL , because it is both a weak acid and a weak base. Neither Reaction 11-7 nor Reaction 11-8 goes very far. For the concentration of HL in Equation 11-10, you can substitute the value 0.050 0 M.”

The missing insight!

Taking her advice, we rewrite Equation 11-10 as follows:

$$[H^+] \approx \sqrt{\frac{K_1 K_2 F + K_1 K_w}{K_1 + F}} \quad (11-11)$$

where F is the formal concentration of HL ($= 0.050 0 \text{ M}$). Equation 11-11 can be further simplified under most conditions. The first term in the numerator is almost always much greater than the second term, so the second term can be dropped:

$$[H^+] \approx \sqrt{\frac{K_1 K_2 F + K_1 K_w}{K_1 + F}}$$

Then, if $K_1 \ll F$, the first term in the denominator can also be neglected.

$$[H^+] \approx \sqrt{\frac{K_1 K_2 F}{K_1 + F}}$$

Canceling F in the numerator and denominator gives

$$[H^+] \approx \sqrt{K_1 K_2} = (K_1 K_2)^{1/2} \quad (11-12)$$

Making use of the identity $\log(x^{1/2}) = \frac{1}{2} \log x$, we rewrite Equation 11-12 in the form

$$\log[H^+] \approx \log(K_1 K_2)^{1/2} = \frac{1}{2} \log(K_1 K_2)$$

Noting that $\log xy = \log x + \log y$, we can rewrite the equation once more:

$$\log[H^+] \approx \frac{1}{2}(\log K_1 + \log K_2)$$

Multiplying both sides by -1 converts the terms into pH and pK:

$$\underbrace{-\log[H^+]}_{\text{pH}} \approx \frac{1}{2}(\underbrace{-\log K_1}_{\text{p}K_1} - \underbrace{\log K_2}_{\text{p}K_2})$$

The pH of the intermediate form of a diprotic acid is close to midway between the two pK_a values and is almost independent of concentration.

*Intermediate form
of diprotic acid:*

$$\text{pH} \approx \frac{1}{2}(pK_1 + pK_2) \quad (11-13)$$

where K_1 and K_2 are the acid dissociation constants ($= K_{a1}$ and K_{a2}) of the diprotic acid.

Equation 11-13 is good to keep in your head. *The pH of the intermediate form of a diprotic acid is approximately midway between pK_1 and pK_2 , regardless of the formal concentration.*

For leucine, Equation 11-13 gives a pH of $\frac{1}{2}(2.328 + 9.744) = 6.036$, or $[H^+] = 10^{-\text{pH}} = 9.20 \times 10^{-7} \text{ M}$. The concentrations of H_2L^+ and L^- can be found from the K_1 and K_2 equilibria, using $[HL] = 0.050 \text{ M}$.

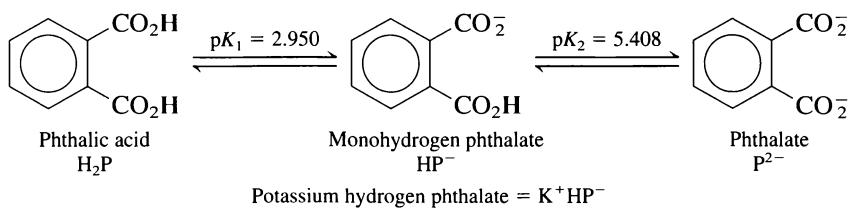
$$[H_2L^+] = \frac{[H^+][HL]}{K_1} = \frac{(9.20 \times 10^{-7})(0.050 \text{ M})}{4.70 \times 10^{-3}} = 9.79 \times 10^{-6} \text{ M}$$

$$[L^-] = \frac{K_2[HL]}{[H^+]} = \frac{(1.80 \times 10^{-10})(0.050 \text{ M})}{9.20 \times 10^{-7}} = 9.78 \times 10^{-6} \text{ M}$$

Was the approximation $[HL] \approx 0.050 \text{ M}$ a good one? It certainly was, because $[H_2L^+] (= 9.79 \times 10^{-6} \text{ M})$ and $[L^-] (= 9.78 \times 10^{-6} \text{ M})$ are small in comparison with $[HL] (\approx 0.050 \text{ M})$. Nearly all the leucine remained in the form HL.

Example pH of the Intermediate Form of a Diprotic Acid

Potassium hydrogen phthalate, KHP, is a salt of the intermediate form of phthalic acid. Calculate the pH of 0.10 M KHP and 0.010 M KHP.



SOLUTION From Equation 11-13, the pH of potassium hydrogen phthalate is estimated as $\frac{1}{2}(pK_1 + pK_2) = 4.18$, regardless of concentration.

Test Yourself Predict the pH of 29 mM serine. (Answer: 5.70)

Diprotic systems:

- Treat H_2A and BH_2^{2+} as monoprotic weak acids.
- Treat A^{2-} and B as monoprotic weak bases.
- Treat HA^- and BH^+ as intermediates: $\text{pH} \approx \frac{1}{2}(pK_1 + pK_2)$.

Summary of Diprotic Acid Calculations

The fully protonated species H_2A is treated as a monoprotic acid with acid dissociation constant K_1 . The fully basic species A^{2-} is treated as a monoprotic base, with

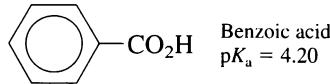
base association constant $K_{b1} = K_w/K_{a2}$. For the intermediate form HA^- , use the equation $\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2)$, where K_1 and K_2 are the acid dissociation constants for H_2A . The same considerations apply to a diprotic base ($\text{B} \longrightarrow \text{BH}^+ \longrightarrow \text{BH}_2^{2+}$): B is treated as monobasic; BH_2^{2+} is treated as monoprotic; and BH^+ is treated as an intermediate with $\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2)$, where K_1 and K_2 are the *acid* dissociation constants of BH_2^{2+} .

Ask Yourself

- 11-B. Find the pH and the concentrations of H_2SO_3 , HSO_3^- , and SO_3^{2-} in each of the following solutions: (a) 0.050 M H_2SO_3 ; (b) 0.050 M NaHSO_3 ; (c) 0.050 M Na_2SO_3 .

11-3 Which Is the Principal Species?

We sometimes need to identify which species of acid, base, or intermediate is predominant under given conditions. For example, what is the principal form of benzoic acid at pH 8? The pH of 8 is the net result of all reagents in the solution. The pH might be 8 because a phosphate buffer was added or because NaOH was added to benzoic acid. It does not matter how the pH came to be 8. That is just where it happens to be.



The $\text{p}K_a$ for benzoic acid is 4.20. Therefore, at pH 4.20, there is a 1:1 mixture of benzoic acid (HA) and benzoate ion (A^-). At $\text{pH} = \text{p}K_a + 1 (= 5.20)$, the quotient $[\text{A}^-]/[\text{HA}]$ is 10:1, which we deduce from the Henderson-Hasselbalch equation:

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

Setting pH equal to $\text{p}K_a + 1$ gives

$$\text{p}K_a + 1 = \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right) \implies 1 = \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

To solve for $[\text{A}^-]/[\text{HA}]$, raise 10 to the power on each side of the equation:

$$10^1 = 10^{\log([\text{A}^-]/[\text{HA}])} \implies \frac{[\text{A}^-]}{[\text{HA}]} = 10$$

At $\text{pH} = \text{p}K_a + 2 (= 6.20)$, the quotient $[\text{A}^-]/[\text{HA}]$ would be 100:1. As the pH increases, the quotient $[\text{A}^-]/[\text{HA}]$ increases still further. At pH 8, the Henderson-Hasselbalch equation is $8 = 4.20 + \log([\text{A}^-]/[\text{HA}]) \implies \log([\text{A}^-]/[\text{HA}]) = 3.8 \implies [\text{A}^-]/[\text{HA}] = 10^{3.8}$. There is almost 10 000 times as much A^- as HA .

For a monoprotic system, the basic species, A^- , is the predominant form when $\text{pH} > \text{p}K_a$. The acidic species, HA , is the predominant form when $\text{pH} < \text{p}K_a$. The predominant form of benzoic acid at pH 8 is the benzoate anion, $\text{C}_6\text{H}_5\text{CO}_3^-$.

At $\text{pH} = \text{p}K_a$, $[\text{A}^-] = [\text{HA}]$ because

$$\begin{aligned} \text{pH} &= \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right) \\ &= \text{p}K_a + \log 1 = \text{p}K_a \end{aligned}$$

pH	Major species	
$<\text{p}K_a$	HA	
$>\text{p}K_a$	A^-	
Predominant form	More acidic	pH
	HA	A^-
	\uparrow	\downarrow
	$\text{p}K_a$	
	$[\text{HA}] = [\text{A}^-]$	

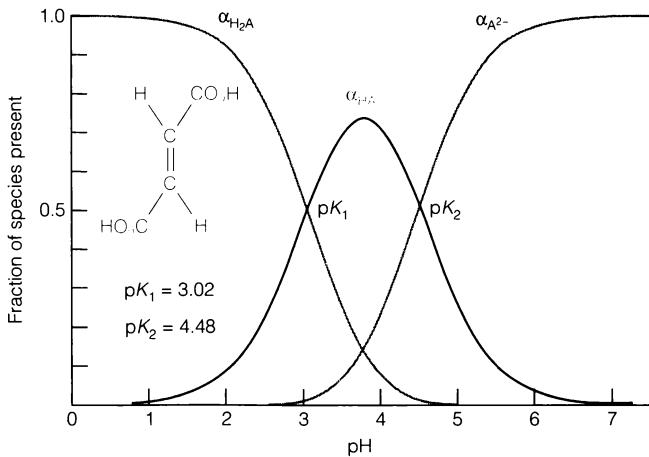


Figure 11-1 Fractional composition diagram for fumaric acid (*trans*-butenedioic acid). α_i is the fraction of species i at each pH. At low pH, H_2A is the dominant form. Between pH = pK_1 and pH = pK_2 , HA^- is dominant. Above pH = pK_2 , A^{2-} dominates. Because pK_1 and pK_2 are not very different, the fraction of HA^- never gets very close to unity.

Example Principal Species—Which One and How Much?

What is the predominant form of ammonia in a solution at pH 7.0? Approximately what fraction is in this form?

SOLUTION In Appendix B, we find $pK_a = 9.24$ for the ammonium ion (NH_4^+), the conjugate acid of ammonia, NH_3 . At pH = 9.24, $[NH_4^+] = [NH_3]$. Below pH 9.24, NH_4^+ will be the predominant form. Because pH = 7.0 is about 2 pH units below pK_a , the quotient $[NH_3]/[NH_4^+]$ will be about 1:100. Approximately 99% is in the form NH_4^+ .

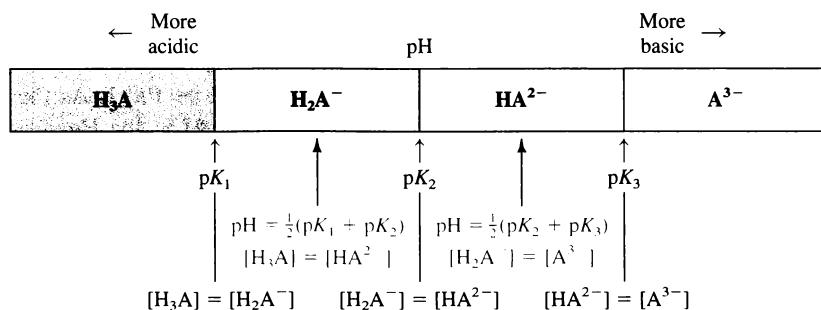
Test Yourself What is the predominant form of cyclohexylamine at pH 9.5? Approximately what fraction is in this form? (Answer: ~10:1 RNH_3^+ : RNH_2)

For diprotic systems, the reasoning is similar, but there are two pK_a values. Consider fumaric acid (*trans*-butenedioic acid), H_2A , with $pK_1 = 3.02$ and $pK_2 = 4.48$. At pH = pK_1 , $[H_2A] = [HA^-]$. At pH = pK_2 , $[HA^-] = [A^{2-}]$. The chart in the margin shows the major species in each pH region. At pH values below pK_1 , H_2A is dominant. At pH values above pK_2 , A^{2-} is dominant. At pH values between pK_1 and pK_2 , HA^- is dominant. Figure 11-1 shows the fraction of each species as a function of pH.

The diagram below shows the major species for a triprotic system and introduces an important extension of what we learned in the previous section.

pH	Major species		
pH < pK_1	H_2A		
$pK_1 < pH < pK_2$		HA^-	
pH > pK_2			A^{2-}

← More acidic	pH	More basic →
H_2A	HA^-	A^{2-}
$\uparrow pK_1$ $[H_2A] = [HA^-]$		
	$\uparrow pK_2$ $[HA^-] = [A^{2-}]$	



H_3A is the dominant species of a triprotic system in the most acidic solution at $\text{pH} < \text{p}K_1$. H_2A^- is dominant between $\text{p}K_1$ and $\text{p}K_2$. HA^{2-} is the major species between $\text{p}K_2$ and $\text{p}K_3$, and A^{3-} dominates in the most basic solution at $\text{pH} > \text{p}K_3$.

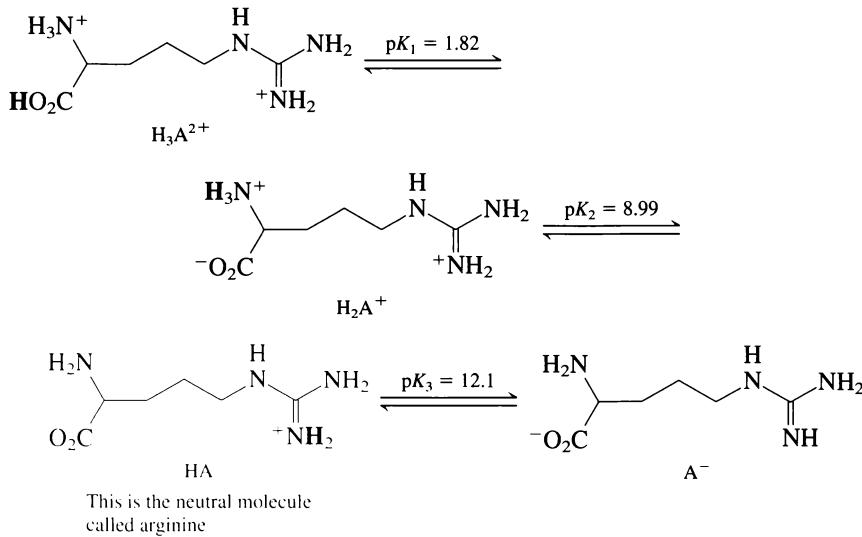
The triprotic acid diagram shows that the pH of the first intermediate species, H_2A^- , is $\frac{1}{2}(\text{p}K_1 + \text{p}K_2)$. At this pH, the concentrations of H_3A and HA^{2-} are small and equal. *The new feature of the diagram is that the pH of the second intermediate species, HA^{2-} , is $\frac{1}{2}(\text{p}K_2 + \text{p}K_3)$.* At this pH, the concentrations of H_2A^- and A^{3-} are small and equal.

Triprotic systems:

- Treat H_3A as monoprotic weak acid.
- Treat A^{3-} as monoprotic weak base.
- Treat H_2A^- as intermediate: $\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2)$.
- Treat HA^{2-} as intermediate: $\text{pH} \approx \frac{1}{2}(\text{p}K_2 + \text{p}K_3)$.

Example Principal Species in a Polyprotic System

The amino acid arginine has the following forms:



The ammonium group next to the carboxyl group is more acidic than the substituent ammonium group at the right. What is the principal form of arginine at pH 10.0? Approximately what fraction is in this form? What is the second most abundant form at this pH?

SOLUTION It helps to draw a diagram showing which species predominates at each pH:

	H_2A^+	HA	A^-
pH	1.82 $\text{p}K_1$	8.99 $\text{p}K_2$	12.1 $\text{p}K_3$

The predominant species between $\text{p}K_2 = 8.99$ and $\text{p}K_3 = 12.1$ is HA . At $\text{p}K_2$, $[\text{H}_2\text{A}^+] = [\text{HA}]$. At $\text{p}K_3$, $[\text{HA}] = [\text{A}^-]$. Because pH 10.0 is about one pH unit higher than $\text{p}K_2$, we can say that $[\text{HA}]/[\text{H}_2\text{A}^+] \approx 10:1$. About 90% of arginine is in the form HA . The second most abundant species is H_2A^+ at $\sim 10\%$.

Test Yourself What is the principal form of arginine at pH 8.0? Approximately what fraction is in this form? (Answer: H_2A^+ , $\sim 90\%$)

Example More on Polyprotic Systems

In the pH range 1.82 to 8.99, H_2A^+ is the principal form of arginine. Which is the second most prominent species at pH 6.0? At pH 5.0?

SOLUTION We know that the pH of the pure intermediate (amphiprotic) species, H_2A^+ , is

$$\text{pH of } \text{H}_2\text{A}^+ \approx \frac{1}{2}(pK_1 + pK_2) = 5.40$$

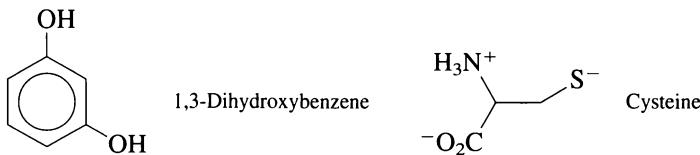
Above pH 5.40 (and below $\text{pH} = \text{p}K_2$), HA is the second most important species. Below pH 5.40 (and above $\text{pH} = \text{p}K_1$), H_3A^{2+} is the second most important species.

 **Test Yourself** What is the second major form of arginine at pH 8.0? What fraction is in this form? (**Answer:** HA, ~10%)

Ask Yourself

11-C. (a) Draw the structure of the predominant form (principal species) of 1,3-dihydroxybenzene at pH 9.00 and at pH 11.00. What is the second most prominent species at each pH?

(b) Cysteine is a triprotic system whose fully protonated form could be designated H_3C^+ . Which form of cysteine is drawn here: H_3C^+ , H_2C , HC^- , or C^{2-} ? What would be the pH of a 0.10 M solution of this form of cysteine?



11-4 Titrations in Polyprotic Systems

Figure 10-2 showed the titration curve for the monoprotic acid HA treated with OH^- . As a brief reminder, the pH at several critical points was computed as follows:

Initial solution:	Has the pH of the weak acid HA
$V_e/2$:	$\text{pH} = \text{p}K_a$ because $[\text{HA}] = [\text{A}^-]$
V_e :	Has the pH of the conjugate base, A^-
Past V_e :	pH is governed by concentration of excess OH^-

The slope of the titration curve is minimum at $V_e/2$ when $\text{pH} = \text{p}K_a$. The slope is steepest at the equivalence point.

Before delving into titration curves for diprotic systems, you should realize that there are two buffer pairs derived from the acid H_2A . H_2A and HA^- constitute one buffer pair and HA^- and A^{2-} constitute a second pair. For the acid H_2A , there are *two* Henderson-Hasselbalch equations, both of which are *always* true. If you happen

to know the concentrations $[H_2A]$ and $[HA^-]$, then use the pK_1 equation. If you know $[HA^-]$ and $[A^{2-}]$, use the pK_2 equation.

$$pH = pK_1 + \log\left(\frac{[HA^-]}{[H_2A]}\right) \quad pH = pK_2 + \log\left(\frac{[A^{2-}]}{[HA^-]}\right)$$

p_a_K in Henderson-Hasselbalch equation always refers to acid in denominator.

All Henderson-Hasselbalch equations are always true for a solution at equilibrium.

So now let's turn our attention to the titration of diprotic acids. Figure 11-2 shows calculated curves for 50.0 mL each of three different 0.0200 M diprotic acids, H_2A , titrated with 0.100 M OH^- . For all three curves, H_2A has $pK_1 = 4.00$. In the lowest curve, pK_2 is 6.00. In the middle curve, pK_2 is 8.00; and, in the upper curve, pK_2 is 10.00. The first equivalence volume (V_{e1}) occurs when the moles of added OH^- equal the moles of H_2A . The second equivalence volume (V_{e2}) is always exactly twice the first equivalence volume, because we must add the same amount of OH^- to convert HA^- to A^{2-} . Let's consider why pH varies as it does during these titrations.

Point A has the same pH in all three cases. It is the pH of the acid H_2A , which is treated as a monoprotic acid with $pK_a = pK_1 = 4.00$ and formal concentration F.

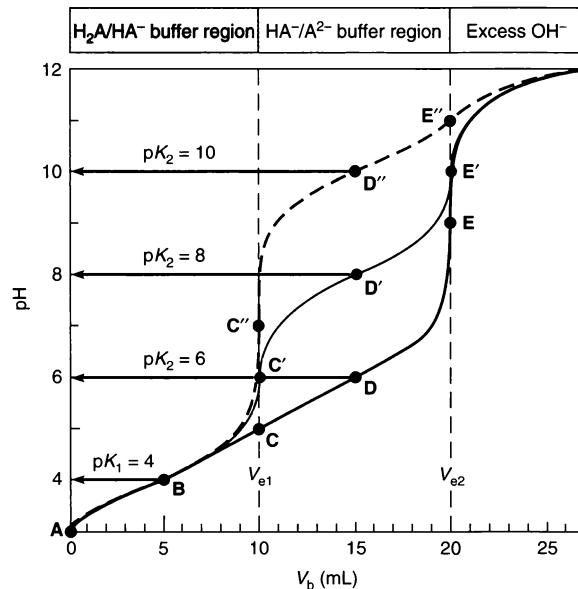
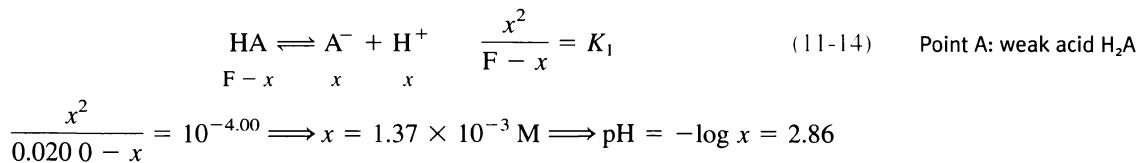


Figure 11-2 Calculated titration curves for three different diprotic acids, H_2A . For each curve, 50.0 mL of 0.0200 M H_2A are titrated with 0.100 M NaOH. Lowest curve: $pK_1 = 4.00$ and $pK_2 = 6.00$. Middle curve: $pK_1 = 4.00$ and $pK_2 = 8.00$. Upper curve: $pK_1 = 4.00$ and $pK_2 = 10.00$.

Point B, which is halfway to the first equivalence point, has the same pH in all three cases. It is the pH of a 1:1 mixture of $\text{H}_2\text{A}:\text{HA}^-$, which is treated as a mono-protic acid with $\text{p}K_{\text{a}} = \text{p}K_1 = 4.00$.

Point B: buffer containing $\text{H}_2\text{A} + \text{HA}^-$

$$\text{pH} = \text{p}K_1 + \log\left(\frac{[\text{HA}^-]}{[\text{H}_2\text{A}]}\right) = \text{p}K_1 + \log 1 = \text{p}K_1 = 4.00 \quad (11-15)$$

Because all three acids have the same $\text{p}K_1$, the pH at this point is the same in all three cases.

Point C (and C' and C'') is the first equivalence point. H_2A has been converted into HA^- , the intermediate form of a diprotic acid. The pH is calculated from Equation 11-13:

Point C: intermediate form HA^-

$$\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2) = \begin{cases} 5.00 & \text{at C} \\ 6.00 & \text{at C'} \\ 7.00 & \text{at C''} \end{cases} \quad (11-13)$$

The three acids have the same $\text{p}K_1$ but different values of $\text{p}K_2$. Therefore the pH at the first equivalence point is different in all three cases.

Point D is halfway from the first equivalence point to the second equivalence point. Half of the HA^- has been converted into A^{2-} . The pH is

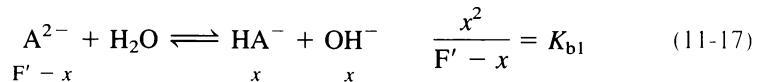
Point D: buffer containing $\text{HA}^- + \text{A}^{2-}$

$$\text{pH} = \text{p}K_2 + \log\left(\frac{[\text{A}^{2-}]}{[\text{HA}^-]}\right) = \text{p}K_2 + \log 1 = \text{p}K_2 = \begin{cases} 6.00 & \text{at D} \\ 8.00 & \text{at D'} \\ 10.00 & \text{at D''} \end{cases} \quad (11-16)$$

In Figure 11-2, points D, D', and D'' come at $\text{p}K_2$ for each of the acids.

Point E is the second equivalence point. All of the acid has been converted into A^{2-} , a weak base with concentration F' . We find the pH by treating A^{2-} as a mono-protic base with $K_{\text{b}1} = K_{\text{w}}/K_{\text{a}2}$:

Point E: weak base A^{2-}



The pH is different for the three acids, because $K_{\text{b}1}$ is different for all three. Here is how we find the pH at the second equivalence point:

$$F' = [\text{A}^{2-}] = \frac{\text{mmol A}^{2-}}{\text{total mL}} = \frac{(0.020\ 0\ \text{M})(50.0\ \text{mL})}{70.0\ \text{mL}} = 0.014\ 3\ \text{M}$$

$$\frac{x^2}{0.0143 - x} = K_{\text{b}1} = \begin{cases} 10^{-8.00} & \text{at E} \\ 10^{-6.00} & \text{at E'} \\ 10^{-4.00} & \text{at E''} \end{cases} \implies x = \begin{cases} 1.20 \times 10^{-5} & \text{at E} \\ 1.19 \times 10^{-4} & \text{at E'} = [\text{OH}^-] \\ 1.15 \times 10^{-3} & \text{at E''} \end{cases}$$

$$\implies \text{pH} = -\log(K_{\text{w}}/x) = \begin{cases} 9.08 & \text{at E} \\ 10.08 & \text{at E'} \\ 11.06 & \text{at E''} \end{cases}$$

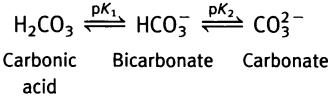
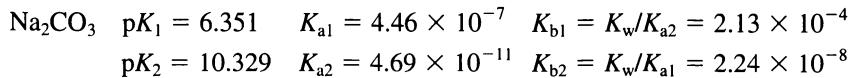
Beyond V_{e2} , pH is governed mainly by the concentration of excess OH^- . pH rapidly converges to the same value for all three titrations.

You can see in Figure 11-2 that, in a favorable case (the middle curve), we observe two obvious, steep equivalence points in the titration curve. When the pK

values are too close together or when the pK values are too low or too high, there may not be a distinct break at each equivalence point.

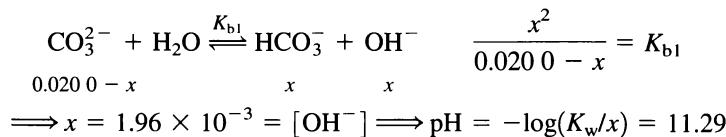
Example Titration of Sodium Carbonate

Let's reverse the process of Figure 11-2 and calculate the pH at points A–E in the titration of a diprotic base. Figure 11-3 shows the calculated titration curve for 50.0 mL of 0.020 0 M Na_2CO_3 treated with 0.100 M HCl. The first equivalence point is at 10.0 mL, and the second is at 20.0 mL. Find the pH at points A–E.



SOLUTION

Point A: This point is just 0.020 0 M Na_2CO_3 , which can be treated as monobasic:



Point B: Halfway to the first equivalence point, half of the carbonate has been converted into bicarbonate, so there is a 1:1 mixture of CO_3^{2-} and HCO_3^- —Aha! A buffer!

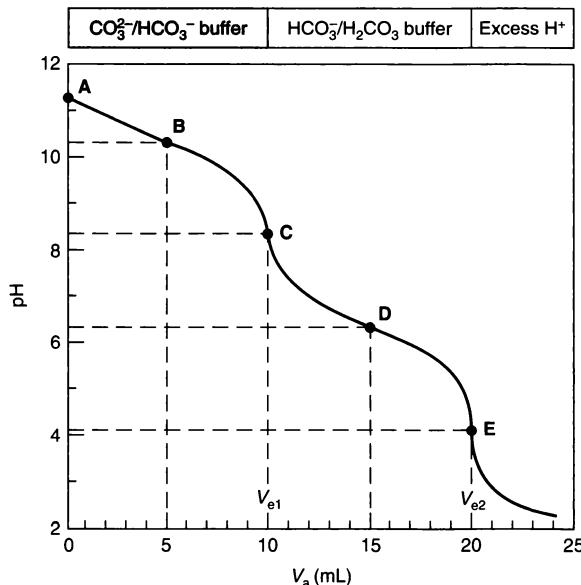
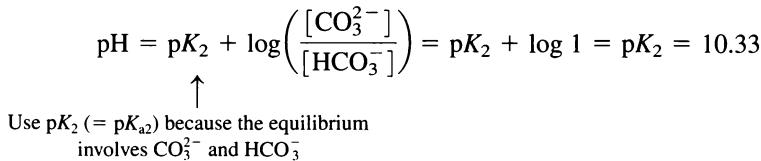
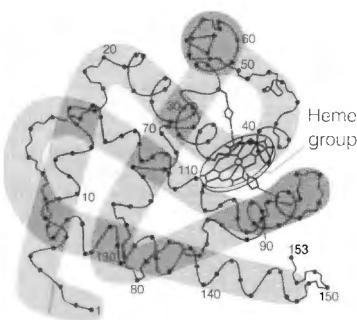


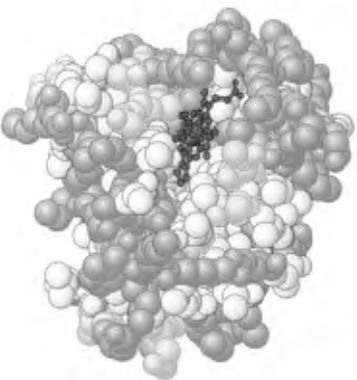
Figure 11-3 Calculated titration curve for 50.0 mL of 0.020 0 M Na_2CO_3 titrated with 0.100 M HCl.

Point C: At the first equivalence point, we have a solution of HCO_3^- , the intermediate form of a diprotic acid. To a good approximation, the pH is independent of concentration and is given by

$$\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2) = \frac{1}{2}(6.351 + 10.329) = 8.34$$



(a)



(b)

Figure 11-4 (a) Amino acid backbone of the protein myoglobin, which stores oxygen in muscle tissue. Substituents (R groups from Table 11-1) are omitted for clarity. The flat *heme* group at the right side of the protein contains an iron atom that can bind O_2 , CO , and other small molecules. [From M. F. Perutz, “The Hemoglobin Molecule.” Copyright © 1964 by Scientific American, Inc.] (b) Space-filling model of myoglobin with charged acidic and basic amino acids in dark color. Lightly colored amino acids are *hydrophilic* (polar, water loving), but not charged. White amino acids are *hydrophobic* (nonpolar, water repelling). The surface of this water-soluble protein is dominated by charged and hydrophilic groups. [From J. M. Berg, J. L. Tymoczko, and L. Stryer, *Biochemistry*, 5th ed. (New York: W. H. Freeman and Company, 2002).]

Point D: Halfway to the second equivalence point, half of the bicarbonate has been converted into carbonic acid, so there is a 1:1 mixture of HCO_3^- and H_2CO_3 —Aha! Another buffer!

$$\text{pH} = \text{p}K_1 + \log\left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}\right) = \text{p}K_1 + \log 1 = \text{p}K_1 = 6.35$$

↑
Use $\text{p}K_1$ because the equilibrium involves HCO_3^- and H_2CO_3

Point E: At the second equivalence point, all carbonate has been converted into carbonic acid, which has been diluted from its initial volume of 50.0 mL to a volume of 70.0 mL.

$$F' = [\text{H}_2\text{CO}_3] = \frac{\text{mmol H}_2\text{CO}_3}{\text{total mL}} = \frac{(0.020\ 0\ \text{M})(50.0\ \text{mL})}{70.0\ \text{mL}} = 0.014\ 3\ \text{M}$$

$$\text{H}_2\text{CO}_3 \xrightleftharpoons{K_1} \text{HCO}_3^- + \text{H}^+ \quad \frac{x^2}{0.014\ 3 - x} = K_1$$

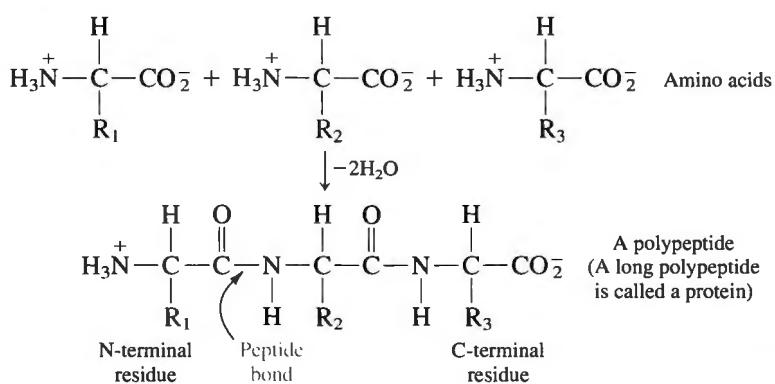
$$F' - x \qquad x \qquad x$$

$$\Rightarrow x = 7.96 \times 10^{-5} = [\text{H}^+] \Rightarrow \text{pH} = -\log x = 4.10$$

 **Test Yourself** What would be the pH at $\frac{1}{2}V_{\text{el}}$, V_{el} , and $\frac{3}{2}V_{\text{el}}$ for the titration of mercaptoacetic acid with NaOH? (Answer: 3.64, 7.12, 10.61)

Proteins Are Polyprotic Acids and Bases

Proteins are polymers made of amino acids:



Proteins have biological functions such as structural support, catalysis of chemical reactions, immune response to foreign substances, transport of molecules across membranes, and control of genetic expression. The three-dimensional structure and function of a protein are determined by the sequence of amino acids from which the protein is made. Figure 11-4 shows the protein myoglobin, whose function is to store

O_2 in muscle cells. Of the 153 amino acids in sperm-whale myoglobin, 35 have basic side groups and 23 are acidic.

At high pH, most proteins have lost so many protons that they have a negative charge. At low pH, most proteins have gained so many protons that they have a positive charge. At some intermediate pH, called the *isoelectric pH* (or **isoelectric point**), each protein has exactly zero net charge. Box 11-2 explains how we use their different isoelectric points to separate proteins from one another.

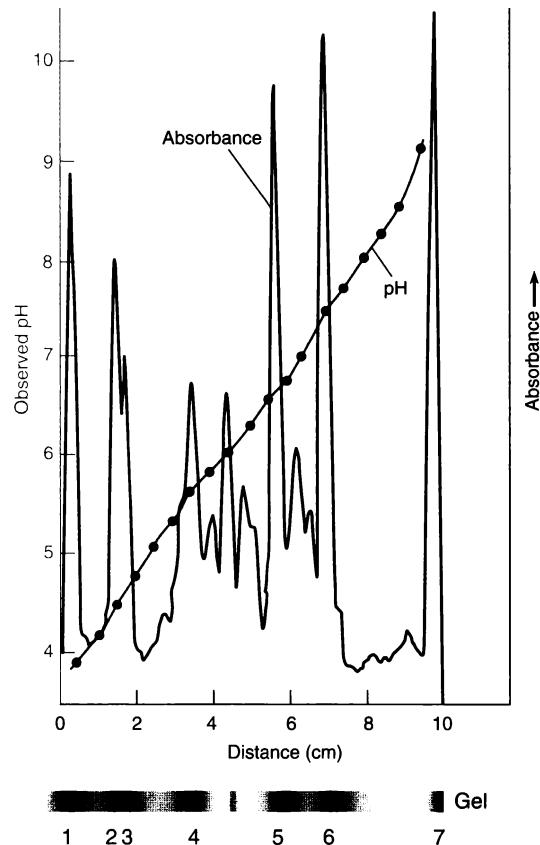
Box 11-2 What Is Isoelectric Focusing?

At its *isoelectric pH*, a protein has zero net charge and will therefore not migrate in an electric field. This effect allows us to separate proteins by **isoelectric focusing**. A mixture of proteins is subjected to a strong electric field in a medium designed to have a pH gradient. Positively charged molecules move toward the negative pole and negatively charged molecules move toward the positive pole. Each protein migrates until it reaches the point where the pH is the same as its isoelectric pH. At this point, the protein has no net charge and no longer moves. If a molecule diffuses out of its isoelectric region, it becomes charged and migrates back to its isoelectric zone. Each protein is focused in one small region at its isoelectric pH.

The figure shows an example of isoelectric focusing. A mixture of proteins was applied to a polyacrylamide gel containing polyprotic compounds called *ampholytes*. Several hundred volts were applied across the length of the gel. The ampholytes migrated until they formed a stable pH gradient ranging from pH 3 at one end of the gel to pH 10 at the other. Each protein migrated until it reached the zone with its isoelectric pH, at which point the protein had no net charge and ceased migrating. When the proteins finished migrating, the electric field was removed and proteins were precipitated in place on the gel and stained with a dye to make them visible.

The stained gel is shown at the bottom of the figure. A spectrophotometric scan of the dye peaks is shown on the graph, and a profile of measured pH is also plotted. Each dark band of stained protein gives an absorbance peak.

Living cells also have isoelectric points and can be separated from one another by isoelectric focusing.



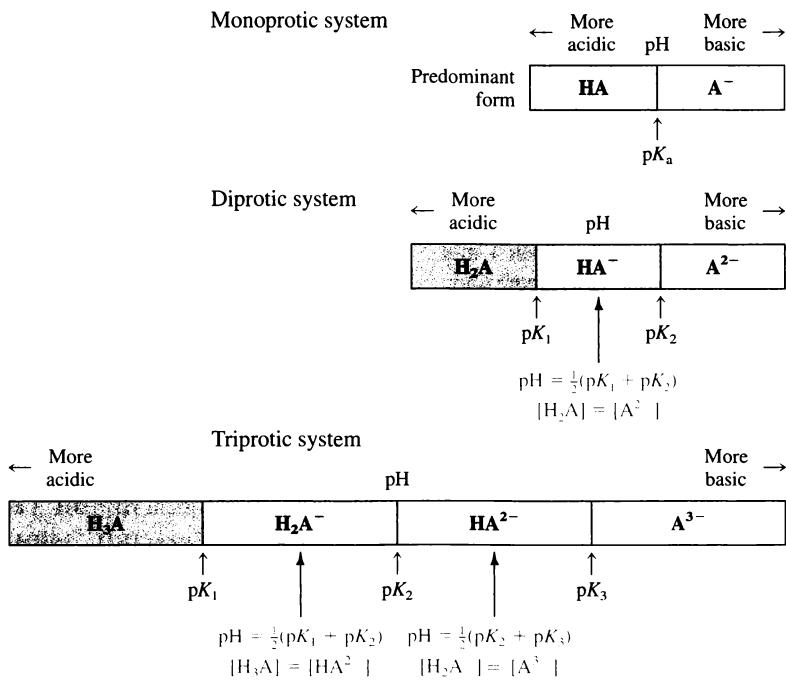
Isoelectric focusing of a mixture of proteins: (1) soybean trypsin inhibitor; (2) β -lactoglobulin A; (3) β -lactoglobulin B; (4) ovotransferrin; (5) horse myoglobin; (6) whale myoglobin; (7) cytochrome c. [Bio-Rad Laboratories, Hercules, CA.]

Ask Yourself

- 11-D. Consider the titration of 50.0 mL of 0.050 M malonic acid with 0.100 M NaOH.
- How many milliliters of titrant are required to reach each equivalence point?
 - Calculate the pH at $V_b = 0.0, 12.5, 25.0, 37.5, 50.0$, and 55.0 mL .
 - Put the points from (b) on a graph and sketch the titration curve.

Key Equations

Diprotic acid equilibria	$\text{H}_2\text{A} \rightleftharpoons \text{HA}^- + \text{H}^+$	$K_{a1} \equiv K_1$
	$\text{HA}^- \rightleftharpoons \text{A}^{2-} + \text{H}^+$	$K_{a2} \equiv K_2$
Diprotic base equilibria	$\text{A}^{2-} + \text{H}_2\text{O} \rightleftharpoons \text{HA}^- + \text{OH}^-$	K_{b1}
	$\text{HA}^- + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{A} + \text{OH}^-$	K_{b2}
Relation between K_a and K_b	Monoprotic system	$K_a K_b = K_w$
	Diprotic system	$K_{a1} K_{b2} = K_w$
		$K_{a2} K_{b1} = K_w$
	Triprotic system	$K_{a1} K_{b3} = K_w$
		$K_{a2} K_{b2} = K_w$
		$K_{a3} K_{b1} = K_w$
pH of H_2A (or BH_2^{2+})	$\frac{\text{H}_2\text{A} \xrightarrow{K_{a1}} \text{H}^+ + \text{HA}^-}{F - x \qquad x \qquad x} \qquad \frac{x^2}{F - x} = K_{a1}$	
	(This calculation gives $[\text{H}^+]$, $[\text{HA}^-]$, and $[\text{H}_2\text{A}]$. You can solve for $[\text{A}^{2-}]$ from the K_{a2} equilibrium.)	
pH of HA^- (or diprotic BH^+)	$\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2)$	
pH of A^{2-} (or diprotic B)	$\frac{\text{A}^{2-} + \text{H}_2\text{O} \xrightarrow{K_{b1}} \text{HA}^- + \text{OH}^-}{F - x \qquad x \qquad x} \qquad \frac{x^2}{F - x} = K_{b1} = \frac{K_w}{K_{a2}}$	
	(This calculation gives $[\text{OH}^-]$, $[\text{HA}^-]$, and $[\text{A}^{2-}]$. You can find $[\text{H}^+]$ from the K_w equilibrium and $[\text{H}_2\text{A}]$ from the K_{a1} equilibrium.)	
Diprotic buffer	$\text{pH} = \text{p}K_1 + \log\left(\frac{[\text{HA}^-]}{[\text{H}_2\text{A}]}\right) \qquad \text{pH} = \text{p}K_2 + \log\left(\frac{[\text{A}^{2-}]}{[\text{HA}^-]}\right)$	
	Both equations are always true and either can be used, depending on which set of concentrations you happen to know.	
Titration of H_2A with OH^-	$V_b = 0$	Find pH of H_2A
	$V_b = \frac{1}{2}V_{e1}$	$\text{pH} = \text{p}K_1$
	$V_b = V_{e1}$	$\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2)$
	$V_b = \frac{3}{2}V_{e1}$	$\text{pH} = \text{p}K_2$
	$V_b = V_{e2}$	Find pH of A^{2-}
	$V_b > V_{e2}$	Find concentration of excess OH^-
Titration of diprotic B with H^+	$V_a = 0$	Find pH of B
	$V_a = \frac{1}{2}V_{e1}$	$\text{pH} = \text{p}K_{a2}$ (for BH_2^{2+})
	$V_a = V_{e1}$	$\text{pH} \approx \frac{1}{2}(\text{p}K_{a1} + \text{p}K_{a2})$
	$V_a = \frac{3}{2}V_{e1}$	$\text{pH} = \text{p}K_{a1}$ (for BH_2^{2+})
	$V_a = V_{e2}$	Find pH of BH_2^{2+}
	$V_a > V_{e2}$	Find concentration of excess H^+



Important Terms

amino acid
amphiprotic
greenhouse gas

hydrolysis
isoelectric focusing
isoelectric point

polyprotic acid
zwitterion

Problems

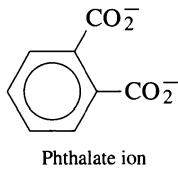
11-1. State what chemistry governs the pH at each point A through E in Figure 11-2.

11-2. Write the K_{a2} reaction of sulfuric acid (H_2SO_4) and the K_{b2} reaction of disodium oxalate ($Na_2C_2O_4$) and find their numerical values.

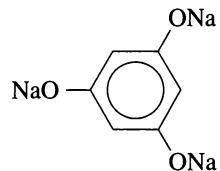
11-3. The base association constants of phosphate are $K_{b1} = 0.024$, $K_{b2} = 1.58 \times 10^{-7}$, and $K_{b3} = 1.41 \times 10^{-12}$. From the K_b values, calculate K_{a1} , K_{a2} , and K_{a3} for H_3PO_4 .

11-4. Write the general structure of an amino acid. Why do some amino acids in Table 11-1 have two pK values and others three?

11-5. Write the stepwise acid-base reactions for the following species in water. Write the correct symbol (for example, K_{b1}) for the equilibrium constant for each reaction and find its numerical value.



11-6. Write the K_{a2} reaction of proline and the K_{b2} reaction of the following trisodium salt.



11-7. From the K_a values for citric acid in Appendix B, find K_{b1} , K_{b2} , and K_{b3} for trisodium citrate.

11-8. Write the chemical reactions whose equilibrium constants are K_{b1} and K_{b2} for the amino acid serine and find their numerical values.

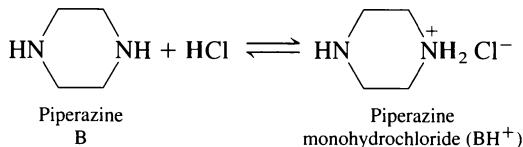
11-9. Abbreviating malonic acid, $CH_2(CO_2H)_2$, as H_2M , find the pH and concentrations of H_2M , HM^- , and M^{2-} in each of the following solutions: (a) 0.100 M H_2M ; (b) 0.100 M $NaHM$; (c) 0.100 M Na_2M . For (b), use the approximation $[HM^-] \approx 0.100\text{ M}$.

11-10. The dibasic compound B forms BH^+ and BH_2^{2+} with $K_{b1} = 1.00 \times 10^{-5}$ and $K_{b2} = 1.00 \times 10^{-9}$. Find the pH and concentrations of B, BH^+ , and BH_2^{2+} in each of the

following solutions: (a) 0.100 M B; (b) 0.100 M BH^+Br^- ; (c) 0.100 M $\text{BH}_2^+(\text{Br}^-)_2$. For (b), use the approximation $[\text{BH}^+] \approx 0.100 \text{ M}$.

11-11. Calculate the pH of a 0.300 M solution of the dibasic compound piperazine, which we will designate B. Calculate the concentration of each form of piperazine (B, BH^+ , BH_2^+).

11-12. Piperazine monohydrochloride is formed when 1 mol of HCl is added to 1 mol of the dibasic compound piperazine:



Find the pH of 0.150 M piperazine monohydrochloride and calculate the concentration of each form of piperazine. Assume $[\text{BH}^+] \approx 0.150 \text{ M}$.

11-13. Draw the structure of the amino acid glutamine and satisfy yourself that it is the intermediate form of a diprotic system. Find the pH of 0.050 M glutamine.

11-14. (a) The diagram shows the pH range in which each species of a diprotic acid is predominant. For each of the three pH values indicated ($\text{pH} = \text{p}K_1$, $\frac{1}{2}(\text{p}K_1 + \text{p}K_2)$, $\text{p}K_2$), state which species are present in equal concentrations.

←Acidic		Basic→	
H_2A	HA^-	A^{2-}	
$\text{p}K_1$		$\text{p}K_2$	
	\uparrow		
	$\frac{1}{2}(\text{p}K_1 + \text{p}K_2)$		

(b) Draw analogous diagrams for monoprotic and triprotic systems. Label key pH values and state which species are present in equal concentrations at each pH.

11-15. The acid HA has $\text{p}K_a = 7.00$.

- (a) Which is the principal species, HA or A^- , at pH 6.00?
- (b) Which is the principal species at pH 8.00?
- (c) What is the quotient $[\text{A}^-]/[\text{HA}]$ at (i) pH 7.00; (ii) at pH 6.00?

11-16. The acid H_2A has $\text{p}K_1 = 4.00$ and $\text{p}K_2 = 8.00$.

- (a) At what pH is $[\text{H}_2\text{A}] = [\text{HA}^-]$?
- (b) At what pH is $[\text{HA}^-] = [\text{A}^{2-}]$?
- (c) Which is the principal species, H_2A , HA^- , or A^{2-} at pH 2.00?
- (d) Which is the principal species at pH 6.00?
- (e) Which is the principal species at pH 10.00?

11-17. Draw a diagram for phosphoric acid analogous to the one in Problem 11-14 showing the principal species as a function of pH. Label numerical values of pH at key points. State the principal species of phosphoric acid and the second most abundant species at pH 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13.

11-18. The base B has $\text{p}K_b = 5.00$.

- (a) What is the value of $\text{p}K_a$ for the acid BH^+ ?
- (b) At what pH is $[\text{BH}^+] = [\text{B}]$?
- (c) Which is the principal species, B or BH^+ , at pH 7.00?
- (d) What is the quotient $[\text{B}]/[\text{BH}^+]$ at pH 12.00?

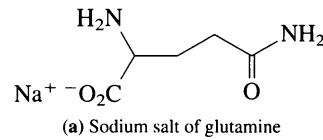
11-19. Ethylenediamine (B) is dibasic with $\text{p}K_{b1} = 4.07$ and $\text{p}K_{b2} = 7.15$.

(a) Find the two $\text{p}K_a$ values for BH_2^+ and draw a diagram like the one in Problem 11-14 showing the major species in each pH region.

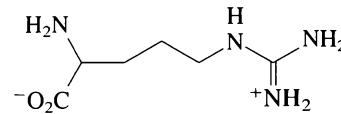
- (b) At what pH is $[\text{BH}^+] = [\text{B}]$?
- (c) At what pH is $[\text{BH}^+] = [\text{BH}_2^+]$?
- (d) Which is the principal species and which is the second most abundant species at pH 4, 5, 6, 7, 8, 9, 10, and 11?
- (e) What is the quotient $[\text{B}]/[\text{BH}^+]$ at pH 12.00?
- (f) What is the quotient $[\text{BH}_2^+]/[\text{BH}^+]$ at pH 2.00?

11-20. Draw the structures of the predominant forms of glutamic acid and tyrosine at pH 9.0 and pH 10.0. What is the second most abundant species at each pH?

11-21. Calculate the pH of a 0.10 M solution of each of the following amino acids in the form drawn:



(a) Sodium salt of glutamine



(b) Arginine

11-22. Draw the structure of the predominant form of pyridoxal-5-phosphate at pH 7.00.

11-23. Find the pH and concentration of each species of arginine in 0.050 M arginine · HCl solution. The notation “arginine · HCl” refers to a neutral arginine molecule that has taken on one extra proton by addition of one mole of HCl. A more meaningful notation shows the salt (arginine H^+) (Cl^-) formed in the reaction.

11-24. What is the predominant form of citric acid at pH 5.00?

11-25. A 100.0-mL aliquot of 0.100 M diprotic acid H_2A ($\text{p}K_1 = 4.00$, $\text{p}K_2 = 8.00$) was titrated with 1.00 M NaOH. At what volumes are the two equivalence points? Find the pH at the following volumes of base added (V_b) and sketch a graph of pH versus V_b : 0, 5.0, 10.0, 15.0, 20.0, and 22.0 mL.

11-26. The dibasic compound B ($\text{p}K_{b1} = 4.00$, $\text{p}K_{b2} = 8.00$) was titrated with 1.00 M HCl. The initial solution of B was 0.100 M and had a volume of 100.0 mL. At what volumes are the two equivalence points? Find the pH at the following volumes of acid added (V_a) and sketch a graph of pH versus V_a : 0, 5.0, 10.0, 15.0, 20.0, and 22.0 mL.

11-27. Select an indicator from Table 9-3 that would be useful for detecting each equivalence point shown in Figure 11-2 and listed below. State the color change you would observe in each case.

- (a) Second equivalence point of the lowest curve
- (b) First equivalence point of the upper curve
- (c) First equivalence point of the middle curve
- (d) Second equivalence point of the middle curve

11-28. Write two consecutive reactions that take place when 40.0 mL of 0.100 M piperazine are titrated with 0.100 M HCl and find the equivalence volumes. Find the pH at $V_a = 0, 20.0, 40.0, 60.0, 80.0$, and 100.0 mL and sketch the titration curve.

11-29. A 25.0-mL volume of 0.040 0 M phosphoric acid was titrated with 0.050 0 M tetramethylammonium hydroxide. Write the series of titration reactions and find the pH at the following volumes of added base: $V_b = 0, 10.0, 20.0, 30.0, 40.0$, and 42.0 mL. Sketch the titration curve and predict what the shape will be beyond 42.0 mL.

11-30. Write the chemical reactions (including structures of reactants and products) that take place when the amino acid histidine is titrated with perchloric acid. (Histidine has no net charge.) A solution containing 25.0 mL of 0.050 0 M histidine was titrated with 0.050 0 M HClO_4 . List the equivalence volumes and calculate the pH at $V_a = 0, 12.5, 25.0$, and 50.0 mL.

11-31. An aqueous solution containing ~ 1 g of oxobutane-dioic acid (FM 132.07) per 100 mL was titrated with 0.094 32 M NaOH to measure the acid molarity.

- (a) Calculate the pH at the following volumes of added base: $\frac{1}{2}V_{\text{e}1}, V_{\text{e}1}, \frac{3}{2}V_{\text{e}1}, V_{\text{e}2}, 1.05V_{\text{e}2}$. Sketch the titration curve.
- (b) Which equivalence point would be best to use in this titration?
- (c) You have the indicators erythrosine, ethyl orange, brom cresol green, bromothymol blue, thymolphthalein, and alizarin yellow. Which indicator will you use and what color change will you look for?

How Would You Do It?

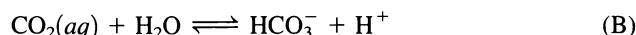
11-32. *Effect of temperature on carbonic acid acidity and the solubility of CaCO_3 .*⁴ Box 11-1 states that marine life with CaCO_3 shells and skeletons will be threatened with extinction in cold polar waters before that will happen in warm tropical waters. The following equilibrium constants apply to seawater

at 0° and 30°C, when concentrations are measured in moles per kilogram of seawater and pressure is in bars:



$$K_{\text{H}} = \frac{[\text{CO}_2(aq)]}{P_{\text{CO}_2}} = 10^{-1.2073} \text{ mol kg}^{-1} \text{ bar}^{-1} \text{ at } 0^\circ\text{C}$$

$$= 10^{-1.6048} \text{ mol kg}^{-1} \text{ bar}^{-1} \text{ at } 30^\circ\text{C}$$



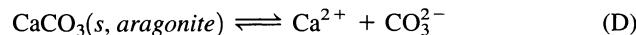
$$K_{\text{a}1} = \frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{CO}_2(aq)]} = 10^{-6.1004} \text{ mol kg}^{-1} \text{ at } 0^\circ\text{C}$$

$$= 10^{-5.8008} \text{ mol kg}^{-1} \text{ at } 30^\circ\text{C}$$



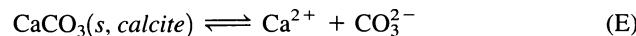
$$K_{\text{a}2} = \frac{[\text{CO}_3^{2-}][\text{H}^+]}{[\text{HCO}_3^-]} = 10^{-9.3762} \text{ mol kg}^{-1} \text{ at } 0^\circ\text{C}$$

$$= 10^{-8.8324} \text{ mol kg}^{-1} \text{ at } 30^\circ\text{C}$$



$$K_{\text{sp}}^{\text{arg}} = [\text{Ca}^{2+}][\text{CO}_3^{2-}] = 10^{-6.1113} \text{ mol}^2 \text{ kg}^{-2} \text{ at } 0^\circ\text{C}$$

$$= 10^{-6.1391} \text{ mol}^2 \text{ kg}^{-2} \text{ at } 30^\circ\text{C}$$



$$K_{\text{sp}}^{\text{cal}} = [\text{Ca}^{2+}][\text{CO}_3^{2-}] = 10^{-6.3652} \text{ mol}^2 \text{ kg}^{-2} \text{ at } 0^\circ\text{C}$$

$$= 10^{-6.3713} \text{ mol}^2 \text{ kg}^{-2} \text{ at } 30^\circ\text{C}$$

The first equilibrium constant is called K_{H} for Henry's law, which states that the solubility of a gas in a liquid is proportional to the pressure of the gas. Units are given to remind you what units you must use.

(a) Combine the expressions for K_{H} , $K_{\text{a}1}$, and $K_{\text{a}2}$ to find an expression for $[\text{CO}_3^{2-}]$ in terms of P_{CO_2} and $[\text{H}^+]$.

(b) From the result of (a), calculate $[\text{CO}_3^{2-}]$ (mol kg^{-1}) at $P_{\text{CO}_2} = 800 \mu\text{bar}$ and $\text{pH} = 7.8$ at temperatures of 0°C (polar ocean) and 30°C (tropical ocean). These are conditions that could be reached around the year 2100 if we continue to release CO_2 at the present rate.

(c) The concentration of Ca^{2+} in the ocean is 0.010 M. Predict whether aragonite and calcite will dissolve under the conditions in (b).

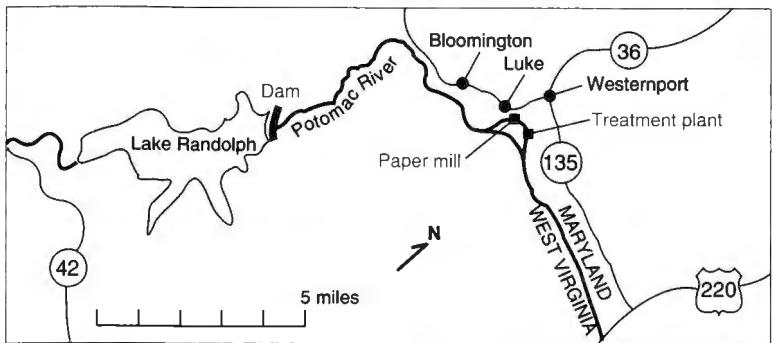
Notes and References

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2. M. J. Hardt and C. Safina, "Threatening Ocean Life from the Inside Out," *Scientific American*, August 2010, p. 66.
3. R. E. Weston, Jr., "Climate Change and Its Effect on Coral Reefs," *J. Chem. Ed.* **2000**, 77, 1574; C. Turley,

<http://www.chinadialogue.net/article/show/single/en/2359-Ocean-acidification-the-other-CO2-problem>.

4. W. Stumm and J. J. Morgan, *Aquatic Chemistry*, 3rd ed. (New York: Wiley, 1996), pp. 343–348; F. J. Millero, "Thermodynamics of the Carbon Dioxide System in the Oceans," *Geochim. Cosmochim. Acta* **1995**, 59, 661.

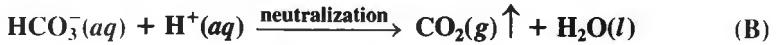
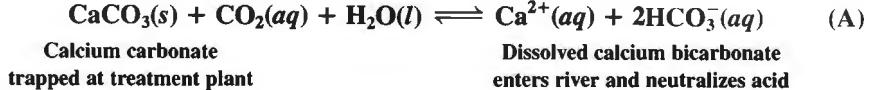
Chemical Equilibrium in the Environment



Paper mill on the Potomac River near Westernport, Maryland, neutralizes acid mine drainage in the water. Upstream of the mill, the river is acidic and lifeless; below the mill, the river teems with life. [C. Dalpra, Potomac River Basin Commission.]

Part of the North Branch of the Potomac River runs crystal clear through the scenic Appalachian Mountains; but it is lifeless—a victim of acid drainage from abandoned coal mines. As the river passes a paper mill and wastewater treatment plant near Westernport, Maryland, the pH rises from a lethal value of 4.5 to a neutral value of 7.2, at which fish and plants thrive. This fortunate accident comes about when calcium carbonate by-product from papermaking exits the paper mill and reacts with massive quantities of carbon dioxide from bacterial respiration at the sewage treatment plant. The resulting soluble bicarbonate neutralizes the acidic river and restores life downstream of the plant. In the absence of CO₂, solid CaCO₃ would be trapped at the treatment plant and would never enter the river.

Reaction A is the same reaction by which ocean acidification (Box 11-1) can destroy marine life in the ocean.



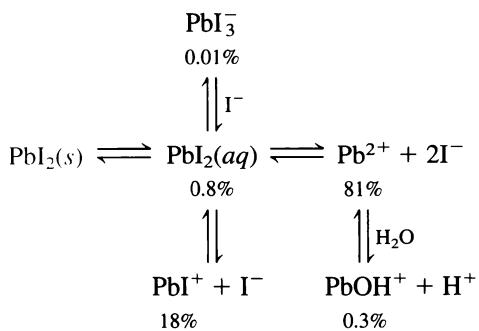
We call these two reactions *coupled equilibria*. Consumption of bicarbonate in the second reaction drives the first reaction to make more product.

A Deeper Look at Chemical Equilibrium

We now pause to look more carefully at chemical equilibrium. This chapter is optional in that later chapters do not depend on it in any critical way. However, many instructors consider the treatment of equilibrium in this chapter to be a fundamental component of an education in chemistry.

12-1 The Effect of Ionic Strength on Solubility of Salts

When slightly soluble lead(II) iodide dissolves in pure water, many species are formed:



Approximately 81% of the lead is found as Pb^{2+} , 18% is PbI^+ , 0.8% is $\text{PbI}_2(aq)$, 0.3% is PbOH^+ , and 0.01% is PbI_3^- . The *solubility product* is the equilibrium constant for the reaction $\text{PbI}_2(s) \rightleftharpoons \text{Pb}^{2+} + 2\text{I}^-$, which tells only part of the story.

Now a funny thing happens when the “inert” salt KNO_3 is added to the saturated PbI_2 solution. “Inert” means that there is no chemical reaction of K^+ or NO_3^- with any of the lead iodide species. As more KNO_3 is added, the total concentration of dissolved iodine increases, as shown in Figure 12-1. (Dissolved iodine includes free iodide and iodide attached to lead.) It turns out that adding any inert salt, such as KNO_3 , to a sparingly soluble salt such as PbI_2 increases the solubility of the sparingly soluble substance. Why does solubility increase when salts are added?

Composition was computed from the following equilibria with activity coefficients:¹

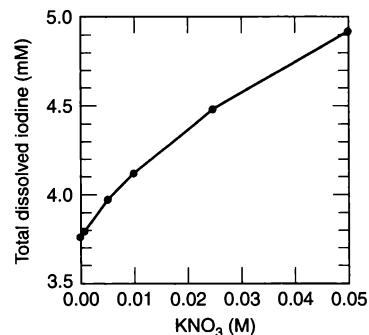
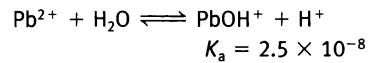
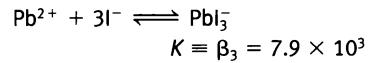
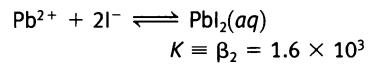
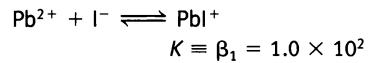
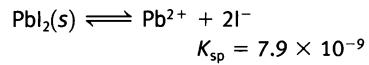


Figure 12-1 Observed effect of KNO_3 on the solubility of PbI_2 . [Data from D. B. Green, G. Rechtsteiner, and A. Honodel, *J. Chem. Ed.* **1996**, 73, 789.]

The Explanation

An anion is surrounded by more cations than anions. A cation is surrounded by more anions than cations.

Consider one particular Pb^{2+} ion and one particular I^- ion in the solution. The I^- ion is surrounded by cations (K^+ , Pb^{2+}) and anions (NO_3^- , I^-) in the solution. However, the typical anion has more cations than anions near it because cations are attracted but anions are repelled. These interactions create a region of net positive charge around any particular anion. We call this region the **ionic atmosphere** (Figure 12-2). Ions continually diffuse into and out of the ionic atmosphere. The net charge in the atmosphere, averaged over time, is less than the charge of the anion at the center. Similarly, an atmosphere of negative charge surrounds any cation in solution.

The ionic atmosphere *attenuates* (decreases) the attraction between ions in solution. The cation plus its negative atmosphere has less positive charge than the cation alone. The anion plus its ionic atmosphere has less negative charge than the anion alone. The net attraction between the cation with its ionic atmosphere and the anion with its ionic atmosphere is smaller than it would be between pure cation and anion in the absence of ionic atmospheres. *The higher the concentration of ions in a solution, the higher the charge in the ionic atmosphere. Each ion-plus-atmosphere contains less net charge and there is less attraction between any particular cation and anion.*

Increasing the concentration of ions in a solution therefore reduces the attraction between any particular Pb^{2+} ion and any I^- ion, relative to their attraction in pure water. The effect is to reduce their tendency to come together, thereby increasing the solubility of PbI_2 .

Increasing the concentration of ions in a solution promotes dissociation of ions. Thus, each of the following reactions is driven to the right if KNO_3 is added (Demonstration 12-1):

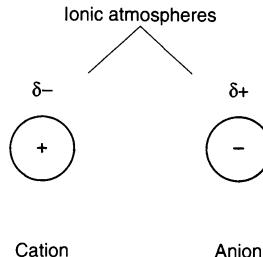
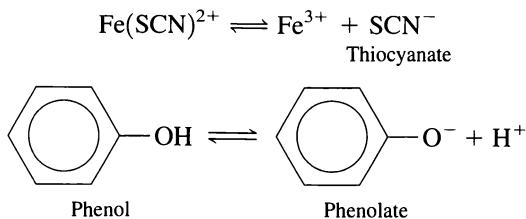
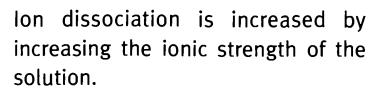


Figure 12-2 An ionic atmosphere, shown as a spherical cloud of charge $\delta+$ or $\delta-$, surrounds each ion in solution. The charge of the atmosphere is less than the charge of the central ion. The greater the ionic strength of the solution, the greater the charge in each ionic atmosphere.

What Do We Mean by “Ionic Strength”?

Ionic strength, μ , is a measure of the total concentration of ions in solution. The more highly charged an ion, the more it is counted.

$$\text{Ionic strength: } \mu = \frac{1}{2}(c_1z_1^2 + c_2z_2^2 + \dots) = \frac{1}{2}\sum_i c_i z_i^2 \quad (12-1)$$

where c_i is the concentration of the i th species and z_i is its charge. The sum extends over all ions in solution.

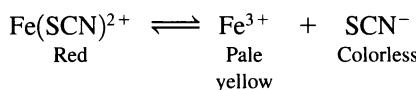
Example Calculation of Ionic Strength

Find the ionic strength of (a) 0.10 M NaNO₃; (b) 0.010 M Na₂SO₄; and (c) 0.020 M KBr plus 0.010 M Na₂SO₄.



Demonstration 12-1 Effect of Ionic Strength on Ion Dissociation²

This experiment demonstrates the effect of ionic strength on the dissociation of the red iron(III) thiocyanate complex:



Prepare a solution of 1 mM FeCl_3 by dissolving 0.27 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 1 L of water containing 3 drops of 15 M (concentrated) HNO_3 . The acid slows the precipitation of Fe(OH)_3 , which occurs in a few days and necessitates the preparation of fresh solution for this demonstration.

To demonstrate the effect of ionic strength on the dissociation reaction, mix 300 mL of the 1 mM FeCl_3 solution with 300 mL of 1.5 mM NH_4SCN or KSCN . Divide the pale red solution into two equal portions and add 12 g of KNO_3 to one of them to increase the ionic strength to 0.4 M. As the KNO_3 dissolves, the red $\text{Fe}(\text{SCN})^{2+}$ complex dissociates and the color fades noticeably (Color Plate 6).

Add a few crystals of NH_4SCN or KSCN to either solution to drive the reaction toward formation of $\text{Fe}(\text{SCN})^{2+}$, thereby intensifying the red color. This reaction demonstrates Le Châtelier's principle—adding a product creates more reactant.

SOLUTION

$$\begin{aligned} \text{(a)} \mu &= \frac{1}{2}\{[\text{Na}^+] \cdot (+1)^2 + [\text{NO}_3^-] \cdot (-1)^2\} \\ &= \frac{1}{2}\{0.10 \cdot 1 + 0.10 \cdot 1\} = 0.10 \text{ M} \end{aligned}$$

$$\begin{aligned} \text{(b)} \mu &= \frac{1}{2}\{[\text{Na}^+] \cdot (+1)^2 + [\text{SO}_4^{2-}] \cdot (-2)^2\} \\ &= \frac{1}{2}\{(0.020 \cdot 1) + (0.010 \cdot 4)\} = 0.030 \text{ M} \end{aligned}$$

Note that $[\text{Na}^+] = 0.020 \text{ M}$ because there are two moles of Na^+ per mole of Na_2SO_4 .

$$\begin{aligned} \text{(c)} \mu &= \frac{1}{2}\{[\text{K}^+] \cdot (+1)^2 + [\text{Br}^-] \cdot (-1)^2 + [\text{Na}^+] \cdot (+1)^2 + [\text{SO}_4^{2-}] \cdot (-2)^2\} \\ &= \frac{1}{2}\{(0.020 \cdot 1) + (0.020 \cdot 1) + (0.020 \cdot 1) + (0.010 \cdot 4)\} = 0.050 \text{ M} \end{aligned}$$

 **Test Yourself** Find the ionic strength of 1.0 mM $\text{Ca}(\text{ClO}_4)_2$. (Answer: 3.0 mM)

NaNO_3 is called a 1:1 electrolyte because the cation and the anion both have a charge of 1. For 1:1 electrolytes, the ionic strength equals the molarity. For any other stoichiometry (such as the 2:1 electrolyte Na_2SO_4), the ionic strength is greater than the molarity.

Electrolyte	Molarity	Ionic strength
1:1	M	M
2:1	M	3M
3:1	M	6M
2:2	M	4M

Ask Yourself

12-A. (a) From the solubility product of PbI_2 , calculate the expected concentration of dissolved iodine in a saturated solution of PbI_2 . Why is your result different from the experimental observation in Figure 12-1, and why does the concentration of dissolved iodine increase with increasing KNO_3 concentration?

(b) If PbI_2 dissolved to give 1.0 mM Pb^{2+} plus 2.0 mM I^- and if there were no other ionic species in the solution, what would be the ionic strength?

12-2 Activity Coefficients

Until now, we have written the equilibrium for the reaction $a\text{A} + b\text{B} \rightleftharpoons c\text{C} + d\text{D}$ in the form $K = [\text{C}]^c[\text{D}]^d / [\text{A}]^a[\text{B}]^b$. This equilibrium constant does not account for

any effect of ionic strength on the chemical reaction. To account for ionic strength, concentrations are replaced by **activities**:

This is a *really good time* to review Section 1-5 on the equilibrium constant.

Do not confuse the terms *activity* and *activity coefficient*.

This is the “real” equilibrium constant.

Activity of C:

$$\mathcal{A}_C = [C]\gamma_C \quad (12-2)$$

The diagram shows three arrows pointing upwards from the text below to the components of the activity equation. The first arrow points to "[C]" with the label "Concentration of C". The second arrow points to "\gamma_C" with the label "Activity coefficient of C". The third arrow points to "\mathcal{A}_C" with the label "Activity of C".

The activity of species C is its concentration multiplied by its **activity coefficient**. The activity coefficient depends on ionic strength. If there were no effect of ionic strength on the chemical reaction, the activity coefficient would be 1. The correct form of the equilibrium constant for the reaction $aA + bB \rightleftharpoons cC + dD$ is

General form of equilibrium constant: $K = \frac{\mathcal{A}_C^c \mathcal{A}_D^d}{\mathcal{A}_A^a \mathcal{A}_B^b} = \frac{[C]^c [D]^d \gamma_C^c \gamma_D^d}{[A]^a [B]^b \gamma_A^a \gamma_B^b}$ (12-3)

For the reaction $\text{PbI}_2(s) \rightleftharpoons \text{Pb}^{2+} + 2\text{I}^-$, the equilibrium constant is

$$K_{sp} = \mathcal{A}_{\text{Pb}^{2+}} \mathcal{A}_{\text{I}^-}^2 = [\text{Pb}^{2+}] \gamma_{\text{Pb}^{2+}} [\text{I}^-]^2 \gamma_{\text{I}^-}^2 \quad (12-4)$$

If the concentrations of Pb^{2+} and I^- are to *increase* when a second salt is added to increase ionic strength, the activity coefficients must *decrease* with increasing ionic strength. Conversely, at low ionic strength, activity coefficients approach unity.

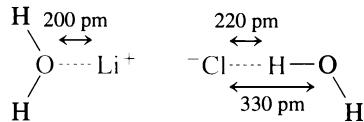
Activity Coefficients of Ions

Detailed consideration of the ionic atmosphere model leads to the **extended Debye-Hückel equation**, relating activity coefficients to ionic strength:

Extended Debye-Hückel equation: $\log \gamma = \frac{-0.51z^2 \sqrt{\mu}}{1 + (\alpha \sqrt{\mu}/305)} \quad (\text{at } 25^\circ\text{C}) \quad (12-5)$

In Equation 12-5, γ is the activity coefficient of an ion of charge $\pm z$ and size α (picometers, pm) in an aqueous solution of ionic strength μ . Table 12-1 gives sizes and activity coefficients for many ions.

The ion size α in Table 12-1 is an empirical parameter that provides agreement between measured activity coefficients and ionic strength up to $\mu \approx 0.1 \text{ M}$. In theory, α is the diameter of the *hydrated ion*, which includes the ion and its tightly bound sheath of water molecules. Cations attract the negatively charged oxygen atom of H_2O and anions attract the positively charged H atoms.



A small or more highly charged ion binds water molecules more tightly and has a *larger* hydrated diameter than does a larger or less charged ion (Figure 12-3).

Sizes in Table 12-1 cannot be taken literally. For example, the diameter of Cs^+ ion in crystals is 340 pm. The hydrated Cs^+ ion in solution must be larger than the unhydrated ion in the crystal, but the size of Cs^+ given in Table 12-1 is only 250 pm. Even though ion sizes in Table 12-1 are empirical parameters, trends among sizes

1 pm (picometer) = 10^{-12} m

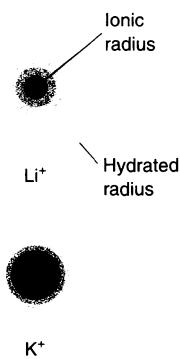


Figure 12-3 The smaller Li^+ ion binds water molecules more tightly than does the larger K^+ ion, so Li^+ has the *larger* hydrated diameter.

Table 12-1 Activity coefficients for aqueous solutions at 25°C^a

Ion	Ion size (α , pm)	Ionic strength (μ , M)				
		0.001	0.005	0.01	0.05	0.1
<i>Charge (z) = ±1</i>		<i>Activity coefficient (γ)</i>				
H ⁺	900	0.967	0.933	0.914	0.86	0.83
(C ₆ H ₅) ₂ CHCO ₂ ⁻ , (C ₃ H ₇) ₄ N ⁺	800	0.966	0.931	0.912	0.85	0.82
(O ₂ N) ₃ C ₆ H ₂ O ⁻ , (C ₃ H ₇) ₃ NH ⁺ , CH ₃ OC ₆ H ₄ CO ₂ ⁻	700	0.965	0.930	0.909	0.845	0.81
Li ⁺ , C ₆ H ₅ CO ₂ ⁻ , HOC ₆ H ₄ CO ₂ ⁻ , ClC ₆ H ₄ CO ₂ ⁻ , C ₆ H ₅ CH ₂ CO ₂ ⁻ , CH ₂ =CHCH ₂ CO ₂ ⁻ , (CH ₃) ₂ CHCH ₂ CO ₂ ⁻ , (CH ₃ CH ₂) ₄ N ⁺ , (C ₃ H ₇) ₂ NH ⁺	600	0.965	0.929	0.907	0.835	0.80
Cl ₂ CHCO ₂ ⁻ , Cl ₃ CCO ₂ ⁻ , (CH ₃ CH ₂) ₃ NH ⁺ , (C ₃ H ₇)NH ⁺	500	0.964	0.928	0.904	0.83	0.79
Na ⁺ , CdCl ⁺ , ClO ₂ ⁻ , IO ₃ ⁻ , HCO ₃ ⁻ , H ₂ PO ₄ ⁻ , HSO ₃ ⁻ , H ₂ AsO ₄ ⁻ , Co(NH ₃) ₄ (NO ₂) ₂ ⁺ , CH ₃ CO ₂ ⁻ , ClCH ₂ CO ₂ ⁻ , (CH ₃) ₄ N ⁺ , (CH ₃ CH ₂) ₂ NH ⁺ , H ₂ NCH ₂ CO ₂ ⁻	450	0.964	0.928	0.902	0.82	0.775
+H ₃ NCH ₂ CO ₂ H, (CH ₃) ₃ NH ⁺ , CH ₃ CH ₂ NH ₃ ⁺	400	0.964	0.927	0.901	0.815	0.77
OH ⁻ , F ⁻ , SCN ⁻ , OCN ⁻ , HS ⁻ , ClO ₃ ⁻ , ClO ₄ ⁻ , BrO ₃ ⁻ , IO ₄ ⁻ , MnO ₄ ⁻ , HCO ₂ ⁻ , H ₂ citrate ⁻ , CH ₃ NH ₃ ⁺ , (CH ₃) ₂ NH ₂ ⁺	350	0.964	0.926	0.900	0.81	0.76
K ⁺ , Cl ⁻ , Br ⁻ , I ⁻ , CN ⁻ , NO ₂ ⁻ , NO ₃ ⁻	300	0.964	0.925	0.899	0.805	0.755
Rb ⁺ , Cs ⁺ , NH ₄ ⁺ , Tl ⁺ , Ag ⁺	250	0.964	0.924	0.898	0.80	0.75
<i>Charge (z) = ±2</i>		<i>Activity coefficient (γ)</i>				
Mg ²⁺ , Be ²⁺	800	0.872	0.755	0.69	0.52	0.45
CH ₂ (CH ₂ CH ₂ CO ₂) ₂ , (CH ₂ CH ₂ CH ₂ CO ₂) ₂	700	0.872	0.755	0.685	0.50	0.425
Ca ²⁺ , Cu ²⁺ , Zn ²⁺ , Sn ²⁺ , Mn ²⁺ , Fe ²⁺ , Ni ²⁺ , Co ²⁺ , C ₆ H ₄ (CO ₂) ₂ , H ₂ C(CH ₂ CO ₂) ₂ , (CH ₂ CH ₂ CO ₂) ₂	600	0.870	0.749	0.675	0.485	0.405
Sr ²⁺ , Ba ²⁺ , Cd ²⁺ , Hg ²⁺ , S ²⁻ , S ₂ O ₄ ²⁻ , WO ₄ ²⁻ , H ₂ C(CO ₂) ₂ , (CH ₂ CO ₂) ₂ , (CHOHCO ₂) ₂	500	0.868	0.744	0.67	0.465	0.38
Pb ²⁺ , CO ₃ ²⁻ , SO ₃ ²⁻ , MoO ₄ ²⁻ , Co(NH ₃) ₅ Cl ²⁺ , Fe(CN) ₅ NO ²⁻ , C ₂ O ₄ ²⁻ , Hcitrate ²⁻	450	0.867	0.742	0.665	0.455	0.37
Hg ₂ ²⁺ , SO ₄ ²⁻ , S ₂ O ₃ ²⁻ , S ₂ O ₆ ²⁻ , S ₂ O ₈ ²⁻ , SeO ₄ ²⁻ , CrO ₄ ²⁻ , HPO ₄ ²⁻	400	0.867	0.740	0.660	0.445	0.355
<i>Charge (z) = ±3</i>		<i>Activity coefficient (γ)</i>				
Al ³⁺ , Fe ³⁺ , Cr ³⁺ , Sc ³⁺ , Y ³⁺ , In ³⁺ , lanthanides ^b	900	0.738	0.54	0.445	0.245	0.18
citrate ³⁻	500	0.728	0.51	0.405	0.18	0.115
PO ₄ ³⁻ , Fe(CN) ₆ ³⁻ , Cr(NH) ₆ ³⁺ , Co(NH ₃) ₆ ³⁺ , Co(NH ₃) ₅ H ₂ O ³⁺	400	0.725	0.505	0.395	0.16	0.095
<i>Charge (z) = ±4</i>		<i>Activity coefficient (γ)</i>				
Th ⁴⁺ , Zr ⁴⁺ , Ce ⁴⁺ , Sn ⁴⁺	1 100	0.588	0.35	0.255	0.10	0.065
Fe(CN) ₆ ⁴⁻	500	0.57	0.31	0.20	0.048	0.021

a. J. Kielland, *J. Am. Chem. Soc.* **1937**, 59, 1675.

b. Lanthanides are elements 57–71 in the periodic table.

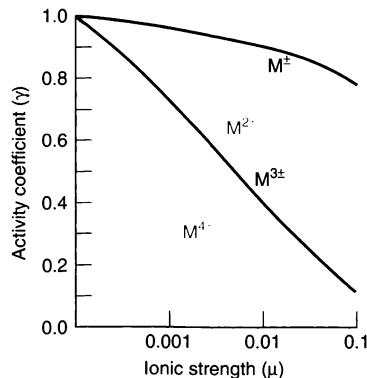


Figure 12-4 Activity coefficients for differently charged ions with a constant size $\alpha = 500$ pm. At zero ionic strength, $\gamma = 1$. The greater the charge of the ion, the more rapidly γ decreases as ionic strength increases. Note that the abscissa is logarithmic.

are sensible. Small, highly charged ions bind solvent more tightly and have larger effective sizes than do larger or less highly charged ions. For example, the order of sizes in Table 12-1 is $\text{Li}^+ > \text{Na}^+ > \text{K}^+ > \text{Rb}^+$, even though crystallographic radii are $\text{Li}^+ < \text{Na}^+ < \text{K}^+ < \text{Rb}^+$.

In Table 12-1, ions of the same size and charge appear in the same group and have the same activity coefficients. For example, Ba^{2+} and succinate [$\text{O}_2\text{CCH}_2\text{CH}_2\text{CO}_2^-$, listed as $(\text{CH}_2\text{CO}_2^-)_2$] each have a size of 500 pm and are listed among charge (z) = ± 2 ions. At an ionic strength of 0.001 M, both ions have an activity coefficient of 0.868.

Effect of Ionic Strength, Ion Charge, and Ion Size on the Activity Coefficient

Over the range of ionic strengths from 0 to 0.1 M, we find that

- As ionic strength increases, the activity coefficient decreases (Figure 12-4). The activity coefficient (γ) approaches unity as the ionic strength (μ) approaches zero.
- As the charge of the ion increases, the departure of its activity coefficient from unity increases. Activity corrections in Figure 12-4 are more important for an ion with a charge of ± 3 than for one with a charge of ± 1 . Note that activity coefficients in Table 12-1 depend on the magnitude of the charge but not on its sign.
- The smaller the size (α) of the ion, the more important activity effects become.

Example Using Table 12-1

Find the activity coefficient of Mg^{2+} in a solution of 3.3 mM $\text{Mg}(\text{NO}_3)_2$.

SOLUTION The ionic strength is

$$\begin{aligned}\mu &= \frac{1}{2} \{ [\text{Mg}^{2+}] \cdot 2^2 + [\text{NO}_3^-] \cdot (-1)^2 \} \\ &= \frac{1}{2} \{ (0.0033) \cdot 4 + (0.0066) \cdot 1 \} = 0.010 \text{ M}\end{aligned}$$

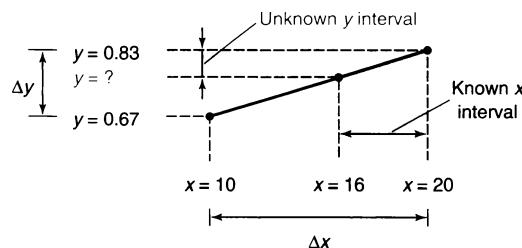
In Table 12-1, Mg^{2+} is listed under the charge ± 2 and has a size of 800 pm. When $\mu = 0.010 \text{ M}$, $\gamma = 0.69$.

 **Test Yourself** Find the activity coefficient of SO_4^{2-} in 1.25 mM MgSO_4 . (Answer: $\mu = 0.005 \text{ M}$, $\gamma = 0.740$)

How to Interpolate

If you need to find an activity coefficient for an ionic strength that is between values in Table 12-1, you can use Equation 12-5. Alternatively, in the absence of a spreadsheet, it is usually easier to *interpolate* than to use Equation 12-5. In *linear interpolation*, we assume that values between two entries of a table lie on a straight line. For example, consider a table in which $y = 0.67$ when $x = 10$ and $y = 0.83$ when $x = 20$. What is the value of y when $x = 16$?

x value:	10	16	20
y value:	0.67	?	0.83



To interpolate a value of γ , we can set up a proportion:

$$\text{Interpolation: } \frac{\text{unknown } y \text{ interval}}{\Delta y} = \frac{\text{known } x \text{ interval}}{\Delta x} \quad (12-6)$$

This calculation is equivalent to saying
16 is 60% of the way from 10 to
20, so the y value will be 60% of
the way from 0.67 to 0.83.

$$\frac{0.83 - \gamma}{0.83 - 0.67} = \frac{20 - 16}{20 - 10} \Rightarrow \gamma = 0.76_6$$

For $x = 16$, our estimate of y is 0.76₆.

Example Interpolating Activity Coefficients

Calculate the activity coefficient of H⁺ when $\mu = 0.025$ M.

SOLUTION H⁺ is the first entry in Table 12-1.

	$\mu = 0.01$	0.025	0.05
H ⁺ :	$\gamma = 0.914$?	0.86

The linear interpolation is set up as follows:

$$\frac{\text{unknown } \gamma \text{ interval}}{\Delta \gamma} = \frac{\text{known } \mu \text{ interval}}{\Delta \mu}$$

$$\frac{0.86 - \gamma}{0.86 - 0.914} = \frac{0.05 - 0.025}{0.05 - 0.01} \Rightarrow \gamma = 0.89_4$$

ANOTHER SOLUTION A more accurate and slightly more tedious calculation uses Equation 12-5, with the size $\alpha = 900$ pm listed for H⁺ in Table 12-1:

$$\log \gamma_{\text{H}^+} = \frac{(-0.51)(1^2)\sqrt{0.025}}{1 + (900\sqrt{0.025}/305)} = -0.054_{98}$$

$$\gamma_{\text{H}^+} = 10^{-0.054_{98}} = 0.88_1$$

The difference between this calculated value and the interpolated value is less than 2%. Equation 12-5 is easy to implement in a spreadsheet.

 **Test Yourself** Find the activity coefficient of Hg²⁺ when $\mu = 0.06$ M by interpolation and with Equation 12-5. (Answer: 0.448, 0.440)

Activity Coefficients of Nonionic Compounds

Neutral molecules, such as benzene and acetic acid, have no ionic atmosphere because they have no charge. To a good approximation, their activity coefficients are unity when the ionic strength is less than 0.1 M. In this text, we set $\gamma = 1$ for neutral molecules. That is, *we assume that the activity of a neutral molecule is equal to its concentration*.

For gases such as H₂, the activity is written

$$\mathcal{A}_{\text{H}_2} = P_{\text{H}_2}\gamma_{\text{H}_2}$$

For neutral species, $\mathcal{A}_C \approx [C]$.

where P_{H_2} is pressure in bars. The activity of a gas is called its *fugacity*, and the activity coefficient is called the *fugacity coefficient*. Deviation of gas behavior from the ideal gas law results in deviation of the fugacity coefficient from unity. For most gases at or below 1 bar, $\gamma \approx 1$. Therefore, for all gases, we will set $\mathcal{A} = P$ (bar).

For gases, $\mathcal{A} \approx P$ (bar).

At high ionic strength, γ increases with increasing μ .

High Ionic Strengths

The extended Debye-Hückel equation 12-5 predicts that the activity coefficient, γ , will decrease as ionic strength, μ , increases. In fact, above an ionic strength of approximately 1 M, activity coefficients of most ions *increase*, as shown for H^+ in $NaClO_4$ solutions in Figure 12-5. We should not be too surprised that activity coefficients in concentrated salt solutions are not the same as those in dilute aqueous solution. The “solvent” is no longer just H_2O but, rather, a mixture of H_2O and $NaClO_4$. Hereafter, we limit our attention to dilute aqueous solutions in which Equation 12-5 applies.

Example A Better Estimate of the Solubility of PbI_2

From the solubility product alone, you estimated in Ask Yourself 12-A that the concentration of dissolved iodine in a saturated solution of PbI_2 is 2.5 mM.

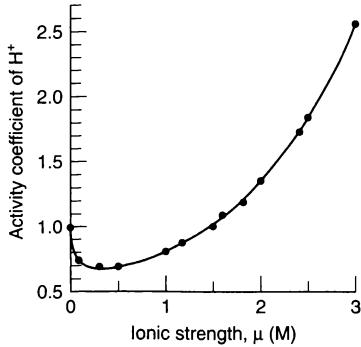
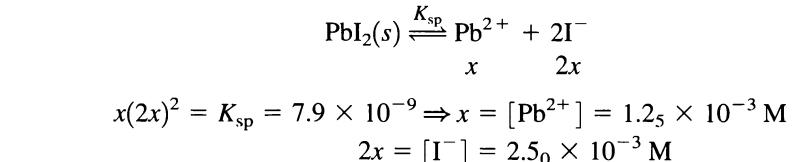


Figure 12-5 Activity coefficient of H^+ in solutions containing 0.010 0 M $NaClO_4$ and various amounts of $NaClO_4$. [Derived from L. Pezza, M. Molina, M. de Moraes, C. B. Melios, and J. O. Tognoli, *Talanta* 1996, 43, 1689.]

Successive approximations: Use one approximation to find a better approximation. Repeat the process until successive approximations are in close agreement.



The observed concentration of dissolved iodine in the absence of KNO_3 in Figure 12-1 is 3.8 mM, which is 50% higher than the predicted concentration of I^- of 2.5 mM. The Pb^{2+} and I^- ions increase the ionic strength of the solution and therefore increase the solubility of PbI_2 . Use activity coefficients to estimate the increased solubility.

SOLUTION The ionic strength of the solution is

$$\begin{aligned} \mu &= \frac{1}{2} \{ [Pb^{2+}] \cdot (+2)^2 + [I^-] \cdot (-1)^2 \} \\ &= \frac{1}{2} \{ (0.00125 \cdot 4) + (0.00250 \cdot 1) \} = 0.00375 \text{ M} \end{aligned}$$

If $\mu = 0.00375$ M, interpolation in Table 12-1 tells us that the activity coefficients are $\gamma_{Pb^{2+}} = 0.781$ and $\gamma_{I^-} = 0.937$. A better estimate of the solubility of PbI_2 is obtained by using these activity coefficients in the solubility product:

$$\begin{aligned} K_{sp} &= [Pb^{2+}] \gamma_{Pb^{2+}} [I^-]^2 \gamma_{I^-}^2 = (x_2)(0.781)(2x_2)^2(0.937)^2 \\ \Rightarrow x_2 &= [Pb^{2+}] = 1.42 \text{ mM} \quad \text{and} \quad [I^-] = 2x_2 = 2.84 \text{ mM} \end{aligned}$$

We wrote a subscript 2 in x_2 to indicate that it is our second approximation. The new concentrations of Pb^{2+} and I^- give a new estimate of the ionic strength, $\mu = 0.00426$ M, which gives new activity coefficients: $\gamma_{Pb^{2+}} = 0.765$ and $\gamma_{I^-} = 0.932$. Repeating the solubility computation gives

$$\begin{aligned} K_{sp} &= [Pb^{2+}] \gamma_{Pb^{2+}} [I^-]^2 \gamma_{I^-}^2 = (x_3)(0.765)(2x_3)^2(0.932)^2 \\ \Rightarrow x_3 &= [Pb^{2+}] = 1.44 \text{ mM} \quad \text{and} \quad [I^-] = 2x_3 = 2.88 \text{ mM} \end{aligned}$$

This third estimate is only slightly different from the second estimate. With activity coefficients, we estimate $[I^-] = 2.9$ mM instead of 2.5 mM calculated without

activity coefficients. The remaining difference between 2.9 mM and the observed solubility of 3.8 mM is that we have not accounted for other species (PbI^+ and $\text{PbI}_2(aq)$) in the solution.

 **Test Yourself** This is not a short question. Use the procedure from this example to calculate the solubility of LiF ($K_{\text{sp}} = 0.0017$) in water. (Answer: first iteration: 0.041 M; second iteration: 0.049 M; third iteration: 0.050 M)

The Real Definition of pH

The pH measured by a pH electrode is not the negative logarithm of the hydrogen ion *concentration*. The ideal quantity that we measure with the pH electrode is the negative logarithm of the hydrogen ion *activity*.

$$\text{Real definition of pH: } \text{pH} = -\log \mathcal{A}_{\text{H}^+} = -\log([\text{H}^+] \gamma_{\text{H}^+}) \quad (12-7) \quad \text{A pH electrode measures } -\log \mathcal{A}_{\text{H}^+}.$$

Effect of Salt on Water Dissociation

Consider the equilibrium $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$, for which $K_w = \mathcal{A}_{\text{H}^+} \mathcal{A}_{\text{OH}^-} = [\text{H}^+] \gamma_{\text{H}^+} [\text{OH}^-] \gamma_{\text{OH}^-}$. What is the pH of pure water and of 0.1 M NaCl?

SOLUTION In pure water, the ionic strength is so low that activity coefficients are close to 1.

Putting $\gamma_{\text{H}^+} = \gamma_{\text{OH}^-} = 1$ into the equilibrium constant gives

$$\begin{aligned} \text{H}_2\text{O} &\rightleftharpoons \underset{x}{\text{H}^+} + \underset{x}{\text{OH}^-} \Rightarrow K_w = [\text{H}^+] \gamma_{\text{H}^+} [\text{OH}^-] \gamma_{\text{OH}^-} \\ 1.0 \times 10^{-14} &= (x)(1)(x)(1) \Rightarrow x = 1.0 \times 10^{-7} \text{ M} \\ \text{pH} &= -\log([\text{H}^+] \gamma_{\text{H}^+}) = -\log([1.0 \times 10^{-7}](1)) = 7.00 \end{aligned}$$

Well, it is no surprise that the pH of pure water is 7.00.

In 0.1 M NaCl, the ionic strength is 0.1 M and the activity coefficients in Table 12-1 are $\gamma_{\text{H}^+} = 0.83$ and $\gamma_{\text{OH}^-} = 0.76$. Putting these values into the equilibrium constant gives

$$\begin{aligned} K_w &= [\text{H}^+] \gamma_{\text{H}^+} [\text{OH}^-] \gamma_{\text{OH}^-} \\ 1.0 \times 10^{-14} &= (x)(0.83)(x)(0.76) \Rightarrow x = 1.26 \times 10^{-7} \text{ M} \end{aligned}$$

The concentrations of H^+ and OH^- increase by 26% when 0.1 M NaCl is added to the water. This result is consistent with the notion that inert salts increase ion dissociation. However, the pH is not changed very much:

$$\text{pH} = -\log([\text{H}^+] \gamma_{\text{H}^+}) = -\log([1.26 \times 10^{-7}](0.83)) = 6.98$$

 **Test Yourself** Find $[\text{H}^+]$ and the pH of 0.05 M LiNO_3 . (Answer: 1.20×10^{-7} M, 6.99)

Ask Yourself

- 12-B. (a) Using activity coefficients with the solubility product, calculate the solubility of HgBr_2 in water. By solubility, we mean the concentration of dissolved Hg^{2+} . The solubility is so small that the ionic strength is approximately 0 and the activity coefficients are approximately 1.

- (b) Considering only the equilibrium $\text{HgBr}_2(s) \rightleftharpoons \text{Hg}^{2+} + 2\text{Br}^-$, and including activity coefficients, calculate the solubility of HgBr_2 in 0.050 M NaBr. The ionic strength is due almost entirely to 0.050 M NaBr.
- (c) If the equilibrium $\text{HgBr}_2(s) + \text{Br}^- \rightleftharpoons \text{HgBr}_3^-$ also occurs, would the solubility of HgBr_2 be greater or less than that computed in (b)?

12-3 Charge and Mass Balances

Difficult equilibrium problems can be tackled by writing all the relevant chemical equilibria plus two more equations: the balances of charge and mass. We now examine these two conditions.

Charge Balance

Solutions must have zero total charge.

The **charge balance** is an algebraic statement of electroneutrality: *The sum of the positive charges in solution equals the sum of the negative charges in solution.*

Suppose that a solution contains the following ionic species: H^+ , OH^- , K^+ , H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-} . The charge balance is

$$\underbrace{[\text{H}^+] + [\text{K}^+]}_{\text{Total positive charge}} = \underbrace{[\text{OH}^-] + [\text{H}_2\text{PO}_4^-] + 2[\text{HPO}_4^{2-}] + 3[\text{PO}_4^{3-}]}_{\text{Total negative charge}} \quad (12-8)$$

The coefficient of each term in the charge balance equals the magnitude of the charge on each ion. The coefficient of $[\text{HPO}_4^{2-}]$ in Equation 12-8 is 2 because the magnitude of its charge is 2. The coefficient of $[\text{PO}_4^{3-}]$ is 3 because the magnitude of its charge is 3.

This statement says that the total charge contributed by H^+ and K^+ equals the magnitude of the charge contributed by all of the anions on the right side of the equation. *The coefficient in front of each species equals the magnitude of the charge on the ion.* A mole of, say, PO_4^{3-} contributes three moles of negative charge. If $[\text{PO}_4^{3-}] = 0.01 \text{ M}$, the negative charge is $3[\text{PO}_4^{3-}] = 3(0.01) = 0.03 \text{ M}$.

Equation 12-8 appears unbalanced to many people. “The right side of the equation has much more charge than the left side!” you might think. But you would be wrong.

For example, consider a solution prepared by weighing out 0.025 0 mol of KH_2PO_4 plus 0.030 0 mol of KOH and diluting to 1.00 L. The concentrations of the species at equilibrium are

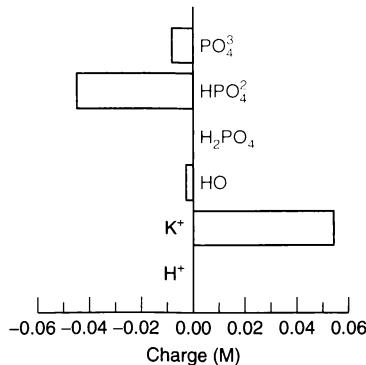


Figure 12-6 Charge contributed by each ion in 1.00 L of solution containing 0.025 0 mol KH_2PO_4 plus 0.030 0 mol KOH. The total positive charge equals the total negative charge.

$$\begin{array}{ll} [\text{H}^+] = 5.1 \times 10^{-12} \text{ M} & [\text{H}_2\text{PO}_4^-] = 1.3 \times 10^{-6} \text{ M} \\ [\text{K}^+] = 0.0550 \text{ M} & [\text{HPO}_4^{2-}] = 0.0220 \text{ M} \\ [\text{OH}^-] = 0.0020 \text{ M} & [\text{PO}_4^{3-}] = 0.0030 \text{ M} \end{array}$$

Are the charges balanced? Yes, indeed. Plugging into Equation 12-8, we find

$$\begin{aligned} & [\text{H}^+] + [\text{K}^+] = [\text{OH}^-] + [\text{H}_2\text{PO}_4^-] + 2[\text{HPO}_4^{2-}] + 3[\text{PO}_4^{3-}] \\ & (5.1 \times 10^{-12}) + 0.0550 = 0.0020 + (1.3 \times 10^{-6}) + 2(0.0220) + 3(0.0030) \\ & 0.0550 = 0.0550 \end{aligned}$$

The total positive charge is 0.055 0 M, and the total negative charge also is 0.055 0 M (Figure 12-6). If charge were not balanced, a beaker with excess positive charge would glide across the lab bench and smash into another beaker with excess negative charge.

The general form of the charge balance for any solution is

$$\text{Charge balance: } n_1[\text{C}_1] + n_2[\text{C}_2] + \dots = m_1[\text{A}_1] + m_2[\text{A}_2] + \dots \quad (12-9)$$

where $[\text{C}]$ is the concentration of a cation, n is the charge of the cation, $[\text{A}]$ is the concentration of an anion, and m is the magnitude of the charge of the anion.

$$\sum[\text{positive charges}] = \sum[\text{negative charges}]$$

Activity coefficients do not appear in the charge balance. The charge contributed by 0.1 M H^+ is exactly 0.1 M. Think about this.

Example Writing a Charge Balance

Write the charge balance for a solution of lead(II) iodide containing the species Pb^{2+} , I^- , PbI^+ , $\text{PbI}_2(aq)$, PbI_3^- , PbOH^+ , H_2O , H^+ , and OH^- .

SOLUTION The species $\text{PbI}_2(aq)$ and H_2O contribute no charge, so the charge balance is

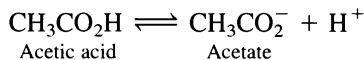
$$2[\text{Pb}^{2+}] + [\text{PbI}^+] + [\text{PbOH}^+] + [\text{H}^+] = [\text{I}^-] + [\text{PbI}_3^-] + [\text{OH}^-]$$

 **Test Yourself** Write a charge balance for H_2SO_4 in water. (Answer: $[\text{H}^+] = 2[\text{SO}_4^{2-}] + [\text{HSO}_4^-] + [\text{OH}^-]$)

Mass Balance

The **mass balance**, also called the *material balance*, is a statement of the conservation of matter. The mass balance states that *the quantity of all species in a solution containing a particular atom (or group of atoms) must equal the amount of that atom (or group) delivered to the solution*. Let's look at some examples.

Suppose that a solution is prepared by dissolving 0.050 mol of acetic acid in water to give a total volume of 1.00 L. The acetic acid partially dissociates into acetate:



The mass balance states that the quantity of dissociated and undissociated acetic acid in the solution must equal the amount of acetic acid put into the solution.

$$\begin{array}{lll} \text{Mass balance for acetic} & 0.050 \text{ M} = [\text{CH}_3\text{CO}_2\text{H}] + [\text{CH}_3\text{CO}_2^-] \\ \text{acid in water:} & \text{What we put} & \text{Undissociated} \\ & \text{into the solution} & \text{product} \\ & & \text{Dissociated} \\ & & \text{product} \end{array}$$

When a compound dissociates in several ways, the mass balance must include all the products. Phosphoric acid (H_3PO_4), for example, dissociates to H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-} . The mass balance for phosphorus atoms in a solution prepared by dissolving 0.025 0 mol of H_3PO_4 in 1.00 L is

$$0.025 \text{ 0 M} = [\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}]$$

Now consider a saturated solution of K_2HPO_4 in water. We do not know the concentration, because we do not know how much K_2HPO_4 dissolves. However, we can say that, for every mole of phosphorus in solution, there are two moles of K^+ . Phosphorus is in the forms H_3PO_4 , H_2PO_4^- , HPO_4^{2-} , and PO_4^{3-} . Therefore the mass balance is

$$[\text{K}^+] = 2\{\underbrace{[\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}]}_{2 \times \text{Total concentration of phosphorus atoms}}\}$$

The mass balance is a statement of the conservation of matter. It really refers to conservation of atoms, not to mass.

Activity coefficients do not appear in the mass balance. The concentration of each species counts *exactly* the number of atoms of that species.

The concentration of K is twice the total concentration of P. Think about which side of the equation should have the 2.

Now consider a solution prepared by dissolving PbI_2 in water to give Pb^{2+} , I^- , PbI^+ , $\text{PbI}_2(aq)$, PbI_3^- , PbOH^+ , H^+ , and OH^- . The source of lead and iodide species is PbI_2 , so there must be two I atoms for every Pb atom in the solution. The mass balance is therefore

We do not know how much PbI_2 dissolved, but we do know that there must be two I atoms for every Pb atom in the solution.

$$\frac{2\{\text{[Pb}^{2+}\text{]} + \text{[PbI}^+\text{]} + \text{[PbOH}^+\text{]} + \text{[PbI}_2\text{(aq)}\text{]} + \text{[PbI}_3^-\text{]}\}}{2 \times \text{total concentration of Pb atoms}} = \frac{\text{[I}^-\text{]} + \text{[PbI}^+\text{]} + 2\text{[PbI}_2\text{(aq)}\text{]} + 3\text{[PbI}_3^-\text{]}}{\text{Total concentration of I atoms}} \quad (12-10)$$

On the right side of Equation 12-10, there is a 2 in front of $[\text{PbI}_2(aq)]$ because each mole of $\text{PbI}_2(aq)$ contains two moles of I atoms. There is a 3 in front of $[\text{PbI}_3^-]$ because each mole of PbI_3^- contains three moles of I atoms.

Ask Yourself

12-C. Consider a buffer solution prepared by mixing 5.00 mmol of $\text{Na}_2\text{C}_2\text{O}_4$ (sodium oxalate) with 2.50 mmol of HCl in 0.100 L.

- (a) List all the chemical species in the solution. Oxalate can accept one or two protons.
- (b) What is the charge balance for the solution?
- (c) Write separate mass balances for Na^+ , oxalate, and Cl^- .
- (d) Decide which species are negligible and simplify the expressions in (b) and (c).

12-4 Systematic Treatment of Equilibrium

Now that we have learned about the charge and mass balances, we are ready for the systematic treatment of equilibrium. The general prescription follows these steps:

Step 1. Write the *pertinent reactions*.

Step 2. Write the *charge balance* equation. There is only one.

Step 3. Write *mass balance* equations. There may be more than one.

Step 4. Write the *equilibrium constant* for each chemical reaction. This step is the only one in which activity coefficients enter.

Step 5. *Count equations and unknowns*. At this point, you should have as many equations as unknowns (chemical concentrations). If not, you must either find more equilibria or fix some concentrations at known values.

Step 6. By hook or by crook, *solve* for all the unknowns.

Steps 1 and 6 are the heart of the problem. Guessing what chemical equilibria exist in a given solution requires a fair degree of chemical intuition. In this text, you will usually be given help with step 1. Unless we know all the relevant equilibria, it is not possible to calculate the composition of a solution correctly. Because we do not know all the chemical reactions, we undoubtedly oversimplify many equilibrium problems.

Step 6 is likely to be your biggest challenge. With n equations involving n unknowns, the problem can always be solved, at least in principle. In the simplest cases, you can do this by hand; but, for most problems, approximations or a spreadsheet are employed.

Activity coefficients enter *only* in step 4.

A Simple Example: The pH of 10^{-8} M KOH

Here is a trick question: What is the pH of 1.0×10^{-8} M KOH? Your first response might be $[\text{OH}^-] = 1.0 \times 10^{-8}$ M, so $[\text{H}^+] = 1.0 \times 10^{-6}$ M, and so the pH is 6.00. However, adding base to a neutral solution could not possibly make it acidic. So let's see how the systematic treatment of equilibrium works in this case.

Step 1. Pertinent reactions: The only one is $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ which exists in every aqueous solution.

Step 2. Charge balance: The ions are K^+ , H^+ , and OH^- , so $[\text{K}^+] + [\text{H}^+] = [\text{OH}^-]$.

Step 3. Mass balance: You might be tempted to write $[\text{K}^+] = [\text{OH}^-]$, but this is false because OH^- comes from both KOH and H_2O . For every mole of K^+ , one mole of OH^- is introduced into the solution. We also know that, for every mole of H^+ from H_2O , one mole of OH^- is introduced. Therefore one mass balance is $[\text{OH}^-] = [\text{K}^+] + [\text{H}^+]$, which is the same as the charge balance in this particular example. A second mass balance is $[\text{K}^+] = 1.00 \times 10^{-8}$ M.

Step 4. Equilibrium constants: The only one is $K_w = [\text{H}^+] \gamma_{\text{H}^+} [\text{OH}^-] \gamma_{\text{OH}^-}$.

Step 5. Count equations and unknowns: At this point, you should have as many equations as unknowns (chemical species). There are three unknowns, $[\text{K}^+]$, $[\text{H}^+]$, and $[\text{OH}^-]$, and three equations:

$$\begin{array}{ll} \text{Charge balance:} & [\text{K}^+] + [\text{H}^+] = [\text{OH}^-] \\ \text{Mass balance:} & [\text{K}^+] = 1.0 \times 10^{-8} \text{ M} \\ \text{Equilibrium constant:} & K_w = [\text{H}^+] \gamma_{\text{H}^+} [\text{OH}^-] \gamma_{\text{OH}^-} \end{array}$$

Step 6. Solve: The ionic strength must be very low in this solution ($\sim 10^{-7}$ M), so it is safe to say that the activity coefficients are 1.00. Therefore the equilibrium constant simplifies to $K_w = [\text{H}^+] [\text{OH}^-]$. Substituting 1.0×10^{-8} M for $[\text{K}^+]$ and $[\text{OH}^-] = K_w / [\text{H}^+]$ into the charge balance gives

$$[1.0 \times 10^{-8}] + [\text{H}^+] = K_w / [\text{H}^+]$$

Multiplying both sides by $[\text{H}^+]$ gives a quadratic equation

$$\begin{aligned} [1.0 \times 10^{-8}][\text{H}^+] + [\text{H}^+]^2 &= K_w \\ [\text{H}^+]^2 + [1.0 \times 10^{-8}][\text{H}^+] - K_w &= 0 \end{aligned}$$

whose two solutions are $[\text{H}^+] = 9.6 \times 10^{-8}$ and -1.1×10^{-7} M. Rejecting the negative solution (because the concentration cannot be negative), we find

$$\text{pH} = -\log([\text{H}^+] \gamma_{\text{H}^+}) = -\log([9.6 \times 10^{-8}](1.00)) = 7.02$$

It should not be too surprising that the pH is close to 7 and very slightly basic.

Coupled Equilibria: Solubility of CaF_2

The mineral fluorite (CaF_2), also called fluorspar, is converted to hydrofluoric acid (HF) for the synthesis of refrigerants and fluoropolymers. A large fraction of the world's supply of fluorspar is controlled by China.

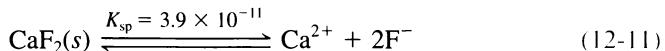
The opening of this chapter gave an example of *coupled equilibria* in which calcium carbonate dissolves and the resulting bicarbonate reacts with H^+ . The second

If you have fewer equations than unknowns, look for another mass balance or a chemical equilibrium that you overlooked.



Fluorite crystal with octahedral shape.

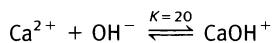
reaction drives the first reaction forward. Now we look at a similar case in which CaF_2 dissolves in water:



Fluoride ion from Reaction 12-11 can react with H^+ to give $\text{HF}(aq)$:



If we were very smart, we might also write the reaction



It turns out that this reaction is important only at high pH.

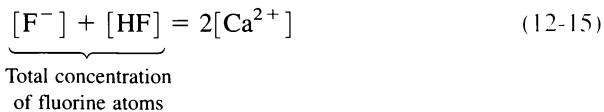
If Reaction 12-12 takes place, then the solubility of CaF_2 is greater than that predicted by the solubility product because F^- produced in Reaction 12-11 is consumed in Reaction 12-12. According to Le Châtelier's principle, Reaction 12-11 will be driven to the right. The systematic treatment of equilibrium allows us to find the net effect of three reactions.

Step 1. Pertinent reactions: Three reactions are 12-11, 12-12, and 12-13, the last of which occurs in every aqueous solution.



Step 2. Charge balance: $[\text{H}^+] + 2[\text{Ca}^{2+}] = [\text{OH}^-] + [\text{F}^-]$ (12-14)

Step 3. Mass balance: If all fluoride remained in the form F^- , we could write $[\text{F}^-] = 2[\text{Ca}^{2+}]$ from the stoichiometry of Reaction 12-11. But some F^- reacts to give HF. The total number of moles of fluorine atoms is equal to the sum of F^- plus HF, and the mass balance is



Step 4. Equilibrium constants:

$$K_{\text{sp}} = [\text{Ca}^{2+}]\gamma_{\text{Ca}^{2+}}[\text{F}^-]^2\gamma_{\text{F}^-}^2 = 3.9 \times 10^{-11} \quad (12-16)$$

$$\frac{1}{K_a} = \frac{[\text{HF}]\gamma_{\text{HF}}}{[\text{F}^-]\gamma_{\text{F}^-}[\text{H}^+]\gamma_{\text{H}^+}} = 1.5 \times 10^3 \quad (12-17)$$

$$K_w = [\text{H}^+]\gamma_{\text{H}^+}[\text{OH}^-]\gamma_{\text{OH}^-} = 1.0 \times 10^{-14} \quad (12-18)$$

For simplicity, we are generally going to ignore the activity coefficients.

Although we wrote activity coefficients in the equilibrium equations, we are not so masochistic as to use them in the rest of the problem. At this point, we are going to ignore the activity coefficients, which is equivalent to saying that they are unity. There will be some inaccuracy in the results, but you could go back after the calculation and compute the ionic strength and the activity coefficients and find a better approximation for the solution if you had to.

Step 5. Count equations and unknowns: There are five equations (12-14 through 12-18) and five unknowns: $[\text{H}^+]$, $[\text{OH}^-]$, $[\text{Ca}^{2+}]$, $[\text{F}^-]$, and $[\text{HF}]$.

Step 6. Solve: This is no simple matter for these five equations. Instead, let us ask a simpler question: What will be the concentrations of $[\text{Ca}^{2+}]$, $[\text{F}^-]$, and $[\text{HF}]$ if the pH is fixed at 3.00 by adding a buffer?

Once we know that $[\text{H}^+] = 1.0 \times 10^{-3}$ M, there is a straightforward procedure for solving the equations. From Equation 12-17, we can write

$$\frac{[\text{HF}]}{[\text{F}^-][\text{H}^+]} = \frac{[\text{HF}]}{[\text{F}^-][1.0 \times 10^{-3}]} = 1.5 \times 10^3 \Rightarrow [\text{HF}] = 1.5[\text{F}^-]$$

Substituting $1.5[F^-]$ for $[HF]$ in the mass balance (Equation 12-15) gives

$$\begin{aligned}[F^-] + [HF] &= 2[Ca^{2+}] \\ [F^-] + 1.5[F^-] &= 2[Ca^{2+}] \\ [F^-] &= 0.80[Ca^{2+}]\end{aligned}\quad (12-15)$$

Finally, we substitute $0.80[Ca^{2+}]$ for $[F^-]$ in the solubility product (Equation 12-16):

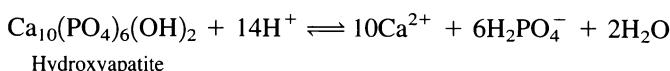
$$\begin{aligned}[Ca^{2+}][F^-]^2 &= K_{sp} \\ [Ca^{2+}](0.80[Ca^{2+}])^2 &= K_{sp} \\ [Ca^{2+}] &= \left(\frac{K_{sp}}{0.80^2}\right)^{1/3} = 3.9 \times 10^{-4} M\end{aligned}$$

You should realize that the charge balance equation (12-14) is no longer valid if the pH is fixed by external means. To adjust the pH, an ionic compound must necessarily have been added to the solution. Equation 12-14 is incomplete because it omits those ions. However, we did not need Equation 12-14 to solve the problem because we eliminated $[H^+]$ as a variable when we fixed the pH.

If we had selected a pH other than 3.00, we would have found a different set of concentrations because of the coupling of Reactions 12-11 and 12-12. Figure 12-7 shows the pH dependence of the concentrations of Ca^{2+} , F^- , and HF. At high pH, there is very little HF, so $[F^-] \approx 2[Ca^{2+}]$. At low pH, there is very little F^- , so $[HF] \approx 2[Ca^{2+}]$. The concentration of Ca^{2+} increases at low pH because Reaction 12-11 is drawn to the right by the reaction of F^- with H^+ to make HF in Reaction 12-12.

In general, many minerals are more soluble at low pH because the anions react with acid.³ Box 12-1 describes an environmental consequence of solubility in acids.

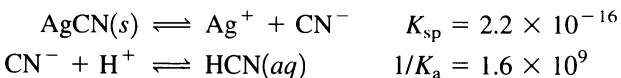
Tooth enamel contains the mineral hydroxyapatite, a calcium hydroxyphosphate. Enamel dissolves in acid because both PO_4^{3-} and OH^- react with H^+ :



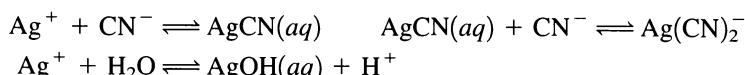
Bacteria on your teeth metabolize sugar into lactic acid—lowering the pH below 5 at the surface of a tooth. Acid causes tooth decay by dissolving enamel.

Ask Yourself

12-D. (a) Ignoring activity coefficients, find $[Ag^+]$, $[CN^-]$, and $[HCN]$ in a saturated solution of AgCN whose pH is fixed at 9.00. Consider the equilibria:



(b) What would be the mass balance if the following equilibria also occur?



Challenge Use the concentration of Ca^{2+} that we just calculated to show that $[F^-] = 3.1 \times 10^{-4} M$ and $[HF] = 4.7 \times 10^{-4} M$.

Fixing the pH invalidates the original charge balance because we added unspecified ions to the solution to fix the pH. There exists a new charge balance, but we do not know enough to write an equation for it.

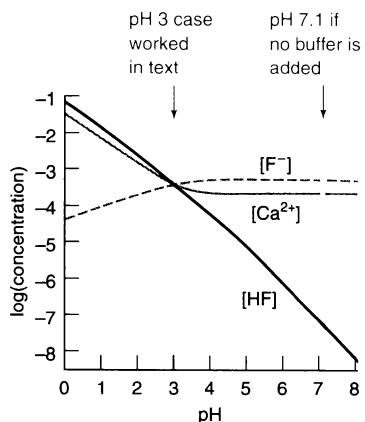
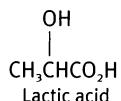


Figure 12-7 pH dependence of the concentrations of Ca^{2+} , F^- , and HF in a saturated solution of CaF_2 . As the pH is lowered, H^+ reacts with F^- to make HF, and the concentration of Ca^{2+} increases. Note the logarithmic ordinate.

12-5 Fractional Composition Equations

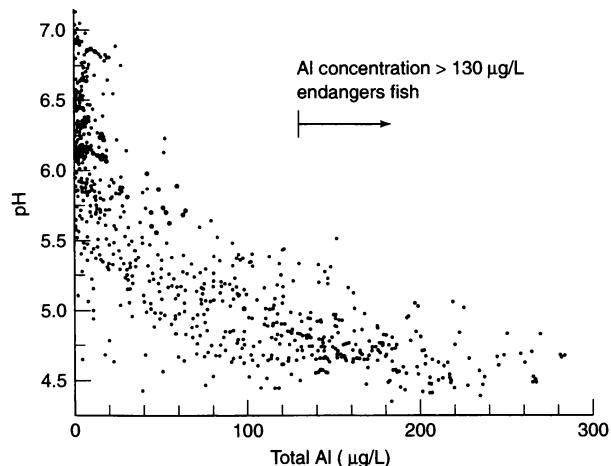
We now derive expressions for the fraction of a weak acid, HA, in each form (HA and A^-). These equations were used in Section 10-7 for an acid-base titration

Box 12-1 Aluminum Mobilization from Minerals by Acid Rain

Aluminum is the third most abundant element on Earth (after oxygen and silicon), but it is tightly locked into insoluble minerals such as kaolinite ($\text{Al}_2(\text{OH})_4\text{Si}_2\text{O}_5$) and bauxite (AlOOH). Acid rain from human activities is a recent change in the history of Earth, and it is introducing soluble forms of aluminum (and lead and mercury) into the environment.⁴ Below pH 5, aluminum is mobilized from minerals and its concentration in lake water rises rapidly. At a concentration of 130 $\mu\text{g/L}$, aluminum kills fish. In humans, high concentrations

Total aluminum (including dissolved and suspended species) in 1 000 Norwegian lakes as a function of the pH of the lake water. The more acidic the water, the greater the aluminum concentration. [From G. Howells, *Acid Rain and Acid Waters*, 2nd ed. (Hertfordshire: Ellis Horwood, 1995).]

of aluminum cause dementia, softening of bones, and anemia.



spreadsheet. In Equation 8-16, we defined the *fraction of dissociation* as

$$\text{fraction of HA in the form } \text{A}^- \equiv \alpha_{\text{A}^-} = \frac{[\text{A}^-]}{[\text{A}^-] + [\text{HA}]} \quad (12-19)$$

Similarly, we define the fraction in the form HA as

$$\text{fraction of HA in the form HA} \equiv \alpha_{\text{HA}} = \frac{[\text{HA}]}{[\text{A}^-] + [\text{HA}]} \quad (12-20)$$

Consider an acid with formal concentration F:



The mass balance is simply

$$F = [\text{HA}] + [\text{A}^-]$$

Rearranging the mass balance gives $[\text{A}^-] = F - [\text{HA}]$, which can be plugged into the K_a equilibrium to give

$$K_a = \frac{[\text{H}^+](F - [\text{HA}])}{[\text{HA}]}$$

or, with a little algebra,

$$[\text{HA}] = \frac{[\text{H}^+]F}{[\text{H}^+] + K_a} \quad (12-21)$$

Dividing both sides by F gives the fraction α_{HA} :

Fraction in the form HA:

$$\alpha_{\text{HA}} = \frac{[\text{HA}]}{F} = \frac{[\text{H}^+]}{[\text{H}^+] + K_a} \quad (12-22)$$

If we substitute $[\text{HA}] = F - [\text{A}^-]$ into the K_a equation, we can rearrange and solve for the fraction α_{A^-} :

Fraction in the form A^- :

$$\alpha_{\text{A}^-} = \frac{[\text{A}^-]}{F} = \frac{K_a}{[\text{H}^+] + K_a} \quad (12-23)$$

Figure 12-8 shows α_{HA} and α_{A^-} for a system with $pK_a = 5.00$. At low pH, almost all of the acid is in the form HA. At high pH, almost everything is in the form A^- . HA is the predominant species when $\text{pH} < pK_a$. A^- is the predominant species when $\text{pH} > pK_a$.

Figure 11-1 is an analogous diagram of species in a diprotic system. At low pH, $\alpha_{\text{H}_2\text{A}}$ approaches 1 and, at high pH, $\alpha_{\text{A}^{2-}}$ approaches 1. At intermediate pH, α_{HA^-} is the largest fraction.

If we were dealing with the conjugate pair BH^+ and B instead of HA and A^- , Equation 12-22 would give the fraction in the form BH^+ and Equation 12-23 would give the fraction in the form B. In this case, K_a is the acid dissociation constant for BH^+ (which is K_w/K_b).

α_{HA} = fraction of species in the form HA

α_{A^-} = fraction of species in the form A^-

$$\alpha_{\text{HA}} + \alpha_{\text{A}^-} = 1$$

For the acid BH^+ :

$$\alpha_{\text{BH}^+} = \frac{[\text{H}^+]}{[\text{H}^+] + K_{\text{BH}^+}}$$

$$\alpha_B = \frac{K_{\text{BH}^+}}{[\text{H}^+] + K_{\text{BH}^+}}$$

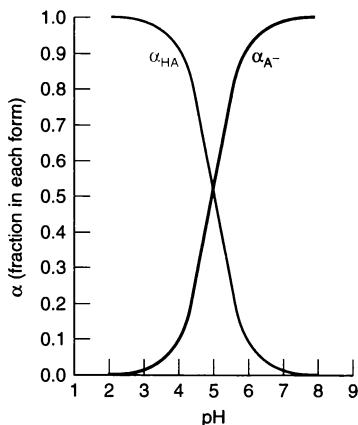


Figure 12-8 Fractional composition diagram of a monoprotic system with $pK_a = 5.00$. Below pH 5, HA is the dominant form, whereas, above pH 5, A^- dominates. Figure 11-1 showed the analogous plot for a diprotic system.

Example Fractional Composition for an Acid

pK_a for benzoic acid (HA) is 4.20. Find the concentration of A^- at pH 5.31 if the formal concentration of HA is 0.021 M.

SOLUTION At pH = 5.31, $[\text{H}^+] = 10^{-5.31} = 4.9 \times 10^{-6}$ M. For $K_a = 10^{-4.20} = 6.3 \times 10^{-5}$,

$$\alpha_{\text{A}^-} = \frac{K_a}{[\text{H}^+] + K_a} = \frac{6.3 \times 10^{-5}}{(4.9 \times 10^{-6}) + (6.3 \times 10^{-5})} = 0.928$$

From Equation 12-23, we can say

$$[\text{A}^-] = \alpha_{\text{A}^-} F = (0.928)(0.021) = 0.020 \text{ M}$$

Test Yourself Find $[\text{A}^-]$ if pH = 4.31. (Answer: 0.012 M)

Example Fractional Composition for a Base

K_a for the ammonium ion, NH_4^+ , is 5.69×10^{-10} ($pK_a = 9.245$). Find the fraction in the form BH^+ at pH 10.38.

SOLUTION At pH = 10.38, $[\text{H}^+] = 10^{-10.38} = 4.17 \times 10^{-11}$ M. Using Equation 12-22, with BH^+ in place of HA, we find

$$\alpha_{\text{BH}^+} = \frac{[\text{H}^+]}{[\text{H}^+] + K_a} = \frac{4.17 \times 10^{-11}}{(4.17 \times 10^{-11}) + (5.69 \times 10^{-10})} = 0.068$$

Test Yourself Find the fraction in the form NH_3 at pH 10.00. (Answer: 0.85)

Problem 12-40 gives fractional composition equations for a diprotic acid, H_2A .



Ask Yourself

12-E. The acid HA has $pK_a = 3.00$. Find the fraction in the form HA and the fraction in the form A^- at pH = 2.00, 3.00, and 4.00. Compute the quotient $[HA]/[A^-]$ at each pH.

Key Equations

Activity

$$\mathcal{A}_C = [C]\gamma_C \quad \mathcal{A} = \text{activity; } \gamma = \text{activity coefficient}$$

Equilibrium constant

For the reaction $aA + bB \rightleftharpoons cC + dD$,

$$K = \frac{\mathcal{A}_C^c \mathcal{A}_D^d}{\mathcal{A}_A^a \mathcal{A}_B^b} = \frac{[C]^c \gamma_C^c [D]^d \gamma_D^d}{[A]^a \gamma_A^a [B]^b \gamma_B^b}$$

Ionic strength

$$\mu = \frac{1}{2}(c_1 z_1^2 + c_2 z_2^2 + \dots) = \frac{1}{2} \sum_i c_i z_i^2$$

c = concentration; z = charge

Extended Debye-Hückel equation

$$\log \gamma = \frac{-0.51z^2 \sqrt{\mu}}{1 + (\alpha \sqrt{\mu}/305)}$$

z = charge; μ = ionic strength; α = ion size

Linear interpolation

$$\frac{\text{unknown } y \text{ interval}}{\Delta y} = \frac{\text{known } x \text{ interval}}{\Delta x}$$

pH

$$\text{pH} = -\log \mathcal{A}_{H^+} = -\log[H^+] \gamma_{H^+}$$

Charge balance

positive charge in solution = negative charge in solution

Mass balance

Quantity of all species in a solution containing a particular atom (or group of atoms) must equal the amount of that atom (or group) delivered to the solution.

Systematic treatment of equilibrium

1. Pertinent reactions
2. Charge balance
3. Mass balance
4. Equilibrium constants
5. Count equations/unknowns
6. Solve

Fraction of HA in acidic form

$$\alpha_{HA} = \frac{[HA]}{F} = \frac{[H^+]}{[H^+] + K_a} = \alpha_{BH^+} = \frac{[BH^+]}{F}$$

Fraction of HA in basic form

$$\alpha_A = \frac{[A^-]}{F} = \frac{[K_a]}{[H^+] + K_a} = \alpha_B = \frac{[B]}{F}$$

Important Terms

activity

extended Debye-Hückel equation

ionic strength

activity coefficient

interpolation

mass balance

charge balance

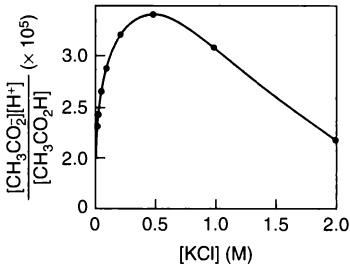
ionic atmosphere

Problems

12-1. What is an ionic atmosphere?

12-2. Explain why the solubility of an ionic compound increases as the ionic strength of the solution increases (at least up to ~ 0.5 M).

12-3. The graph shows the quotient of concentrations $[CH_3CO_2^-][H^+]/[CH_3CO_2H]$ for the dissociation of acetic acid as a function of the concentration of KCl added to the solution. Explain the shape of the curve.



12-4. Which statements are true? In the ionic strength range 0–0.1 M, activity coefficients decrease with (a) increasing ionic strength; (b) increasing ionic charge; (c) decreasing hydrated size (α).

12-5. Explain the following observations:

- (a) Mg^{2+} has a greater hydrated diameter than Ba^{2+} .
- (b) Hydrated diameters decrease in the order $Sn^{4+} > In^{3+} > Cd^{2+} > Rb^+$.
- (c) H^+ (which is really H_3O^+) has one of the largest hydrated sizes in Table 12-1. Consider the possibilities of hydrogen bonding to H_3O^+ .

12-6. State in words the meaning of the charge balance equation.

12-7. State the meaning of the mass balance equation.

12-8. Why does the solubility of a salt of a basic anion increase with decreasing pH? Write chemical reactions for the minerals galena (PbS) and cerussite ($PbCO_3$) to explain how acid rain mobilizes trace quantities of toxic metallic elements from relatively inert forms into the environment, where the metals can be taken up by plants and animals. Why are the minerals kaolinite and bauxite in Box 12-1 more soluble in acidic solution than in neutral solution?

12-9. Assuming complete dissociation of the salts, calculate the ionic strength of (a) 0.2 mM KNO_3 ; (b) 0.2 mM Cs_2CrO_4 ; (c) 0.2 mM $MgCl_2$ plus 0.3 mM $AlCl_3$.

12-10. Find the activity coefficient of each ion at the indicated ionic strength:

- (a) SO_4^{2-} ($\mu = 0.01$ M)
- (b) Sc^{3+} ($\mu = 0.005$ M)
- (c) Eu^{3+} ($\mu = 0.1$ M)
- (d) $(CH_3CH_2)_3NH^+$ ($\mu = 0.05$ M)

12-11. Find the activity (not the activity coefficient) of $(C_3H_7)_4N^+$ (tetrapropylammonium ion) in a solution containing 0.005 0 M $(C_3H_7)_4N^+Br^-$ plus 0.005 0 M $(CH_3)_4N^+Cl^-$.

12-12. Interpolate in Table 12-1 to find the activity coefficient of H^+ when $\mu =$ (a) 0.030 M and (b) 0.042 M.

12-13. Calculate the activity coefficient of Zn^{2+} when $\mu = 0.083$ M by using (a) Equation 12-5; (b) linear interpolation with Table 12-1.

12-14. Using activities, find the concentration of Ag^+ in a saturated solution of $AgSCN$ in (a) 0.060 M KNO_3 ; (b) 0.060 M $KSCN$.

12-15. Find the activity coefficient of H^+ in a solution containing 0.010 M HCl plus 0.040 M $KClO_4$. What is the pH of the solution?

12-16. Using activities, calculate the pH and concentration of H^+ in pure water containing 0.050 M $LiBr$ at 25°C.

12-17. Using activities, calculate the pH of a solution containing 0.010 M $NaOH$ plus 0.012 0 M $LiNO_3$. What would be the pH if you neglected activities?

12-18. (a) Using the solubility product and neglecting activity, find the concentrations of Mn^{2+} and OH^- and the pH of a saturated solution of $Mn(OH)_2$.

(b) Consider a solution of 0.075 M $NaClO_4$ saturated with $Mn(OH)_2$. Using activities, find $[Mn^{2+}]$ and $[OH^-]$ and the pH. It is convenient to use $pH = -\log \mathcal{A}_{H^+} = -\log(K_w / \mathcal{A}_{OH^-})$.

12-19. Using activities, find the concentration of Ba^{2+} in a 0.100 M $(CH_3)_4N^+IO_3^-$ solution saturated with $Ba(IO_3)_2$. Assume that $Ba(IO_3)_2$ makes a negligible contribution to the ionic strength and verify your assumption when you are done.

12-20. *Successive approximations.* With activities, find $[Pb^{2+}]$ in a saturated solution of PbF_2 . Consider only the equilibrium $PbF_2 \rightleftharpoons Pb^{2+} + 2F^-$. Follow the Example “A Better Estimate of the Solubility of PbI_2 ” in Section 12-2 to find the ionic strength by successive approximations.

12-21. (a) Using activities and K_{sp} for $CaSO_4$, find $[Ca^{2+}]$ in a saturated aqueous solution of $CaSO_4$. You don’t know the ionic strength, so you need to find it by successive approximations. Begin with $\mu = 0$ to find a first estimate of $[Ca^{2+}]$ and $[SO_4^{2-}]$. Then compute the ionic strength and find a new estimate for $[Ca^{2+}]$ and $[SO_4^{2-}]$. Repeat the process until the concentrations stop changing.

(b) The observed total concentration of dissolved calcium is ~ 15 mM.⁵ Explain.

12-22. Write a charge balance for a solution containing H^+ , OH^- , Ca^{2+} , HCO_3^- , CO_3^{2-} , $Ca(HCO_3)^+$, $Ca(OH)^+$, K^+ , and ClO_4^- .

12-23. Write a charge balance for a solution of H_2SO_4 in water if the H_2SO_4 ionizes to HSO_4^- and SO_4^{2-} .

12-24. Write the charge balance for an aqueous solution of arsenic acid, H_3AsO_4 , in which the acid can dissociate to $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-} . Look up the structure of arsenic acid in Appendix B and write the structure of $HAsO_4^{2-}$.

12-25. (a) Suppose that $MgBr_2$ dissolves to give Mg^{2+} and Br^- . Write a charge balance for this aqueous solution.

(b) What is the charge balance if, in addition to Mg^{2+} and Br^- , $MgBr^+$ is formed?

12-26. For a 0.1 M aqueous solution of sodium acetate, $\text{Na}^+\text{CH}_3\text{CO}_2^-$, one mass balance is simply $[\text{Na}^+] = 0.1 \text{ M}$. Write a mass balance involving acetate.

12-27. Suppose that MgBr_2 dissolves to give Mg^{2+} and Br^- .

(a) Write the mass balance for Mg^{2+} for 0.20 M MgBr_2 .

(b) Write a mass balance for Br^- for 0.20 M MgBr_2 .

Now suppose that MgBr^+ is formed in addition to Mg^{2+} and Br^- .

(c) Write a mass balance for Mg^{2+} for 0.20 M MgBr_2 .

(d) Write a mass balance for Br^- for 0.20 M MgBr_2 .

12-28. (a) Write the mass balance for CaF_2 in water if the reactions are $\text{CaF}_2(s) \rightleftharpoons \text{Ca}^{2+} + 2\text{F}^-$ and $\text{F}^- + \text{H}^+ \rightleftharpoons \text{HF}(aq)$.

(b) Write a mass balance for CaF_2 in water if, in addition to the preceding reactions, the following reaction takes place: $\text{HF}(aq) + \text{F}^- \rightleftharpoons \text{HF}_2^-$.

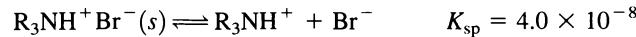
12-29. (a) Write a mass balance for an aqueous solution of $\text{Ca}_3(\text{PO}_4)_2$ if the aqueous species are Ca^{2+} , PO_4^{2-} , HPO_4^{2-} , H_2PO_4^- , and H_3PO_4 .

(b) Write a mass balance for a solution of $\text{Fe}_2(\text{SO}_4)_3$ if the species are Fe^{3+} , $\text{Fe}(\text{OH})^{2+}$, $\text{Fe}(\text{OH})_2^+$, FeSO_4^+ , SO_4^{2-} , and HSO_4^- .

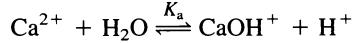
12-30. Consider the dissolution of the compound X_2Y_3 , which gives $\text{X}_2\text{Y}_2^{2+}$, $\text{X}_2\text{Y}_4^{4+}$, $\text{X}_2\text{Y}_3(aq)$, and Y^{2-} . Use the mass balance to find an expression for $[\text{Y}^{2-}]$ in terms of the other concentrations. Simplify your answer as much as possible.

12-31. Ignoring activity coefficients, follow the example of 10^{-8} M KOH in Section 12-4 to calculate the concentration of each ion in a solution of $4.0 \times 10^{-8} \text{ M Mg(OH)}_2$, which is completely dissociated to Mg^{2+} and OH^- .

12-32. Consider a saturated solution of $\text{R}_3\text{NH}^+\text{Br}^-$, where R is an organic group. Follow the example of CaF_2 in Section 12-4 to find the solubility (mol/L) of $\text{R}_3\text{NH}^+\text{Br}^-$ in a solution maintained at pH 9.50. Use the equilibria



12-33. At neutral pH, $\text{CaSO}_4(s)$ dissolves in water to give Ca^{2+} , SO_4^{2-} , and the ion pair, $\text{CaSO}_4(aq)$. Bisulfate (HSO_4^-) has a pK_a of 2.0. Ca^{2+} has an “acid dissociation” constant of $pK_a = 12.70$ for the reaction

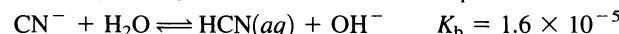


(a) It is observed that the solubility of $\text{CaSO}_4(s)$ is nearly constant over the pH range 3 to 8.5.⁵ Explain why.

(b) What do you expect to happen to the solubility at low pH (below 3) and at high pH (above 12)?

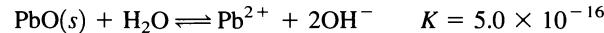
12-34. (a) Ignoring activity coefficients, find the concentrations of Ag^+ , CN^- , and $\text{HCN}(aq)$ in a saturated solution of

AgCN whose pH is somehow fixed at 9.00. Consider the following equilibria:



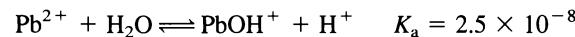
(b) *Activity problem.* Use activity coefficients to answer (a). Assume that the ionic strength is fixed at 0.10 M by addition of an inert salt. When activities are used, the statement that the pH is 9.00 means that $-\log([\text{H}^+]\gamma_{\text{H}^+}) = 9.00$.

12-35. (a) Consider the equilibrium



Without worrying about activities, how many moles of PbO will dissolve in a 1.00-L solution if the pH is fixed at 10.50?

(b) Answer the same question asked in (a), but also consider the reaction



(c) *Activity problem.* Answer (a) by using activity coefficients, assuming the ionic strength is fixed at 0.050 M.

12-36. Find the fraction of 1-naphthoic acid in the form HA and the fraction in the form A^- at pH = (a) 2.00; (b) 3.00; (c) 3.50.

12-37. Find the fraction of pyridine (B) in the form B and the fraction in the form BH^+ at pH = (a) 4.00; (b) 5.00; (c) 6.00.

12-38. The base B has $pK_b = 4.00$. Find the fraction in the form B and the fraction in the form BH^+ at pH = (a) 9.00; (b) 10.00; (c) 10.30.

12-39. Create a spreadsheet that uses Equations 12-22 and 12-23 to compute and plot the concentrations of HA and A^- in a 0.200 M solution of hydroxybenzene as a function of pH from pH 2 to pH 12.

12-40. In this problem, we construct the fractional composition graph in Figure 11-1. From the mass balance and the equilibrium expressions, we can derive fractional composition equations for a diprotic system, H_2A :

Fraction in the form H_2A :

$$\alpha_{\text{H}_2\text{A}} = \frac{[\text{H}_2\text{A}]}{F} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + [\text{H}^+]K_1 + K_1K_2}$$

Fraction in the form HA^- :

$$\alpha_{\text{HA}^-} = \frac{[\text{HA}^-]}{F} = \frac{K_1[\text{H}^+]}{[\text{H}^+]^2 + [\text{H}^+]K_1 + K_1K_2}$$

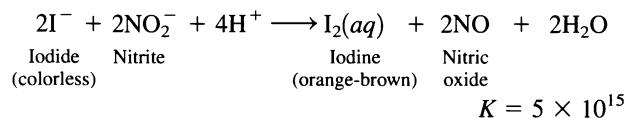
Fraction in the form A^{2-} :

$$\alpha_{\text{A}^{2-}} = \frac{[\text{A}^{2-}]}{F} = \frac{K_1K_2}{[\text{H}^+]^2 + [\text{H}^+]K_1 + K_1K_2}$$

in which $F = [H_2A] + [HA^-] + [A^{2-}]$. Enter these equations into a spreadsheet to construct a graph of α_{H_2A} , α_{HA^-} , and $\alpha_{A^{2-}}$ for *trans*-butenedioic acid ($pK_1 = 3.02$, $pK_2 = 4.48$) as a function of pH from pH 0 to 8 in 0.2 pH units.

How Would You Do It?

12-41. Figure 12-1 shows student data for the total concentration of dissolved iodine in solutions saturated with $PbI_2(s)$ in the presence of added KNO_3 . Dissolved iodine is present as iodide ion (I^-) or iodide attached to Pb^{2+} . Dissolved iodine was measured by adding nitrite to convert iodide into iodine (I_2):



Only the product I_2 is colored, so it can be measured by its absorption of visible light. To collect the data in Figure 12-1, excess $PbI_2(s)$ was shaken with various concentrations of KNO_3 . Solutions were then centrifuged and the clear supernatant liquid was removed for analysis by reaction with nitrite.

By assuming that the only dissolved species were Pb^{2+} and I^- , and taking activity coefficients into account, the people

who measured the concentration of dissolved iodine calculated that the solubility product for PbI_2 is 1.64×10^{-8} . This number is higher than the value 7.9×10^{-9} in Appendix A because no account was made for species such as PbI^+ , $PbI_2(aq)$, PbI_3^- , and $PbOH^+$.

(a) Write the chemical reaction that produces $PbOH^+$ and propose an experiment to measure the concentration of this species, using a pH meter.

(b) I can't think of a way to distinguish the species PbI^+ from I^- by measuring total dissolved iodine. Can you propose a different kind of experiment to measure the equilibrium constant for formation of PbI^+ ?

12-42. We use the approximation that the activity coefficient (γ) of neutral molecules is 1.00. A more accurate relation is $\log \gamma = k\mu$, where μ is ionic strength and $k \approx 0.11$ for NH_3 and CO_2 and $k \approx 0.2$ for organic molecules. Using activity coefficients for HA , A^- , and H^+ , predict the quotient

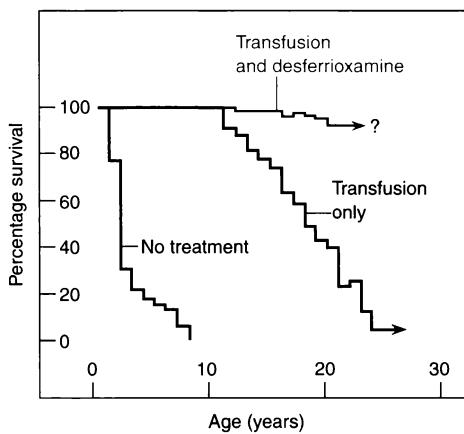
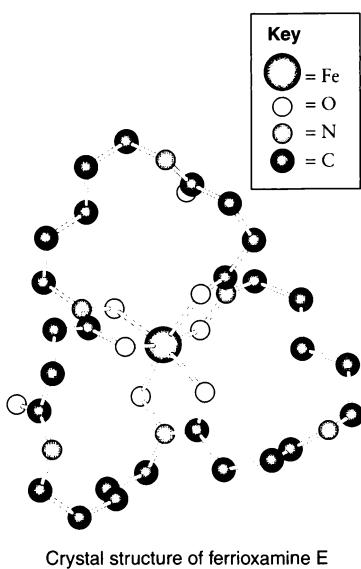
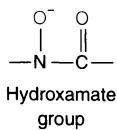
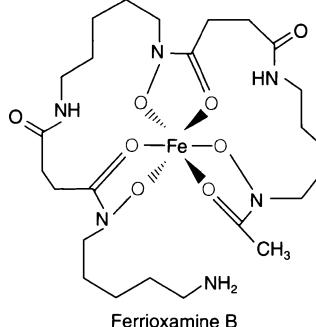
$$\frac{[H^+][A^-]/[HA] \text{ (at } \mu = 0.1 \text{ M})}{[H^+][A^-]/[HA] \text{ (at } \mu = 0 \text{ M})}$$

for benzoic acid ($HA = C_6H_5CO_2H$). The observed quotient is 0.63 ± 0.03 .⁶

Notes and References

- The primary source of equilibrium constants for serious work is the computer database compiled by A. E. Martell, R. M. Smith, and R. J. Motekaitis, *NIST Standard Reference Database 46: Critically Selected Stability Constants of Metal Complexes* (Gaithersburg, MD: National Institute of Standards and Technology, 2004).
- D. R. Driscoll, *J. Chem. Ed.* **1979**, 56, 603. See also R. W. Ramette, *J. Chem. Ed.* **1963**, 40, 252.
- For a general spreadsheet approach to computing the solubility of salts in which the anion and cation can react with water, see J. L. Guiñón, J. García-Antón, and V. Pérez-Herranz, *J. Chem. Ed.* **1999**, 76, 1157.
- R. B. Martin, *Acc. Chem. Res.* **1994**, 27, 204.
- J. Shukla, V. P. Mohandas, and A. Kumar, *J. Chem. Eng. Data* **2008**, 53, 2797.
- E. Koort, P. Gans, K. Herodes, V. Pihl, and I. Leito, *Anal. Bioanal. Chem.* **2006**, 385, 1124.

Chelation Therapy and Thalassemia



Fe^{3+} complex ferrioxamine B and the related compound, ferrioxamine E, in which the chelate has a cyclic structure. Graph shows success of transfusions and transfusions plus chelation therapy. [Crystal structure provided by M. Neu, Los Alamos National Laboratory, based on D. Van der Helm and M. Poling, *J. Am. Chem. Soc.* **1976**, 98, 82. Graph from P. S. Dobbin and R. C. Hider, *Chem. Br.* **1990**, 26, 565.]

Oxygen (O_2) in the human circulatory system is bound to iron in the protein hemoglobin, which consists of two pairs of subunits, designated α and β . β -Thalassemia major is a genetic disease in which β subunits are not synthesized in adequate quantities. A child afflicted with this disease survives only with frequent transfusions of normal red blood cells. However, the child accumulates 4–8 g of iron per year from hemoglobin in the transfused cells. Our bodies have no mechanism for excreting large quantities of iron, and most patients die by age 20 from toxic effects of iron overload.

A ligand that binds to a metal ion through multiple ligand atoms is called a *chelate*, pronounced *KEE-late*. Chelation therapy enhances iron excretion from thalassemia patients. The most successful drug is *desferrioxamine B*. Its Fe^{3+} complex, ferrioxamine B, has a formation constant of $10^{30.6}$. Used in conjunction with ascorbic acid (vitamin C), which reduces Fe^{3+} to soluble Fe^{2+} , desferrioxamine clears grams of iron per year from an overloaded patient. The Fe^{3+} -complex is excreted in the urine. Too high a dose of this expensive drug stunts a child's growth. Desferrioxamine is not absorbed through the gut. It must be taken by overnight subcutaneous infusion five to seven times per week.

Many chelators have been tested to find one that can be taken orally.¹ Deferiprone has been used with positive effect in more than 50 countries since 1987, but it is not licensed in the U.S. and Canada. Combined use of desferrioxamine and deferiprone increases survival and reduces the incidence of cardiac disease. An orally administered chelator called deferasirox was approved by the U.S. in 2005. The continued search for chelators indicates that no current treatment is fully effective. Eventually, bone marrow transplants or gene therapy might cure the disease.

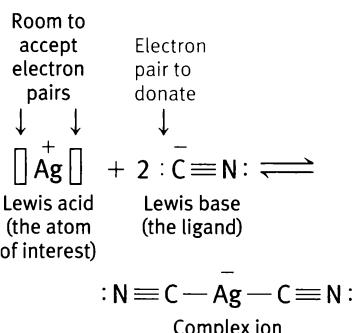
EDTA Titrations

EDTA is a merciful abbreviation for *ethylenediaminetetraacetic acid*, a synthetic compound that can be used to titrate most metal ions by forming strong 1:1 complexes. In addition to its place in chemical analysis, EDTA is used as a metal-binding agent in industrial processes and in household products such as soaps, cleaning agents, and food additives that prevent metal-catalyzed oxidation of food. EDTA also participates in environmental chemistry. For example, much of the nickel, iron, lead, copper, and zinc discharged into San Francisco Bay is in the form of EDTA complexes that pass unscathed through wastewater treatment plants.

13-1 Metal-Chelate Complexes

An atom or group of atoms bound to whatever atom you are interested in is called a **ligand**. A ligand with a pair of electrons to share can bind to a metal ion that can accept a pair of electrons. Electron pair acceptors are called **Lewis acids** and electron pair donors are called **Lewis bases**. Cyanide is said to be a **monodentate** ("one-toothed") **ligand** because it binds to a metal ion through only one atom (the carbon atom). A **multidentate ligand** binds to a metal ion through more than one ligand atom. EDTA in Figure 13-1 is *hexadentate*, binding to a metal through two N atoms and four O atoms.

A multidentate ligand is also called a **chelating ligand**, or just a *chelate*, pronounced **KEE-late**. The term "chelate" is derived from the great claw, or *chela*, of the lobster. The ligand engulfs a metal ion the way a lobster might grab an object with its claw.



Lewis acid: electron pair acceptor

Lewis base: electron pair donor

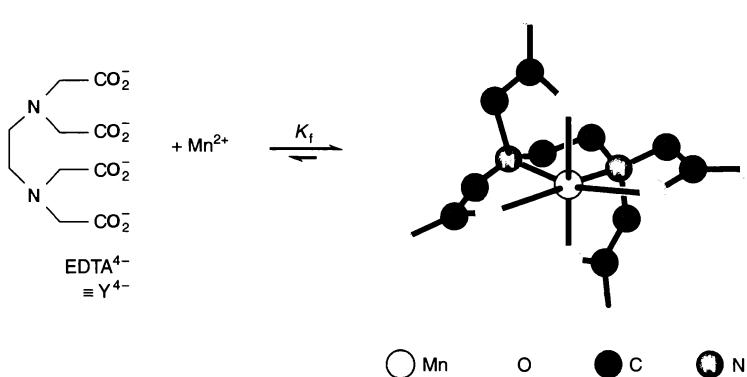
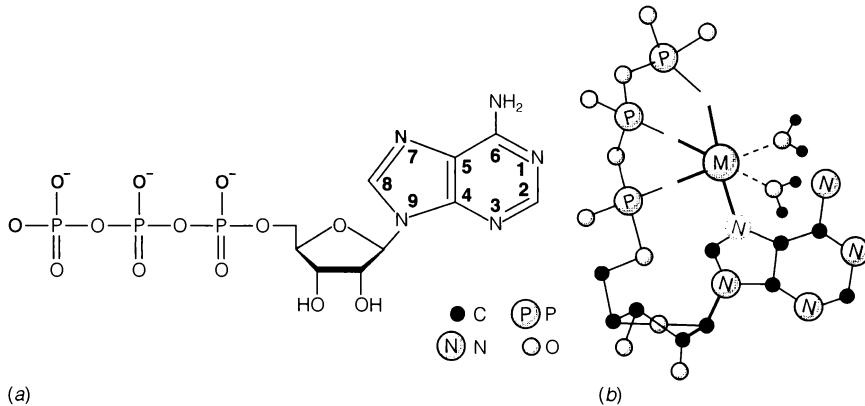


Figure 13-1 EDTA forms strong 1:1 complexes with most metal ions, binding through four oxygen and two nitrogen atoms. The six-coordinate structure of Mn^{3+} -EDTA is found in the compound $\text{KMnEDTA} \cdot 2\text{H}_2\text{O}$. [From J. Stein, J. P. Fackler, Jr., G. J. McClune, J. A. Fee, and L. T. Chan, *Inorg. Chem.* 1979, 18, 3511.]

Figure 13-2 (a) Structure of adenosine triphosphate (ATP), with ligand atoms shown in color. (b) Possible structure of a metal-ATP complex, with four bonds to ATP and two bonds to H_2O ligands.



Most transition metal ions bind six ligand atoms. An important *tetradeятate* ligand is adenosine triphosphate (ATP), which binds to divalent metal ions (such as Mg^{2+} , Mn^{2+} , Co^{2+} , and Ni^{2+}) through four of their six coordination positions (Figure 13-2). The fifth and sixth positions are occupied by water molecules. The biologically active form of ATP is generally the Mg^{2+} complex.

The synthetic *octadентate* ligand in Figure 13-3 is being evaluated as an anti-cancer agent. This chelate binds a metal through four N atoms and four O atoms. The chelate is covalently attached to a *monoclonal antibody*, which is a protein produced by one specific type of cell in response to one specific foreign substance called an *antigen*. In this case, the antibody binds to a specific feature of a tumor cell. The chelate carries a short-lived radioisotope such as $^{90}\text{Y}^{3+}$ or $^{177}\text{Lu}^{3+}$, which delivers lethal doses of radiation to the tumor.

Metal-chelate complexes are ubiquitous in biology. Bacteria such as *Escherichia coli* and *Salmonella enterica* in your gut excrete the chelator enterobactin (Figure 13-4) to scavenge iron that is essential for their growth.² Chelates excreted by microbes to gather iron are called *siderophores*. The iron-enterobactin complex binds to the bacterial cell surface and is taken into the cell. Iron is then released by enzymatic disassembly of the chelate. To fight bacterial infection, your immune system produces a protein called siderocalin to sequester and inactivate enterobactin.³

The drug desferrioxamine mentioned at the opening of this chapter is produced by the microbe *Streptomyces pilosus*. Ferrioxamines are found in the ocean at

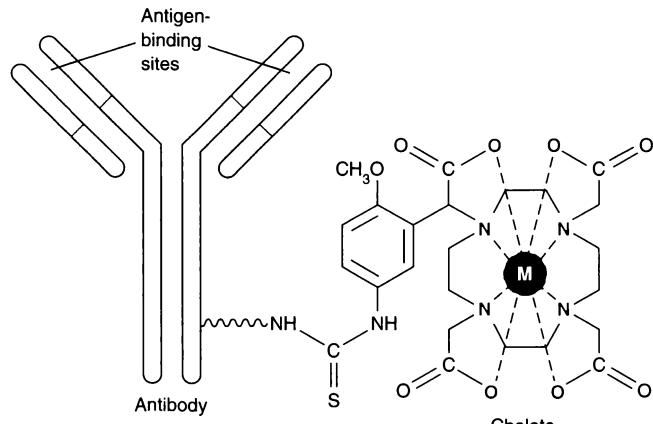


Figure 13-3 Synthetic chelate covalently attached to an antibody carries a metal isotope (M) to deliver lethal doses of radiation to tumor cells.

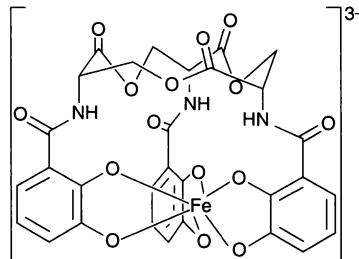


Figure 13-4 Iron(III)-enterobactin complex. Certain bacteria secrete enterobactin to capture iron and bring it into the cell. Enterobactin is one of several known chelates—designated *siderophores*—released by microbes to capture iron to be used by the cell.

concentrations of 0.1–10 pM. They are presumably excreted by microorganisms to accumulate scarce iron from the ocean.⁴

The aminocarboxylic acids in Figure 13-5 are synthetic chelating agents whose nitrogen and carboxylate oxygen atoms can lose protons and bind to metal ions. Molecules in Figure 13-5 form strong 1:1 complexes with all metal ions, except univalent ions such as Li^+ , Na^+ , and K^+ . *The stoichiometry is 1:1 regardless of the charge on the ion.* A medical application of the ligand DTPA in Figure 13-5 is illustrated by the tightly bound complex Gd^{3+} -DTPA, which is injected into humans at a concentration of $\sim 0.5 \text{ mM}$ to provide contrast in magnetic resonance imaging.⁵

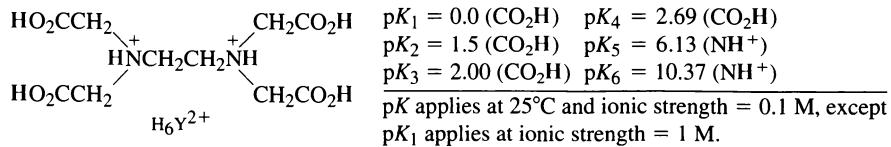
Ask Yourself

13-A. What is the difference between a monodentate and a multidentate ligand? Is a chelating ligand monodentate or multidentate? How many and what kind of ligand atoms are in desferrioxamine B at the opening of this chapter?

13-2 EDTA

EDTA is the most widely used chelator in analytical chemistry. By direct titration or through a sequence of reactions, virtually every element of the periodic table can be analyzed with EDTA. A titration based on complex formation is called a **complexometric titration**.

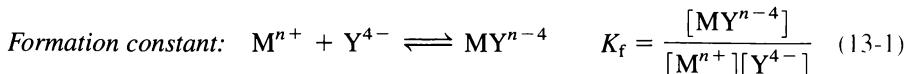
EDTA is a hexaproto system, designated H_6Y^{2+} . The highlighted acidic hydrogen atoms are the ones that are lost on metal-complex formation:



The first four $\text{p}K$ values apply to carboxyl protons, and the last two are for the ammonium protons. Below a pH of 10.24, most EDTA is protonated and is not in the form Y^{4-} that binds to metal ions (Figure 13-1).

Neutral EDTA is H_4Y . A common reagent is the disodium salt, $\text{Na}_2\text{H}_2\text{Y} \cdot 2\text{H}_2\text{O}$, which attains the dihydrate composition upon heating at 80°C .

The equilibrium constant for the reaction of a metal with a ligand is called the **formation constant**, K_f , or the **stability constant**:



Formation constants for EDTA complexes in Table 13-1 are large and tend to be larger for more positively charged metal ions. Note that K_f is defined for reaction of the species Y^{4-} with the metal ion. At low pH, most EDTA is in one of its protonated forms, not Y^{4-} .

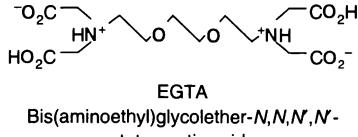
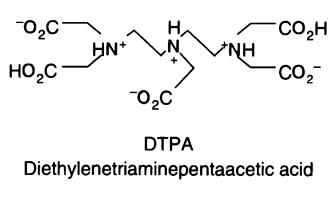
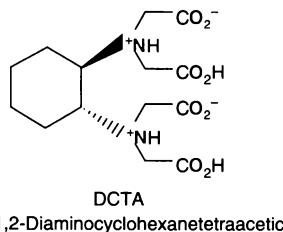
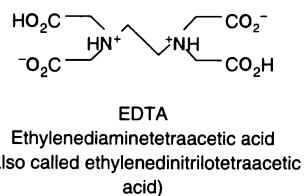


Figure 13-5 Analytically useful synthetic chelating agents that form strong 1:1 complexes with most metal ions.

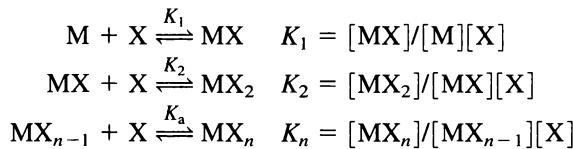
One mole of EDTA reacts with one mole of metal ion.

Only some of the EDTA is in the form Y^{4-} .

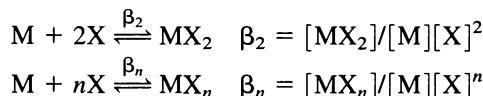
Box 13-1 describes the notation used for formation constants.

Box 13-1 Notation for Formation Constants

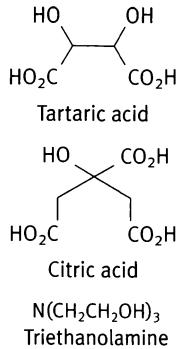
Formation constants are equilibrium constants for complex formation. The **stepwise formation constants**, designated K_i , are defined as follows:



where M is a metal ion and X is a ligand. The **overall, or cumulative, formation constants** are denoted β_i :



A useful relation is that $\beta_n = K_1 K_2 \cdots K_n$. Page 257 shows cumulative formation constants for lead-iodide complexes used to calculate the composition of a saturated solution of PbI_2 .



A metal-EDTA complex becomes unstable at low pH because H^+ competes with the metal ion for EDTA. At too high a pH, OH^- competes with EDTA for the metal ion and may precipitate the metal hydroxide or form unreactive hydroxide complexes. Figure 13-6 shows the pH ranges over which some common metal ions can be titrated. Pb^{2+} , for example, reacts “quantitatively” with EDTA between pH 3 and pH 12. Between pH 9 and 12, it is necessary to use an **auxiliary complexing agent**, which forms a weak complex with Pb^{2+} and keeps it in solution (Color Plate 7). The auxiliary complexing agent is displaced by EDTA during the titration. Auxiliary complexing agents, such as ammonia, tartrate, citrate, or triethanolamine, prevent metal ions from precipitating in the absence of EDTA. The titration of Pb^{2+} is carried out at pH 10 in the presence of tartrate, which complexes the metal ion and does not

Table 13-1 Formation constants for metal-EDTA complexes^{a,b}

Ion	$\log K_f$						
Li^+	2.95	V^{2+}	12.7 ^c	Fe^{3+}	25.1	Sn^{2+}	18.3 ^d
Na^+	1.86	Cr^{2+}	13.6 ^c	Co^{3+}	41.4	Pb^{2+}	18.0
K^+	0.8	Mn^{2+}	13.89	Zr^{4+}	29.3	Al^{3+}	16.4
Be^{2+}	9.7	Fe^{2+}	14.30	VO^{2+}	18.7	Ga^{3+}	21.7
Mg^{2+}	8.79	Co^{2+}	16.45	VO_2^+	15.5	In^{3+}	24.9
Ca^{2+}	10.65	Ni^{2+}	18.4	Ag^+	7.20	Tl^{3+}	35.3
Sr^{2+}	8.72	Cu^{2+}	18.78	Tl^+	6.41	Bi^{3+}	27.8 ^c
Ba^{2+}	7.88	Ti^{3+}	21.3	Pd^{2+}	25.6 ^c	Ce^{3+}	15.93
Ra^{2+}	7.4	V^{3+}	25.9 ^c	Zn^{2+}	16.5	Gd^{3+}	17.35
Sc^{3+}	23.1 ^c	Cr^{3+}	23.4 ^c	Cd^{2+}	16.5	Th^{4+}	23.2
Y^{3+}	18.08	Mn^{3+}	25.2	Hg^{2+}	21.5	U^{4+}	25.7
La^{3+}	15.36						

a. From A. E. Martell, R. M Smith, and R. J. Motekaitis, *NIST Critically Selected Stability Constants of Metal Complexes*, NIST Standard Reference Database 46, Gaithersburg, MD, 2001.

b. The formation constant is the equilibrium constant for the reaction $M^{n+} + Y^{4-} \rightleftharpoons MY^{n-4}$. Values in table apply at 25°C and ionic strength 0.1 M unless otherwise indicated.

c. 20°C, ionic strength = 0.1 M.

d. 20°C, ionic strength = 1 M.

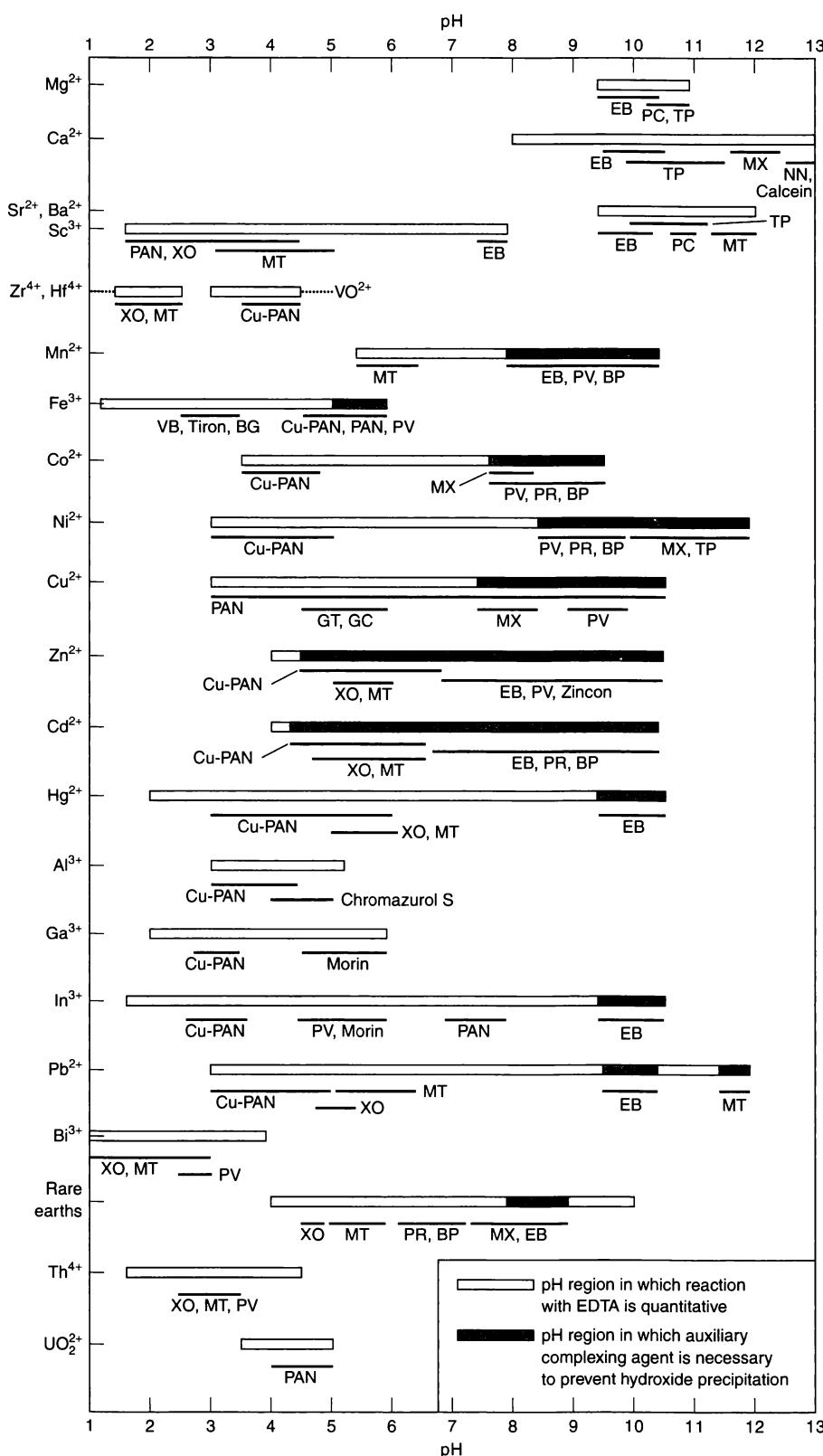


Figure 13-6 Guide to EDTA titrations of some common metals. Light color shows pH range in which reaction with EDTA is quantitative. Dark color shows pH range in which auxiliary complexing agent such as ammonia is required to prevent metal from precipitating. [Adapted from K. Ueno, *J. Chem. Ed.* **1965**, 42, 432.]

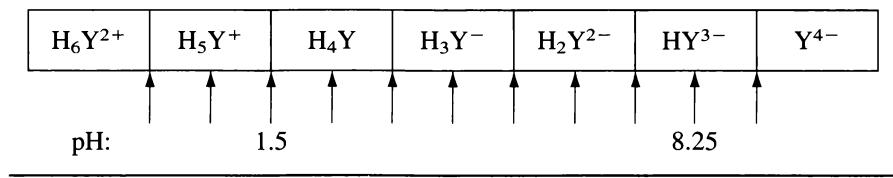
Abbreviations for indicators:
 BG, Bindschedler's green leuco base
 BP, Bromopyrogallol red
 Cu-PAN, PAN plus Cu-EDTA
 EB, Eriochrome black T
 GC, Glycinecresol red
 GT, Glycerethymol blue
 MT, Methylthymol blue
 MX, Murexide
 NN, Patton & Reeder's dye
 PAN, Pyridylazonaphthol
 PC, *o*-Cresolphthalein complexone
 PR, Pyrogallol red
 PV, Pyrocatechol violet
 TP, Thymolphthalein complexone
 VB, Variamine blue B base
 XO, Xylenol orange

allow $\text{Pb}(\text{OH})_2$ to precipitate. The lead-tartrate complex must be less stable than the lead-EDTA complex or the titration would not be feasible.

Figure 13-6 shows pH ranges in which various *metal ion indicators* (discussed in the next section) are useful for finding end points. The chart also provides a strategy for selective titration of one ion in the presence of another. For example, a solution containing both Fe^{3+} and Ca^{2+} could be titrated with EDTA at pH 4. At this pH, Fe^{3+} is titrated without interference from Ca^{2+} .

Ask Yourself

- 13-B.** (a) Write the reaction whose equilibrium constant is the formation constant for EDTA complex formation and write the algebraic form of K_f .
(b) Why is EDTA complex formation less complete at lower pH?
(c) What is the purpose of an auxiliary complexing agent?
(d) The following diagram is analogous to those in Section 11-3 for polyprotic acids showing the pH range at which each species is predominant. Fill in the pH at each arrow marking the border or center of each pH range. Explain the significance of the pH at each arrow.



13-3 Metal Ion Indicators

A metal ion indicator is a compound whose color changes when it binds to a metal ion. Two common indicators are shown in Table 13-2. *For an indicator to be useful, it must bind metal less strongly than EDTA does.*

A typical analysis is illustrated by the titration of Mg^{2+} with EDTA, with Calmagite as the indicator:

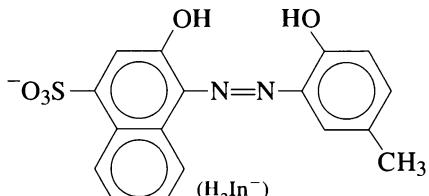
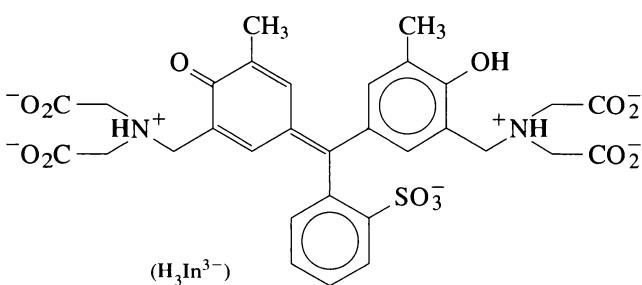
The indicator must release its metal to EDTA.



At the start of the experiment, a small amount of indicator (In) is added to the colorless solution of Mg^{2+} to form a red complex. As EDTA is added, it reacts first with free, colorless Mg^{2+} . When free Mg^{2+} is used up, the last EDTA added before the equivalence point displaces indicator from the red MgIn complex. The change from the red of MgIn to the blue of unbound In signals the end point of the titration (Demonstration 13-1).

Most metal ion indicators are also acid-base indicators. Because the color of free indicator is pH dependent, most indicators can be used only in certain pH ranges. For example, xylene orange (pronounced zy-leen-ol) in Table 13-2 changes from yellow to red when it binds to a metal ion at pH 5.5. This color change is easy to observe. At pH 7.5, the change from violet to red is difficult to see.

Table 13-2 Some common metal ion indicators

Name	Structure	pK_a	Formula and color of free indicator	Color of metal ion complex
Calmagite	 (H_2In^-)	$pK_2 = 8.1$ $pK_3 = 12.4$	H_2In^- Red HIn^{2-} Blue In^{3-} Orange	Wine red
Xylenol orange	 (H_3In^{3-})	$pK_2 = 2.32$ $pK_3 = 2.85$ $pK_4 = 6.70$ $pK_5 = 10.47$ $pK_6 = 12.23$	H_5In^- Yellow H_4In^{2-} Yellow H_3In^{3-} Yellow H_2In^{4-} Violet HIn^{5-} Violet In^{6-} Violet	Red



Demonstration 13-1 Metal Ion Indicator Color Changes

This demonstration illustrates the color change associated with Reaction 13-2.

Stock solutions

Calmagite: Dissolve 0.05 g of indicator in 100 mL of water. Alternatively, dissolve 0.1 g Eriochrome black T indicator in 7.5 mL of triethanolamine plus 2.5 mL of absolute ethanol. Color changes are the same for both indicators.

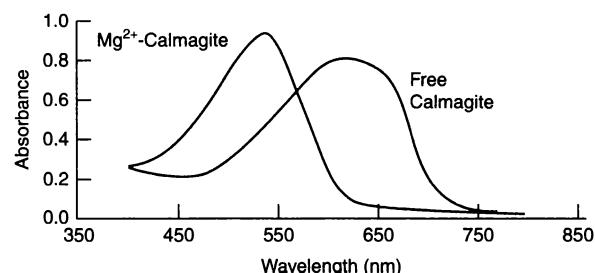
Buffer (pH 10): Add 142 mL of concentrated (14.5 M) aqueous ammonia to 17.5 g of ammonium chloride and dilute to 250 mL with water.

$MgCl_2$: 0.05 M

EDTA: 0.05 M $Na_2H_2EDTA \cdot 2H_2O$

Prepare a solution containing 25 mL of $MgCl_2$, 5 mL of buffer, and 300 mL of water. Add 6 drops of indicator and titrate with EDTA. Note the color change from wine red to pale blue at the end point (Color Plate 8). The spectroscopic change accompanying the color change is shown in the figure.

Visible spectra of Mg^{2+} -Calmagite and free Calmagite at pH 10 in ammonia buffer. [From C. E. Dahm, J. W. Hall, and B. E. Mattioni, *J. Chem. Ed.* **2004**, 81, 1787.]



Question What will the color change be when the back titration is performed?

For an indicator to be useful in an EDTA titration, the indicator must give up its metal ion to EDTA. If a metal ion does not freely dissociate from the indicator, the metal is said to **block** the indicator. Calmagite is blocked by Cu^{2+} , Ni^{2+} , Co^{2+} , Cr^{3+} , Fe^{3+} , and Al^{3+} . It cannot be used for the direct titration of any of these metals. However, it can be used for a back titration. For example, excess standard EDTA can be added to Cu^{2+} . Then indicator is added and excess EDTA is back titrated with Mg^{2+} .

Ask Yourself

- 13-C. (a) Explain why the change from red to blue in Reaction 13-2 occurs suddenly at the equivalence point instead of gradually throughout the entire titration. (b) EDTA buffered to pH 5 was titrated with standard Pb^{2+} , with xylenol orange as indicator (Table 13-2). (i) Which is the principal species of the indicator at pH 5? (ii) What color was observed before the equivalence point? (iii) What color was observed after the equivalence point? (iv) What would the color change be if the titration were conducted at pH 8 instead of pH 5?

13-4 EDTA Titration Techniques

EDTA can be used directly or indirectly to analyze most elements of the periodic table. In this section, we discuss several important techniques.

Direct Titration

In a **direct titration**, analyte is titrated with standard EDTA. Analyte is buffered to a pH at which the reaction with EDTA is essentially complete and the free indicator has a color distinctly different from that of the metal-indicator complex. An auxiliary complexing agent might be required to maintain the metal ion in solution in the absence of EDTA.

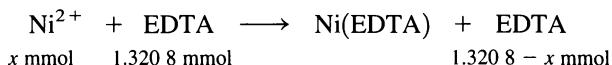
Back Titration

In a **back titration**, a known excess of EDTA is added to the analyte. Excess EDTA is then titrated with a standard solution of metal ion. A back titration is necessary if analyte precipitates in the absence of EDTA, if analyte reacts too slowly with EDTA, or if analyte blocks the indicator. The metal used in the back titration must not displace analyte from EDTA.

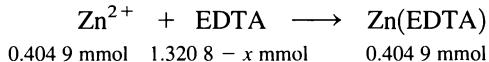
Example A Back Titration

Ni^{2+} can be analyzed by a back titration with standard Zn^{2+} at pH 5.5 and xylenol orange indicator. A solution containing 25.00 mL of Ni^{2+} in dilute HCl was treated with 25.00 mL of 0.052 83 M Na_2EDTA . The solution was neutralized with NaOH, and the pH was adjusted to 5.5 with acetate buffer. The solution turned yellow when a few drops of indicator were added. Titration with 0.022 99 M Zn^{2+} required 17.61 mL of Zn^{2+} to reach the red end point. What was the molarity of Ni^{2+} in the unknown?

SOLUTION The unknown was treated with 25.00 mL of 0.05283 M EDTA, which contains $(25.00 \text{ mL})(0.05283 \text{ M}) = 1.3208 \text{ mmol}$ of EDTA.



Back titration required $(17.61 \text{ mL})(0.02299 \text{ M}) = 0.4049 \text{ mmol}$ of Zn^{2+} .



The moles of Zn^{2+} required in the second reaction must equal the moles of excess EDTA from the first reaction

$$\begin{aligned} 0.4049 \text{ mmol Zn}^{2+} &= 1.3208 \text{ mmol EDTA} - x \text{ mmol Ni}^{2+} \\ x &= 0.9159 \text{ mmol Ni}^{2+} \end{aligned}$$

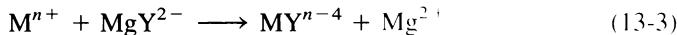
The concentration of Ni^{2+} is $0.9159 \text{ mmol}/25.00 \text{ mL} = 0.03664 \text{ M}$.

 **Test Yourself** Suppose that 0.04000 M EDTA was used and back titration required 15.00 mL of Zn^{2+} . Find the concentration of Ni^{2+} . (Answer: 0.02621 M)

An EDTA back titration can prevent precipitation of analyte. For example, Al(OH)_3 precipitates at pH 7 in the absence of EDTA. An acidic solution of Al^{3+} can be treated with excess EDTA, adjusted to pH 7 with sodium acetate, and boiled to ensure complete complexation. The Al^{3+} -EDTA complex is stable at pH 7. The solution is then cooled, Calmagite indicator is added, and back titration with standard Zn^{2+} is performed.

Displacement Titration

For some metal ions, there is no satisfactory indicator, but a **displacement titration** is feasible. In this procedure, analyte is usually treated with excess Mg(EDTA)^{2-} to displace Mg^{2+} , which is later titrated with standard EDTA.



Hg^{2+} is determined in this manner. The formation constant of Hg(EDTA)^{2-} must be greater than the formation constant of Mg(EDTA)^{2-} or else the displacement of Mg^{2+} from Mg(EDTA)^{2-} would not occur.

There is no suitable indicator for Ag^+ . However, Ag^+ will displace Ni^{2+} from the tetracyanonickelate(II) ion:



The liberated Ni^{2+} can then be titrated with EDTA to find out how much Ag^+ was added.

Challenge Calculate the equilibrium constant for Reaction 13-3 if $\text{M}^{n+} = \text{Hg}^{2+}$. Why is Mg(EDTA)^{2-} used for a displacement titration?

Indirect Titration of Anions

Anions that precipitate metal ions can be analyzed with EDTA by **indirect titration**. For example, sulfate can be analyzed by precipitation with excess Ba^{2+} at pH 1. The resulting $\text{BaSO}_4(s)$ is filtered, washed, and boiled with excess EDTA at pH 10 to bring Ba^{2+} back into solution as $\text{Ba}(\text{EDTA})^{2-}$. The excess EDTA is back titrated with Mg^{2+} .

Alternatively, an anion can be precipitated with excess metal ion. The precipitate is filtered and washed, and the excess metal ion in the filtrate is titrated with EDTA. CO_3^{2-} , CrO_4^{2-} , S^{2-} , and SO_4^{2-} can be determined in this manner.

Masking

Masking prevents one element from interfering in the analysis of another element. Box 13-2 describes an important application of masking.

A **masking agent** protects some component of a mixture from reaction with EDTA. For example, Mg^{2+} in a mixture of Mg^{2+} and Al^{3+} can be titrated by masking Al^{3+} with F^- to generate AlF_6^{3-} , which does not react with EDTA. Only the Mg^{2+} reacts with EDTA.

Cyanide is a masking agent that forms complexes with Cd^{2+} , Zn^{2+} , Hg^{2+} , Co^{2+} , Cu^+ , Ag^+ , Ni^{2+} , Pd^{2+} , Pt^{2+} , Fe^{2+} , and Fe^{3+} , but not with Mg^{2+} , Ca^{2+} , Mn^{2+} , or Pb^{2+} . When CN^- is added to a solution containing Cd^{2+} and Pb^{2+} , only the Pb^{2+} can react with EDTA. (CAUTION Cyanide forms toxic gaseous HCN below pH 11. Cyanide solutions should be strongly basic and handled in a hood.) Fluoride masks Al^{3+} , Fe^{3+} , Ti^{4+} , and Be^{2+} . (CAUTION HF formed by F^- in acidic solution is

Box 13-2 What Is Hard Water?

Hardness refers to the total concentration of alkaline earth ions in water. The concentrations of Ca^{2+} and Mg^{2+} are usually much greater than those of other Group 2 ions, so hardness can be equated to $[\text{Ca}^{2+}] + [\text{Mg}^{2+}]$. Hardness is commonly expressed as the equivalent number of milligrams of CaCO_3 per liter. Thus, if $[\text{Ca}^{2+}] + [\text{Mg}^{2+}] = 1 \text{ mM}$, we would say that the hardness is 100 mg CaCO_3 per liter, because 100 mg $\text{CaCO}_3 = 1 \text{ mmol CaCO}_3$. Water whose hardness is less than 60 mg CaCO_3 per liter is considered to be “soft.”

Hard water reacts with soap to form insoluble curds:

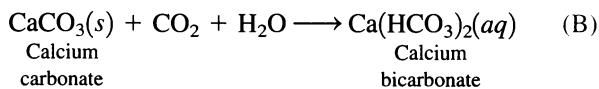


Enough soap to consume the Ca^{2+} and Mg^{2+} must be used before the soap is useful for cleaning. Hard water is not thought to be unhealthy. Hardness is beneficial in irrigation because alkaline earth ions tend to *flocculate* (cause to aggregate) *colloidal* particles in soil and thereby increase the permeability of the soil to water. Colloids are soluble particles that are 1–500 nm in

diameter (Demonstration 7-1). Such small particles tend to plug the paths by which water can drain through soil.

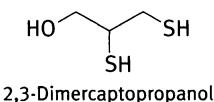
To measure hardness, water is treated with ascorbic acid to reduce Fe^{3+} to Fe^{2+} and with cyanide to mask Fe^{2+} , Cu^+ , and several other minor metal ions. Titration with EDTA at pH 10 in ammonia buffer gives $[\text{Ca}^{2+}] + [\text{Mg}^{2+}]$. $[\text{Ca}^{2+}]$ can be determined separately if the titration is carried out at pH 13 without ammonia. At this pH, $\text{Mg}(\text{OH})_2$ precipitates and is inaccessible to EDTA.

Insoluble carbonates are converted into soluble bicarbonates by excess carbon dioxide:



Heating reverses Reaction B to form a solid scale of CaCO_3 that clogs boiler pipes. The fraction of hardness due to $\text{Ca}(\text{HCO}_3)_2(aq)$ is called *temporary hardness*, because this calcium is lost (by precipitation of CaCO_3) on heating. Hardness arising from other salts (mainly dissolved CaSO_4) is called *permanent hardness*, because it is not removed by heating.

extremely hazardous and should not contact skin or eyes. It may not be immediately painful, but the affected area should be flooded with water for 5 min and then treated with 2.5 wt% calcium gluconate gel that you have on hand *before* the accident. First aiders must wear rubber gloves to protect themselves. Damage from HF exposure can continue for several days after exposure. Exposure of 2% of your body to concentrated HF can kill you.⁶ Triethanolamine (page 282) masks Al³⁺, Fe³⁺, and Mn²⁺; and 2,3-dimercaptopropanol masks Bi³⁺, Cd²⁺, Cu²⁺, Hg²⁺, and Pb²⁺. Selectivity afforded by masking and pH control allows individual components of complex mixtures to be analyzed by EDTA titration.



Ask Yourself

- 13-D. (a) A 50.0-mL sample containing Ni²⁺ was treated with 25.0 mL of 0.050 0 M EDTA to complex all the Ni²⁺ and leave excess EDTA in solution. How many millimoles of EDTA are contained in 25.0 mL of 0.050 0 M EDTA?
 (b) The excess EDTA in (a) was then back titrated, requiring 5.00 mL of 0.050 0 M Zn²⁺. How many millimoles of Zn²⁺ are in 5.00 mL of 0.050 0 M Zn²⁺?
 (c) The number of millimoles of Ni²⁺ in the unknown is the difference between the EDTA added in (a) and the Zn²⁺ required in (b). Find the number of millimoles of Ni²⁺ and the concentration of Ni²⁺ in the unknown.

13-5 The pH-Dependent Metal-EDTA Equilibrium

At this point in the chapter, those who want to spend more time on instrumental methods of analysis might want to move to a new chapter. We now consider equilibrium calculations required to understand the shape of an EDTA titration curve.

Fractional Composition of EDTA Solutions

The fraction of EDTA in each of its protonated forms is plotted in Figure 13-7. The fraction, α , is defined as it was in Section 12-5 for any weak acid. For example, $\alpha_{Y^{4-}}$ is

Fraction of EDTA in the form Y⁴⁻:

$$\alpha_{Y^{4-}} = \frac{[Y^{4-}]}{[H_6Y^{2+}] + [H_5Y^+] + [H_4Y] + [H_3Y^-] + [H_2Y^{2-}] + [HY^{3-}] + [Y^{4-}]}$$

$$\alpha_{Y^{4-}} = \frac{[Y^{4-}]}{[\text{EDTA}]} \quad (13-4)$$

where [EDTA] is the total concentration of all *free* EDTA species in the solution. “Free” means EDTA that is not complexed to metal ions. Following a derivation

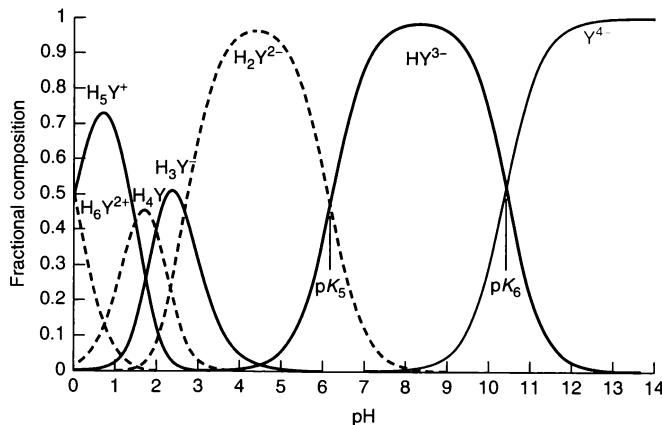


Figure 13-7 Fraction of EDTA in each of its protonated forms as a function of pH. This figure should remind you of Figure 11-1 for a diprotic acid. The fraction of Y^{4-} , shown by the colored curve at the right, is very small below pH 8.

similar to the one in Section 12-5, it can be shown that $\alpha_{\text{Y}^{4-}}$ is given by

$$\alpha_{\text{Y}^{4-}} = \frac{K_1 K_2 K_3 K_4 K_5 K_6}{\{[\text{H}^+]^6 + [\text{H}^+]^5 K_1 + [\text{H}^+]^4 K_1 K_2 + [\text{H}^+]^3 K_1 K_2 K_3 + [\text{H}^+]^2 K_1 K_2 K_3 K_4 + [\text{H}^+] K_1 K_2 K_3 K_4 K_5 + K_1 K_2 K_3 K_4 K_5 K_6\}} \quad (13-5)$$

Table 13-3 gives values for $\alpha_{\text{Y}^{4-}}$ as a function of pH.

Table 13-3 Values of $\alpha_{\text{Y}^{4-}}$ for EDTA at 25°C and $\mu = 0.10 \text{ M}$

pH	$\alpha_{\text{Y}^{4-}}$
0	1.3×10^{-23}
1	1.4×10^{-18}
2	2.6×10^{-14}
3	2.1×10^{-11}
4	3.0×10^{-9}
5	2.9×10^{-7}
6	1.8×10^{-5}
7	3.8×10^{-4}
8	4.2×10^{-3}
9	0.041
10	0.30
11	0.81
12	0.98
13	1.00
14	1.00

Example What Does $\alpha_{\text{Y}^{4-}}$ Mean?

The fraction of free EDTA in the form Y^{4-} , called $\alpha_{\text{Y}^{4-}}$, is shown by the colored curve in Figure 13-7. At pH 6.00 and a formal concentration of 0.10 M, the composition of an EDTA solution is

$$\begin{aligned} [\text{H}_6\text{Y}^{2+}] &= 8.9 \times 10^{-20} \text{ M} & [\text{H}_5\text{Y}^+] &= 8.9 \times 10^{-14} \text{ M} & [\text{H}_4\text{Y}] &= 2.8 \times 10^{-9} \text{ M} \\ [\text{H}_3\text{Y}^-] &= 2.8 \times 10^{-5} \text{ M} & [\text{H}_2\text{Y}^{2-}] &= 0.057 \text{ M} & [\text{HY}^{3-}] &= 0.043 \text{ M} \\ &&&& [\text{Y}^{4-}] &= 1.8 \times 10^{-6} \text{ M} \end{aligned}$$

Find $\alpha_{\text{Y}^{4-}}$.

SOLUTION $\alpha_{\text{Y}^{4-}}$ is the fraction in the form Y^{4-} :

$$\begin{aligned} \alpha_{\text{Y}^{4-}} &= \frac{[\text{Y}^{4-}]}{[\text{H}_6\text{Y}^{2+}] + [\text{H}_5\text{Y}^+] + [\text{H}_4\text{Y}] + [\text{H}_3\text{Y}^-] + [\text{H}_2\text{Y}^{2-}] + [\text{HY}^{3-}] + [\text{Y}^{4-}]} \\ &= \frac{[1.8 \times 10^{-6}]}{[8.9 \times 10^{-20}] + [8.9 \times 10^{-14}] + [2.8 \times 10^{-9}] + [2.8 \times 10^{-5}] + [0.057] \\ &\quad + [0.043] + [1.8 \times 10^{-6}]} \\ &= 1.8 \times 10^{-5} \end{aligned}$$

 **Test Yourself** From Figure 13-7, estimate the fractions of HY^{3-} and H_2Y^{2-} at pH 7. (**Answer:** 0.9 and 0.1)

Conditional Formation Constant

The formation constant in Equation 13-1 describes the reaction between Y^{4-} and a metal ion. As you can see in Figure 13-7, most of the EDTA is not Y^{4-} below pH = $\text{p}K_6 = 10.37$. The species HY^{3-} , H_2Y^{2-} , and so on, predominate at lower pH. It is convenient to express the fraction of free EDTA in the form Y^{4-} by rearranging Equation 13-4 to give

$$[\text{Y}^{4-}] = \alpha_{\text{Y}^{4-}}[\text{EDTA}] \quad (13-6)$$

where [EDTA] refers to the total concentration of all EDTA species not bound to metal ion.

The equilibrium constant for Reaction 13-1 can now be rewritten as

$$K_f = \frac{[\text{MY}^{n-4}]}{[\text{M}^{n+}][\text{Y}^{4-}]} = \frac{[\text{MY}^{n-4}]}{[\text{M}^{n+}]\alpha_{\text{Y}^{4-}}[\text{EDTA}]}$$

If the pH is fixed by a buffer, then $\alpha_{\text{Y}^{4-}}$ is a constant that can be combined with K_f :

Conditional formation constant: $K'_f = \alpha_{\text{Y}^{4-}}K_f = \frac{[\text{MY}^{n-4}]}{[\text{M}^{n+}][\text{EDTA}]} \quad (13-7)$

K'_f ($= \alpha_{\text{Y}^{4-}}K_f$) is called the **conditional formation constant** or the *effective formation constant*. It describes the formation of MY^{n-4} at any particular pH.

The conditional formation constant allows us to look at EDTA complex formation as if the uncomplexed EDTA were all in one form:



At any given pH, we can find $\alpha_{\text{Y}^{4-}}$ and evaluate K'_f .

Equation 13-1 does not imply that Y^{4-} is the only species that reacts with M^{n+} . It only says that the equilibrium constant is expressed in terms of the concentration of Y^{4-} .

The product $\alpha_{\text{Y}^{4-}}[\text{EDTA}]$ accounts for the fact that only some free EDTA is in the form Y^{4-} .

With the conditional formation constant, we can treat EDTA complex formation as if all free EDTA were in one form.

Example Using the Conditional Formation Constant

The formation constant in Table 13-1 for FeY^- is $10^{25.1} = 1.3 \times 10^{25}$. Calculate the concentration of free Fe^{3+} in a solution of 0.10 M FeY^- at pH 4.00 and at pH 1.00.

SOLUTION The complex formation reaction is



where EDTA on the left side of the equation refers to all forms of unbound EDTA ($= \text{Y}^{4-}$, HY^{3-} , H_2Y^{2-} , H_3Y^- , and so on). Using $\alpha_{\text{Y}^{4-}}$ from Table 13-3, we find

$$\begin{aligned}\text{At pH 4.00: } K'_f &= (3.0 \times 10^{-9})(1.3 \times 10^{25}) = 3.9 \times 10^{16} \\ \text{At pH 1.00: } K'_f &= (1.4 \times 10^{-18})(1.3 \times 10^{25}) = 1.8 \times 10^7\end{aligned}$$

Because dissociation of FeY^- must produce equal quantities of Fe^{3+} and EDTA, we can write

	Fe^{3+}	+	EDTA	\rightleftharpoons	FeY^-
Initial concentration (M)	0		0		0.10
Final concentration (M)	x		x		$0.10 - x$

$$\frac{[\text{FeY}^-]}{[\text{Fe}^{3+}][\text{EDTA}]} = \frac{0.10 - x}{x^2} = K'_f = 3.9 \times 10^{16} \quad \text{at pH 4.00}$$

$$= 1.8 \times 10^7 \quad \text{at pH 1.00}$$

Solving for x , we find $[\text{Fe}^{3+}] = x = 1.6 \times 10^{-9} \text{ M}$ at pH 4.00 and $7.4 \times 10^{-5} \text{ M}$ at pH 1.00. *Using the conditional formation constant at a fixed pH, we treat the dissociated EDTA as if it were a single species.*

 **Test Yourself** Calculate $[\text{Fe}^{3+}]$ in 0.10 M FeY^- at pH 5.00. (Answer: $1.6 \times 10^{-10} \text{ M}$)

We see that a metal-EDTA complex becomes less stable at lower pH. A titration reaction must go “to completion,” so the equilibrium constant must be large—the analyte and titrant are essentially completely reacted (say, 99.9%) at the equivalence point. Figure 13-8 shows how pH affects the titration of Ca^{2+} with EDTA. Below pH \approx 8, the break at the end point is not sharp enough to allow accurate determination. At pH 5, the inflection point disappears because the conditional formation constant for CaY^{2-} is too small.

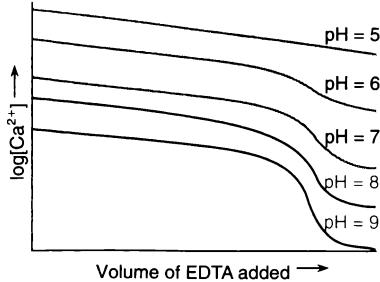


Figure 13-8 Titration of Ca^{2+} with EDTA as a function of pH. The experimental ordinate is the voltage difference between two electrodes (mercury and calomel) immersed in the titration solution. This voltage is a measure of $\log[\text{Ca}^{2+}]$. [From C. N. Reilley and R. W. Schmid, *Anal. Chem.* 1958, 30, 947.]

Ask Yourself

13-E. The solution at the equivalence point in the titration of Ca^{2+} with EDTA is the same as a solution of pure CaY^{2-} . Suppose that the formal concentration $[\text{CaY}^{2-}] = 0.010 \text{ M}$ at the equivalence point in Figure 13-8. Let’s see how complete the reaction is at low and high pH.

- (a) Find the concentration of free Ca^{2+} at pH 5.00 at the equivalence point.
- (b) What is the fraction of bound Ca^{2+} ($= [\text{CaY}^{2-}] / ([\text{CaY}^{2-}] + [\text{Ca}^{2+}])$)?
- (c) Find the concentration of free Ca^{2+} at pH 9.00 and the fraction of bound Ca^{2+} .

13-6 EDTA Titration Curves

Now we calculate the concentration of free metal ion in the course of the titration of metal with EDTA. The titration reaction is



If K'_f is large, we can consider the reaction to be complete at each point in the titration.

The titration curve is a graph of pM ($\equiv -\log[M]$) versus the volume of added EDTA. The curve is analogous to that obtained by plotting pH versus volume of titrant in an acid-base titration. There are three natural regions of the titration curve in Figure 13-9:

Region 1: Before the Equivalence Point

In this region, there is excess M^{n+} after EDTA has been consumed. The concentration of free metal ion is equal to the concentration of excess, unreacted M^{n+} . The dissociation of MY^{n-4} is negligible.

Region 2: At the Equivalence Point

There is exactly as much EDTA as metal in the solution. We can treat the solution as if it were made by dissolving pure MY^{n-4} . Some free M^{n+} is generated by the slight dissociation of MY^{n-4} :



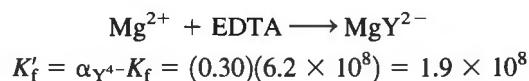
In this reaction, EDTA refers to the total concentration of free EDTA in all of its forms. At the equivalence point, $[M^{n+}] = [\text{EDTA}]$.

Region 3: After the Equivalence Point

Now there is excess EDTA, and virtually all the metal ion is in the form MY^{n-4} . The concentration of free EDTA can be equated to the concentration of excess EDTA added after the equivalence point.

Titration Calculations

Let's calculate the titration curve for the reaction of 50.0 mL of 0.050 0 M Mg^{2+} (buffered to pH 10.00) with 0.050 0 M EDTA. The equivalence volume is 50.0 mL.



Because K'_f is large, it is reasonable to say that the reaction goes to completion with each addition of titrant. We want to make a graph in which pMg^{2+} ($\equiv -\log[Mg^{2+}]$) is plotted versus milliliters of added EDTA.

K'_f is the effective formation constant at the fixed pH of the solution.

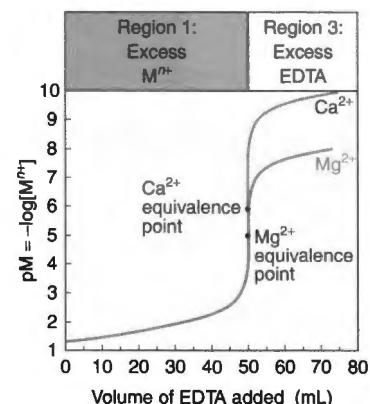


Figure 13-9 Three regions in an EDTA titration of 50.0 mL of 0.050 0 M Mg^{2+} or Ca^{2+} with 0.050 0 M EDTA at pH 10. Region 2 is the equivalence point. The concentration of free M^{n+} decreases as the titration proceeds.

$\alpha_{Y^{4-}}$ comes from Table 13-3 and K_f comes from Table 13-1.

Region 1: Before the Equivalence Point

Before the equivalence point, there is excess unreacted M^{n+} .

Consider the addition of 5.00 mL of EDTA. Because the equivalence point is 50.0 mL, one-tenth of the Mg^{2+} will be consumed and nine-tenths remains.

$$\text{initial mmol } Mg^{2+} = (0.050\ 0\ M\ Mg^{2+})(50.0\ mL) = 2.50\ \text{mmol}$$

$$\text{mmol remaining} = (0.900)(2.50\ \text{mmol}) = 2.25\ \text{mmol}$$

$$[Mg^{2+}] = \frac{2.25\ \text{mmol}}{55.0\ mL} = 0.040\ 9\ M \Rightarrow pMg^{2+} = -\log[Mg^{2+}] = 1.39$$

In a similar manner, we could calculate pMg^{2+} for any volume of EDTA less than 50.0 mL.

Region 2: At the Equivalence Point

At the equivalence point, the major species is MY^{n-4} , in equilibrium with small, equal amounts of free M^{n+} and EDTA.

Virtually all the metal is in the form MgY^{2-} . We began with 2.50 mmol Mg^{2+} , which is now close to 2.50 mmol MgY^{2-} in a volume of $50.0 + 50.0 = 100.0\ mL$.

$$[MgY^{2-}] = \frac{2.50\ \text{mmol}}{100.0\ mL} = 0.025\ 0\ M$$

The concentration of free Mg^{2+} is small and unknown. We can write

	Mg^{2+}	+	EDTA	\rightleftharpoons	MgY^{2-}
Initial concentration (M)	—		—		0.025 0
Final concentration (M)	x		x		$0.025\ 0 - x$

[EDTA] refers to the total concentration of all forms of EDTA not bound to metal.

$$\frac{[MgY^{2-}]}{[Mg^{2+}][\text{EDTA}]} = K_f' = 1.9 \times 10^8$$

$$\frac{0.025\ 0 - x}{x^2} = 1.9 \times 10^8 \Rightarrow x = 1.15 \times 10^{-5}\ M$$

$$pMg^{2+} = -\log x = 4.94$$

Region 3: After the Equivalence Point

After the equivalence point, virtually all metal is in the form MY^{n-4} . There is a known excess of EDTA. A small amount of free M^{n+} exists in equilibrium with MY^{n-4} and EDTA.

In this region, virtually all the metal is in the form MgY^{2-} , and there is excess, unreacted EDTA. The concentrations of MgY^{2-} and excess EDTA are easily calculated. For example, when we have added 51.00 mL of EDTA, there is 1.00 mL of excess EDTA = $(0.050\ 0\ M)(1.00\ mL) = 0.050\ 0\ \text{mmol}$.

$$[\text{EDTA}] = \frac{0.050\ 0\ \text{mmol}}{101.0\ mL} = 0.000\ 495\ M$$

$$[MgY^{2-}] = \frac{2.50\ \text{mmol}}{101.0\ mL} = 0.024\ 8\ M$$

The concentration of Mg^{2+} is governed by

$$\frac{[MgY^{2-}]}{[Mg^{2+}][EDTA]} = K_f' = 1.9 \times 10^8$$
$$\frac{[0.024\ 8]}{[Mg^{2+}](0.000\ 495)} = 1.9 \times 10^8$$
$$[Mg^{2+}] = 2.6 \times 10^{-7} M \Rightarrow pMg^{2+} = 6.58$$

The same sort of calculation can be used for any volume past the equivalence point.

The Titration Curve

The calculated titration curves for Mg^{2+} and for Ca^{2+} in Figure 13-9 show a distinct break at the equivalence point, where the slope is greatest. The break is greater for Ca^{2+} than for Mg^{2+} because the formation constant for CaY^{2-} is greater than K_f for MgY^{2-} . Notice the analogy between Figure 13-9 and acid-base titration curves. The greater the metal-EDTA formation constant, the more pronounced the break at the equivalence point. The stronger the acid HA, the greater the break at the equivalence point in the titration with OH^- .

The completeness of reaction (and hence the sharpness of the equivalence point) is determined by the conditional formation constant, $\alpha_{Y^{4-}}K_f$, which is pH dependent. Because $\alpha_{Y^{4-}}$ decreases as pH is lowered, pH is an important variable determining whether a titration is feasible. The end point is more distinct at high pH. However, the pH must not be so high that metal hydroxide precipitates. The effect of pH on the titration of Ca^{2+} was shown in Figure 13-8.

The lower the pH, the less distinct the end point.

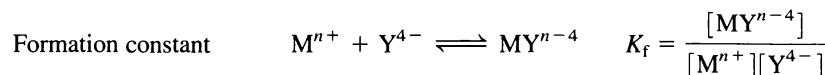
Beware

The calculation that we just did was oversimplified because we neglected any other chemistry of M^{2+} , such as formation of MOH^+ , $M(OH)_2(aq)$, $M(OH)_2(s)$, and $M(OH)_3^-$. These species decrease the concentration of available M^{2+} and decrease the sharpness of the titration curve. Mg^{2+} is normally titrated in ammonia buffer at pH 10 in which $Mg(NH_3)^{2+}$ also is present. The accurate calculation of metal-EDTA titration curves requires full knowledge of the chemistry of the metal with water and any other ligands present in the solution.

Ask Yourself

13-F. Find pCa^{2+} ($= -\log[Ca^{2+}]$) in the titration in Figure 13-9 at $V_{EDTA} = 5.00$, 50.00, and 51.00 mL. See that your answers agree with Figure 13-9.

Key Equations



Fraction of EDTA as Y^{4-}

$$\alpha_{\text{Y}^{4-}} = \frac{[\text{Y}^{4-}]}{[\text{EDTA}]}$$

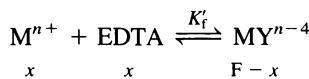
$[\text{EDTA}]$ = total concentration of EDTA not bound to metal

Conditional formation constant

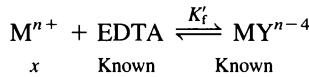
$$K'_f = \alpha_{\text{Y}^{4-}} K_f = \frac{[\text{MY}^{n-4}]}{[\text{M}^{n+}][\text{EDTA}]}$$

Titration calculations Before V_e , there is a known excess of M^{n+}
 $\text{pM} = -\log[\text{M}]$

At V_e , some M^{n+} is generated by the dissociation of MY^{n-4}



After V_e , $[\text{EDTA}]$ and $[\text{MY}^{n-4}]$ are known



Important Terms

auxiliary complexing agent	direct titration	masking agent
back titration	displacement titration	metal ion indicator
blocking	formation constant	monodentate ligand
chelating ligand	indirect titration	multidentate ligand
complexometric titration	Lewis acid	overall formation constant
conditional formation constant	Lewis base	stability constant
cumulative formation constant	ligand	stepwise formation constant

Problems

13-1. How many milliliters of 0.050 0 M EDTA are required to react with 50.0 mL of (a) 0.010 0 M Ca^{2+} or (b) 0.010 0 M Al^{3+} ?

13-2. Give three circumstances in which an EDTA back titration might be necessary.

13-3. Describe what is done in a displacement titration and give an example.

13-4. Give an example of the use of a masking agent.

13-5. What is meant by water hardness? Explain the difference between temporary and permanent hardness.

13-6. State the purpose of an auxiliary complexing agent and give an example of its use.

13-7. Draw a reasonable structure for a complex between Fe^{3+} and nitrilotriacetic acid, $\text{N}(\text{CH}_2\text{CO}_2\text{H})_3$.

13-8. A 25.00-mL sample containing Fe^{3+} was treated with 10.00 mL of 0.036 7 M EDTA to complex all the Fe^{3+} and

leave excess EDTA in solution. The excess EDTA was then back titrated, requiring 2.37 mL of 0.046 1 M Mg^{2+} . What was the concentration of Fe^{3+} in the original solution?

13-9. A 50.0-mL solution containing Ni^{2+} and Zn^{2+} was treated with 25.0 mL of 0.045 2 M EDTA to bind all the metal. The excess unreacted EDTA required 12.4 mL of 0.012 3 M Mg^{2+} for complete reaction. An excess of the reagent 2,3-dimercapto-1-propanol was then added to displace the EDTA from zinc. Another 29.2 mL of Mg^{2+} were required for reaction with the liberated EDTA. Calculate the molarities of Ni^{2+} and Zn^{2+} in the original solution.

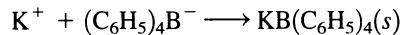
13-10. Hg^{2+} can be measured in the presence of many other metal ions by selective masking with I^- . A 100-mL unknown solution containing Hg^{2+} , Ca^{2+} , Al^{3+} , Mg^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Ba^{2+} , Cr^{3+} , Fe^{3+} , and Bi^{3+} was adjusted to pH 5.5 with acetate buffer and treated with more than enough EDTA (10.00 mL of 0.040 0 M EDTA) to bind all metal ions. Xylenol orange indicator was added, and the

excess EDTA required 5.05 mL of 0.026 2 M ZnSO₄ for back titration of EDTA. This procedure measures all metal ions in the unknown. To measure Hg²⁺, the solution at the end point of the titration was treated with 100 mg of solid KI to form HgI₄²⁻ and liberate EDTA from Hg²⁺, but not from any other metal. Liberated EDTA required an additional 5.81 mL of 0.026 2 M ZnSO₄ to reach the end point.

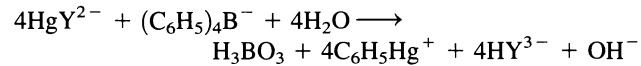
- (a) From the first titration, find the total millimoles of metal ions in the unknown.
- (b) From the second titration, find mmol Hg²⁺ in the unknown.
- (c) From Table 13-2, determine what color you would expect to observe before Zn²⁺ is added in the first titration? At the first end point? After adding excess KI? At the second end point? (The observed end point is dark orange.)

13-11. Sulfide ion was determined by indirect titration with EDTA. To a solution containing 25.00 mL of 0.043 32 M Cu(ClO₄)₂ plus 15 mL of 1 M acetate buffer (pH 4.5) were added 25.00 mL of unknown sulfide solution with vigorous stirring. The CuS precipitate was filtered and washed with hot water. Ammonia was added to the filtrate (which contains excess Cu²⁺) until the blue color of Cu(NH₃)₄²⁺ was observed. Titration of the filtrate with 0.039 27 M EDTA required 12.11 mL of EDTA to reach the end point with the indicator murexide. Find the molarity of sulfide in the unknown.

13-12. *Propagation of uncertainty.* The potassium ion in a 250.0 (± 0.1)-mL water sample was precipitated with sodium tetraphenylborate:



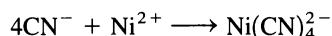
The precipitate was filtered, washed, and dissolved in an organic solvent. Treatment of the organic solution with an excess of Hg²⁺-EDTA then gave the following reaction:



The liberated EDTA was titrated with 28.73 (± 0.03) mL of 0.043 7 ($\pm 0.000 1$) M Zn²⁺. Find the concentration (and uncertainty) of K⁺ in the original sample.

13-13. A 25.00-mL sample of unknown containing Fe³⁺ and Cu²⁺ required 16.06 mL of 0.050 83 M EDTA for complete titration. A 50.00-mL sample of the unknown was treated with NH₄F to protect the Fe³⁺. Then the Cu²⁺ was reduced and masked by addition of thiourea. On addition of 25.00 mL of 0.050 83 M EDTA, the Fe³⁺ was liberated from its fluoride complex and formed an EDTA complex. The excess EDTA required 19.77 mL of 0.018 83 M Pb²⁺ to reach a xylenol orange end point. Find [Cu²⁺] in the unknown.

13-14. Cyanide recovered from the refining of gold ore can be determined indirectly by EDTA titration. A known excess of Ni²⁺ is added to the cyanide to form tetracyanonickelate(II):



When the excess Ni²⁺ is titrated with standard EDTA, Ni(CN)₄²⁻ does not react. In a cyanide analysis, 12.7 mL of cyanide solution were treated with 25.0 mL of standard solution containing excess Ni²⁺ to form tetracyanonickelate. The excess Ni²⁺ required 10.1 mL of 0.013 0 M EDTA for complete reaction. In a separate experiment, 39.3 mL of 0.013 0 M EDTA were required to react with 30.0 mL of the standard Ni²⁺ solution. Calculate the molarity of CN⁻ in the 12.7-mL sample of unknown.

13-15. A mixture of Mn²⁺, Mg²⁺, and Zn²⁺ was analyzed as follows: The 25.00-mL sample was treated with 0.25 g of NH₃OH⁺Cl⁻ (hydroxylammonium chloride, a reducing agent that maintains manganese in the +2 state), 10 mL of ammonia buffer (pH 10), and a few drops of Calmagite indicator and then diluted to 100 mL. It was warmed to 40°C and titrated with 39.98 mL of 0.045 00 M EDTA to the blue end point. Then 2.5 g of NaF were added to displace Mg²⁺ from its EDTA complex. The liberated EDTA required 10.26 mL of standard 0.020 65 M Mn²⁺ for complete titration. After this second end point was reached, 5 mL of 15 wt% aqueous KCN were added to displace Zn²⁺ from its EDTA complex. This time the liberated EDTA required 15.47 mL of standard 0.020 65 M Mn²⁺. Calculate the number of milligrams of each metal (Mn²⁺, Zn²⁺, and Mg²⁺) in the 25.00-mL sample of unknown.

13-16. The sulfur content of insoluble sulfides that do not readily dissolve in acid can be measured by oxidation with Br₂ to SO₄²⁻.⁸ Metal ions are then replaced with H⁺ by an ion-exchange column (Chapter 23), and sulfate is precipitated as BaSO₄ with a known excess of BaCl₂. The excess Ba²⁺ is then titrated with EDTA to determine how much was present. (To make the indicator end point clearer, a small, known quantity of Zn²⁺ is also added. The EDTA titrates both Ba²⁺ and Zn²⁺.) Knowing the excess Ba²⁺, we can calculate how much sulfur was in the original material. To analyze the mineral sphalerite (ZnS, FM 97.474), 5.89 mg of powdered solid were suspended in a mixture of carbon tetrachloride and water containing 1.5 mmol Br₂. After 1 h at 20° and 2 h at 50°C, the powder dissolved and the solvent and excess Br₂ were removed by heating. The residue was dissolved in 3 mL of water and passed through an ion-exchange column to replace Zn²⁺ with H⁺. Then 5.000 mL of 0.014 63 M BaCl₂ were added to precipitate all sulfate as BaSO₄. After the addition of 1.000 mL of 0.010 00 M ZnCl₂ and 3 mL of ammonia buffer, pH 10, the excess Ba²⁺ and Zn²⁺ required 2.39 mL of 0.009 63 M EDTA to reach the Calmagite end point. Find the weight percent of sulfur in the sphalerite. What is the theoretical value?

13-17. State (in words) what $\alpha_{Y^{4-}}$ means. Calculate $\alpha_{Y^{4-}}$ for EDTA at (a) pH 3.50 and (b) pH 10.50.

13-18. The cumulative formation constants for the reaction of Co^{2+} with ammonia are $\log \beta_1 = 1.99$, $\log \beta_2 = 3.50$, $\log \beta_3 = 4.43$, $\log \beta_4 = 5.07$, $\log \beta_5 = 5.13$, and $\log \beta_6 = 4.39$.

- (a) Write the chemical reaction whose equilibrium constant is β_4 .
(b) Write the reaction whose stepwise formation constant is K_4 and find its numerical value.

13-19. (a) Find the conditional formation constant for $\text{Mg}(\text{EDTA})^{2-}$ at pH 9.00.

(b) Find the concentration of free Mg^{2+} at pH 9.00 in 0.050 M $\text{Na}_2[\text{Mg}(\text{EDTA})]$.

13-20. A 100.0-mL solution of the ion M^{n+} at a concentration of 0.050 0 M buffered to pH 9.00 was titrated with 0.050 0 M EDTA.

- (a) What is the equivalence volume, V_e , in milliliters?
(b) Calculate the concentration of M^{n+} at $V = \frac{1}{2}V_e$.
(c) What fraction ($\alpha_{Y^{4-}}$) of free EDTA is in the form Y^{4-} at pH 9.00?
(d) The formation constant (K_f) is $10^{12.00}$. Calculate the value of the conditional formation constant K'_f ($= \alpha_{Y^{4-}} K_f$).
(e) Calculate the concentration of M^{n+} at $V = V_e$.
(f) What is the concentration of M^{n+} at $V = 1.100V_e$?

13-21. Consider the titration of 25.0 mL of 0.020 0 M MnSO_4 with 0.010 0 M EDTA in a solution buffered to pH 6.00. Calculate pMn^{2+} at the following volumes of added EDTA and sketch the titration curve: 0, 20.0, 40.0, 49.0, 49.9, 50.0, 50.1, 55.0, and 60.0 mL.

13-22. Using volumes from Problem 13-21, calculate pCa^{2+} for the titration of 25.00 mL of 0.020 00 M EDTA with 0.010 00 M CaSO_4 at pH 10.00 and sketch the titration curve.

13-23. Explain the analogies between the titration of a metal with EDTA and the titration of a strong acid (H^+) with a weak base (A^-). Make comparisons in all three regions of the titration curve.

13-24. Calculate pCu^{2+} at each of the following points in the titration of 25.0 mL of 0.080 0 M $\text{Cu}(\text{NO}_3)_2$ with 0.040 0 M EDTA at pH 5.00: 0, 20.0, 40.0, 49.0, 50.0, 51.0, and 55.0 mL. Sketch a graph of pCu^{2+} versus volume of titrant.

13-25. Effect of pH on titration curve. Repeat the calculations of Problem 13-24 for pH = 7.00. Sketch the two titration curves on one graph and give a chemical explanation for the difference between the two curves.

13-26. Metal ion buffers. By analogy to a hydrogen ion buffer, a metal ion buffer tends to maintain a particular metal ion concentration in solution. A mixture of the acid HA and its conjugate base A^- is a hydrogen ion buffer that maintains a pH defined by the equation $K_a = [\text{A}^-][\text{H}^+]/[\text{HA}]$. A mixture of CaY^{2-} and Y^{4-} serves as a Ca^{2+} buffer governed by the equation $1/K'_f = [\text{EDTA}][\text{Ca}^{2+}]/[\text{CaY}^{2-}]$. How many grams of $\text{Na}_2\text{H}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ (FM 372.23) should be mixed with 1.95 g of $\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ (FM 200.12) in a 500-mL volumetric flask to give a buffer with $\text{pCa}^{2+} = 9.00$ at pH 9.00?

How Would You Do It?

13-27. Consider the titration curve for Mg^{2+} in Figure 13-9. The curve was calculated under the simplifying assumption that all the magnesium not bound to EDTA is free Mg^{2+} . In fact, at pH 10 in a buffer containing 1 M NH_3 , approximately 63% of the magnesium not bound to EDTA is $\text{Mg}(\text{NH}_3)^{2+}$ and 4% is MgOH^+ . Both NH_3 and OH^- are readily displaced from Mg^{2+} by EDTA in the course of the titration.

(a) Sketch how the titration curve in Figure 13-9 would be different prior to the equivalence point if two-thirds of the magnesium not bound to EDTA were bound to the ligands NH_3 and OH^- .

(b) Consider a point that is 10% past the equivalence point. We know that almost all magnesium is bound to EDTA at this point. We also know that there is 10% excess unbound EDTA at this point. Is the concentration of free Mg^{2+} the same or different from what we calculated in Figure 13-9? Will the titration curve be the same or different from Figure 13-9 past the equivalence point?

13-28. A reprecipitation was employed to remove occluded nitrate from BaSO_4 precipitate prior to isotopic analysis of oxygen for geologic studies.⁹ Approximately 30 mg of BaSO_4 crystals were mixed with 15 mL of 0.05 M DTPA (Figure 13-5) in 1 M NaOH. After dissolving the solid with vigorous shaking at 70°C, it was reprecipitated by adding 10 M HCl dropwise to obtain pH 3–4 and allowing the mixture to stand for 1 h. The solid was isolated by centrifugation, removal of the mother liquor, and resuspension in deionized water. Centrifugation and washing was repeated a second time to reduce the molar ratio $\text{NO}_3^-/\text{SO}_4^{2-}$ from 0.25 in the original precipitate to 0.001 in the purified material. What will be the predominant species of sulfate and DTPA at pH 14 and pH 3? Explain why BaSO_4 dissolves in DTPA in 1 M NaOH and then reprecipitates when the pH is lowered to 3–4.

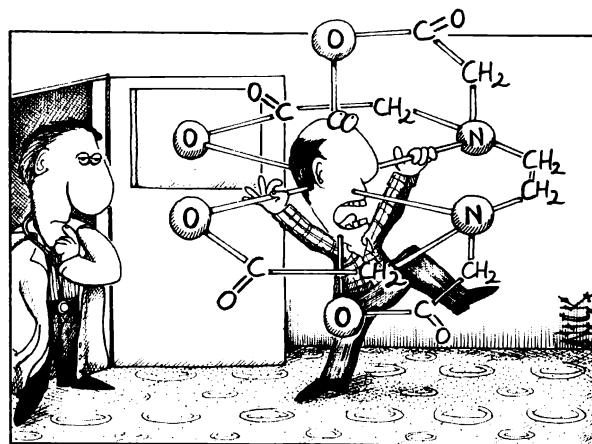
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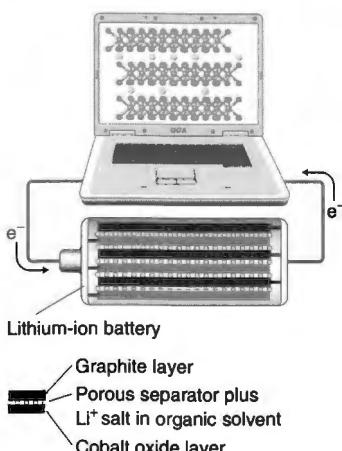
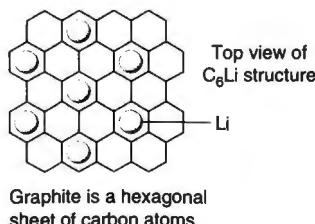
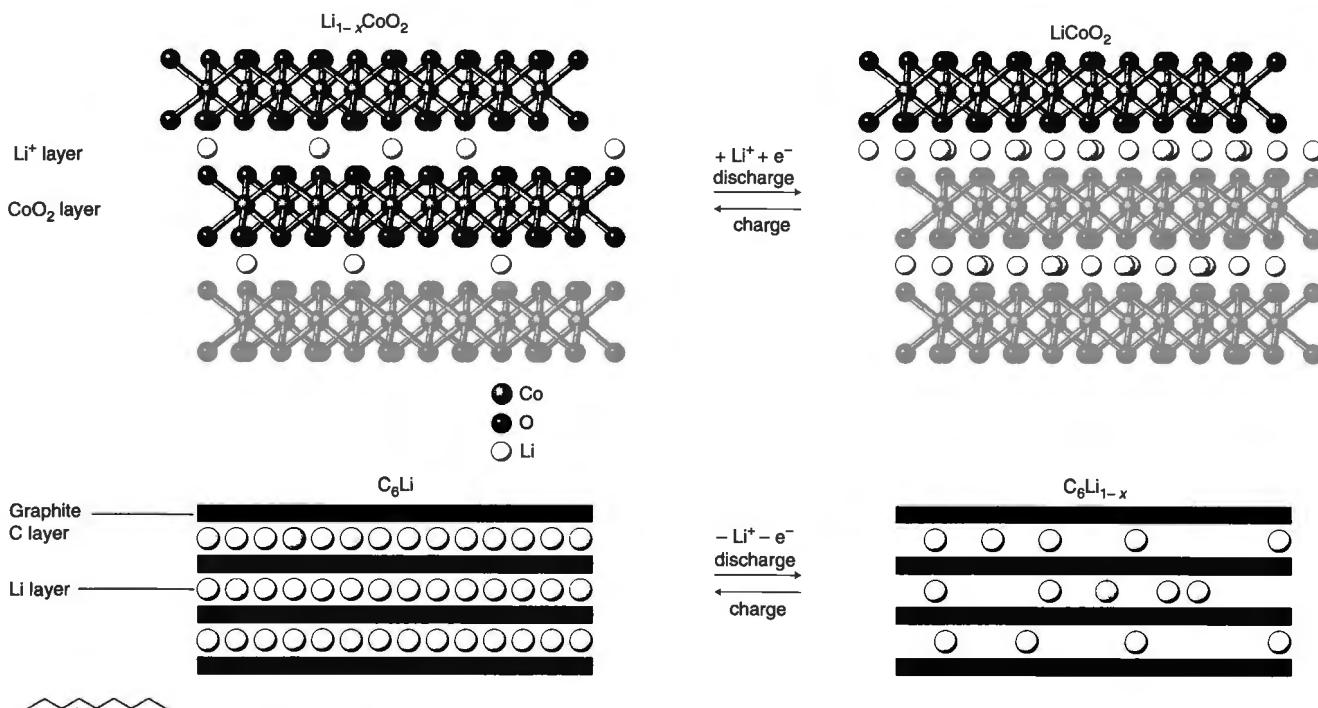
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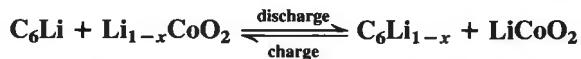
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When Chelation Therapy goes wrong.

Lithium-Ion Battery



High-capacity, rechargeable lithium-ion batteries, such as those in cell phones and laptop computers, are a shining example of the fruits of materials chemistry research. The idealized chemistry is



In C_6Li , lithium atoms reside between layers of carbon in graphite. Atoms or molecules located between layers of a structure are said to be *intercalated*. During battery discharge, lithium spontaneously migrates from graphite to cobalt oxide. Lithium atoms leave electrons behind in the graphite, and Li^+ ions become intercalated between CoO_2 layers. To go from graphite to cobalt oxide, Li^+ passes through an electrolyte consisting of a lithium salt dissolved in a high-boiling organic solvent. A porous polymer separator impregnated with electrolyte between graphite and cobalt oxide permits Li^+ ions to pass. Electrons travel through the external circuit from graphite to cobalt oxide. During recharging, an externally applied electric field drives electrons back into the graphite. Li^+ migrates from LiCoO_2 back to graphite to restore electroneutrality.

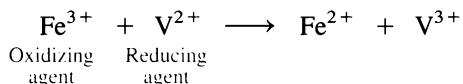
A single-cell lithium-ion battery produces ~ 3.7 volts. These batteries store twice as much energy per unit mass as the nickel-metal hydride batteries they replaced. Ongoing research is aimed at improved materials and high-area microstructures for the electrodes and the separator layer. Goals include higher energy density, longer life, and safer operation.

Electrode Potentials

Measurements of the acidity of rainwater, the fuel-air mixture in an automobile engine, and the concentrations of gases and electrolytes in your bloodstream are all made with electrochemical sensors. This chapter provides the foundation needed to discuss some common electrochemical sensors in Chapter 15.

14-1 Redox Chemistry and Electricity

In a **redox reaction**, electrons are transferred from one species to another. A molecule is said to be **oxidized** when it *loses electrons*. It is **reduced** when it *gains electrons*. An **oxidizing agent**, also called an **oxidant**, takes electrons from another substance and becomes reduced. A **reducing agent**, also called a **reductant**, gives electrons to another substance and is oxidized in the process. In the reaction



Fe^{3+} is the oxidizing agent because it takes an electron from V^{2+} . V^{2+} is the reducing agent because it gives an electron to Fe^{3+} . As the reaction proceeds from left to right, Fe^{3+} is reduced (its oxidation state goes down from +3 to +2), and V^{2+} is oxidized (its oxidation state goes up from +2 to +3). Appendix D discusses oxidation numbers and how to balance redox equations. You should be able to write a balanced redox reaction as the sum of two *half-reactions*, one an oxidation and the other a reduction.

Chemistry and Electricity

Electric charge (q) is measured in **coulombs** (C). The magnitude of the charge of a single electron (or proton) is 1.602×10^{-19} C. A mole of electrons therefore has a charge of $(1.602 \times 10^{-19}$ C)(6.022×10^{23} /mol) = 9.649×10^4 C/mol, which is called the **Faraday constant**, F . For N moles of a species with n charges per molecule, the moles of charge are nN . For example, for Fe^{3+} , $n = 3$ because each ion carries three units of charge. The electric charge in coulombs is

$$\begin{array}{cccccc} \text{Relation between} & q & = & n & \cdot & N & \cdot & F \\ \text{charge and moles.} & \text{Coulombs} & & \text{Unit charges} & & \text{Moles} & & \frac{\text{Coulombs}}{\text{Mole}} \end{array} \quad (14-1)$$

Oxidation: loss of electrons
Reduction: gain of electrons

Oxidizing agent: takes electrons
Reducing agent: gives electrons

Michael Faraday (1791–1867) was a self-educated English “natural philosopher” (the old term for “scientist”) who discovered that the extent of an electrochemical reaction is proportional to the electric charge passing through a cell. Faraday discovered many fundamental laws of electromagnetism. He gave us the electric motor, electric generator, and electric transformer, as well as the terms *ion*, *cation*, *anion*, *electrode*, *cathode*, *anode*, and *electrolyte*. His gift for lecturing is best remembered from his Christmas lecture demonstrations for children at the Royal Institution. Faraday “took great delight in talking to [children], and easily won their confidence. . . . They felt as if he belonged to them; and indeed he sometimes, in his joyous enthusiasm, appeared like an inspired child.”¹

The units work because the number of unit charges per molecule, n , is dimensionless. The electric charge on one mole of Fe^{3+} is $q = n\text{F} = (3)(1 \text{ mol})(9.649 \times 10^4 \text{ C/mol}) = 2.89 \times 10^5 \text{ C}$.

In the reaction $\text{Fe}^{3+} + \text{V}^{2+} \rightarrow \text{Fe}^{2+} + \text{V}^{3+}$, one electron is transferred to oxidize one atom of V^{2+} and to reduce one atom of Fe^{3+} . If we know how many moles of electrons are transferred from V^{2+} to Fe^{3+} , then we know how many moles of product have been formed.

Example Relating Coulombs to Quantity of Reaction

If 5.585 g of Fe^{3+} were reduced in the reaction $\text{Fe}^{3+} + \text{V}^{2+} \rightarrow \text{Fe}^{2+} + \text{V}^{3+}$, how many coulombs of charge must have been transferred from V^{2+} to Fe^{3+} ?

SOLUTION Each Fe^{3+} accepts one electron in the reaction. The moles of Fe^{3+} are $(5.585 \text{ g})/(55.845 \text{ g/mol}) = 0.100 \text{ mol Fe}^{3+}$. Equation 14-1 converts moles to coulombs of charge:

$$q = n\text{F} = (1)(0.100 \text{ mol})\left(9.649 \times 10^4 \frac{\text{C}}{\text{mol}}\right) = 9.649 \times 10^3 \text{ C}$$

 **Test Yourself** How many coulombs are released when H_2O is oxidized to liberate 1.00 mol O_2 in the reaction $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$? (**Answer:** $3.86 \times 10^5 \text{ C}$)

Electric Current Is Proportional to the Rate of a Redox Reaction

$$1 \text{ A} = 1 \text{ C/s}$$

$$1 \text{ ampere} = 1 \frac{\text{coulomb}}{\text{second}}$$

Electric **current** (I) is the quantity of charge flowing each second past a point in an electric circuit. The unit of current is the **ampere** (A), which is a flow of one coulomb per second.

Consider Figure 14-1, in which a Pt wire is dipped into a solution containing Sn^{4+} ions. The wire conducts electrons to allow the reduction of Sn^{4+} to Sn^{2+} :

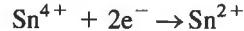


Figure 14-1 Electrons flowing into a coil of Pt wire at which Sn^{4+} ions in solution are reduced to Sn^{2+} ions. This process could not happen by itself. If Sn^{4+} is reduced at this Pt electrode, some other species must be oxidized at some other place.

The Pt wire is an **electrode**—a device to conduct electrons into or out of the chemicals involved in the redox reaction. Platinum is an *inert* electrode; it does not participate in the reaction except as a conductor of electrons. We call a molecule that can donate or accept electrons at an electrode an **electroactive species**. The rate at which electrons flow into the electrode is a measure of the rate of reduction of Sn^{4+} .

Example Relating Current to the Rate of Reaction

Suppose that Sn^{4+} is reduced to Sn^{2+} at a constant rate of 4.24 mmol/h in Figure 14-1. How much current flows into the solution?

SOLUTION Two electrons are required to reduce *one* Sn^{4+} ion to Sn^{2+} . If Sn^{4+} is reacting at a rate of 4.24 mmol/h, electrons flow at a rate of $2(4.24) = 8.48$ mmol/h, which corresponds to

$$\frac{8.48 \text{ mmol/h}}{3600 \text{ s/h}} = 2.356 \times 10^{-3} \text{ mmol/s} = 2.356 \times 10^{-6} \text{ mol/s}$$

To find the current, we use the Faraday constant to convert moles of electrons per second into coulombs per second:

$$\begin{aligned}\text{current} &= \frac{\text{coulombs}}{\text{second}} = \frac{\text{moles}}{\text{second}} \cdot \frac{\text{coulombs}}{\text{mole}} \\ &= \left(2.356 \times 10^{-6} \frac{\text{mol}}{\text{s}}\right) \left(9.649 \times 10^4 \frac{\text{C}}{\text{mol}}\right) = 0.227 \text{ C/s} = 0.227 \text{ A}\end{aligned}$$

 **Test Yourself** What current is required to oxidize water at an electrode to liberate 1.00 mol O₂/day in the reaction 2H₂O → O₂ + 4H⁺ + 4e⁻? (Answer: 4.47 A)

Voltage and Electrical Work

Electrons are attracted to positively charged regions and repelled from negatively charged regions. If electrons are attracted from one point to another, they can do useful work along the way. To force electrons into a region from which they are repelled, we must do work on the electrons to push them along. *Work* has the dimensions of energy, whose units are *joules* (J).

The difference in **electric potential** between two points measures the work that can be done (or is needed) when electrons move from one point to another. The greater the potential difference between points A and B, the more work can be done (or must be done) when electrons travel from point A to point B. Potential difference is measured in **volts** (V).

A good analogy for understanding current and potential is to think of water flowing through a garden hose (Figure 14-2). Electric current is the quantity of electric charge flowing per second past a point in the wire. Electric current is analogous to the volume of water flowing per second past a point in the hose. Electric potential difference is a measure of the force pushing on the electrons. The greater the force, the more current flows. Electric potential difference is analogous to the pressure on the water in the hose. The greater the pressure, the faster the water flows.

When a charge, *q*, moves through a potential difference, *E*, the work done is

$$\begin{array}{llll} \text{Relation between} & \text{work} & = & E \cdot q \\ \text{work and voltage:} & \text{Joules} & \text{Volts} & \text{Coulombs} \end{array} \quad (14-2)$$

One joule of energy is gained or lost when one coulomb of charge moves through a potential difference of one volt. Equation 14-2 tells us that the dimensions of volts are J/C.

Here is the garden hose analogy for work: Suppose that one end of a hose is raised 1 m above the other end and 1 L of water flows through the hose. The water could pass through a mechanical device like a paddle wheel to do a certain amount of work. If one end of the hose is raised 2 m above the other, the amount of work that can be done by the same volume of water is twice as great. The elevation difference between the ends of the hose is analogous to electric potential difference and the volume of water is analogous to electric charge. The greater the electric potential difference between two points in a circuit, the more work can be done by the charge flowing between those two points.

Example Electrical Work

How much work can be done when 2.36 mmol of electrons move “downhill” through a potential difference of 1.05 V?

It costs energy to move like charges toward each other. Energy is released when opposite charges move toward each other.

$$1 \text{ volt} = 1 \text{ J/C}$$

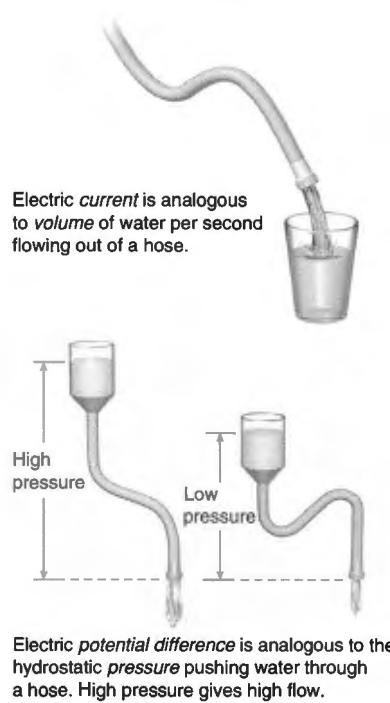


Figure 14-2 Analogy between the flow of water through a hose and the flow of electricity through a wire.

SOLUTION To use Equation 14-2, we need to find the coulombs of charge in 2.36 mmol of electrons. Each electron has $n = 1$ unit of charge, so Equation 14-1 gives:

$$q = nNF = (1)(2.36 \times 10^{-3} \text{ mol})(9.649 \times 10^4 \text{ C/mol}) = 2.27 \times 10^2 \text{ C}$$

The work that can be done by the moving electrons is

$$\text{work} = E \cdot q = (1.05 \text{ V})(2.27 \times 10^2 \text{ C}) = 239 \text{ J}$$

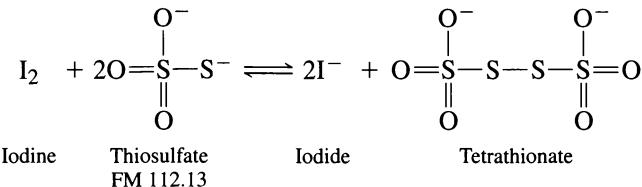
 **Test Yourself** An electric current of 1.5 mA does 0.25 J of work in 1.00 min. How much voltage is driving the electrons? (**Answer:** 2.78 V)

Electrolytic production of aluminum consumes 4.5% of the electrical output of the United States! Al^{3+} in a molten solution of Al_2O_3 and cryolite (Na_3AlF_6) is reduced to aluminum metal at the cathode of a cell that typically draws 250 000 A. This process was invented by Charles Hall in 1886 when he was 22 years old, just after graduating from Oberlin College.

Electrolysis is a chemical reaction in which we apply a voltage to drive a redox reaction that is not spontaneous (not energetically favorable) and would not otherwise occur. For example, electrolysis is used to make aluminum metal from Al^{3+} and to make chlorine gas (Cl_2) from Cl^- in seawater. Demonstration 14-1 illustrates electrolysis.

Ask Yourself

14-A. Consider the redox reaction

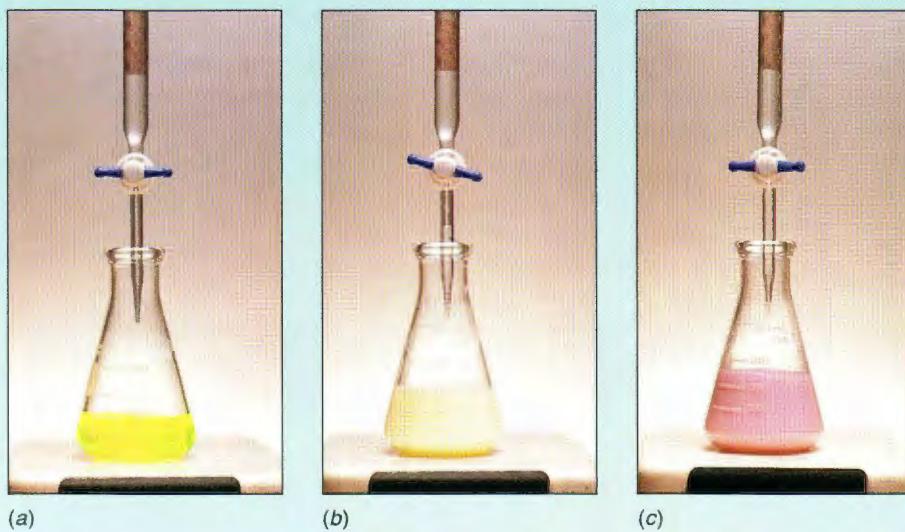


- (a) Identify the oxidizing agent on the left side of the reaction and write its balanced half-reaction.
- (b) Identify the reducing agent on the left side of the reaction and write its balanced half-reaction.
- (c) How many electrons (n) are transferred by each thiosulfate ion?
- (d) How many coulombs of charge are passed from reductant to oxidant when 1.00 g of thiosulfate reacts?
- (e) If the rate of reaction is 1.00 g of thiosulfate consumed per minute, what current (in amperes) flows from reductant to oxidant?
- (f) If the charge flows “downhill” through a potential difference of 0.200 V, how much work (in joules) can be done by the electric current?

14-2 Galvanic Cells

In a *spontaneous reaction*, it is energetically favorable for reactants to be converted into products. Energy released from the chemicals could be available as electrical energy.

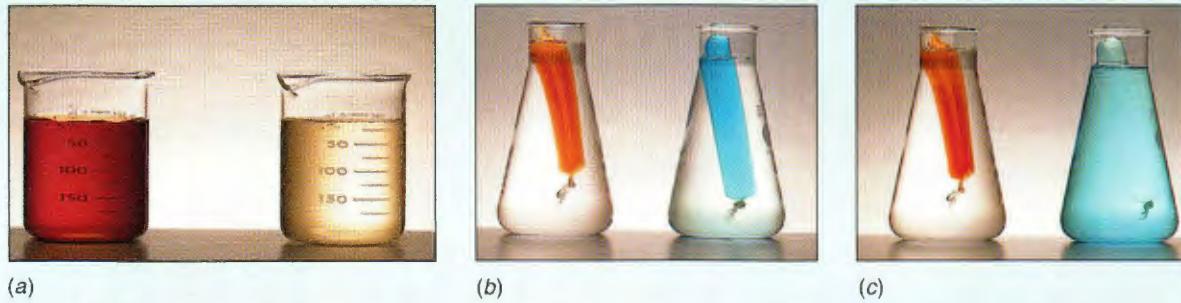
In a **galvanic cell**, a *spontaneous* chemical reaction generates electricity. To accomplish this, one reagent must be oxidized and another must be reduced. The two cannot be in contact, or electrons would flow directly from the reducing agent to the oxidizing agent without going through the external circuit. Therefore, the oxidizing and reducing agents are physically separated, and electrons flow through a wire to get from one reactant to the other.



(a) (b) (c)

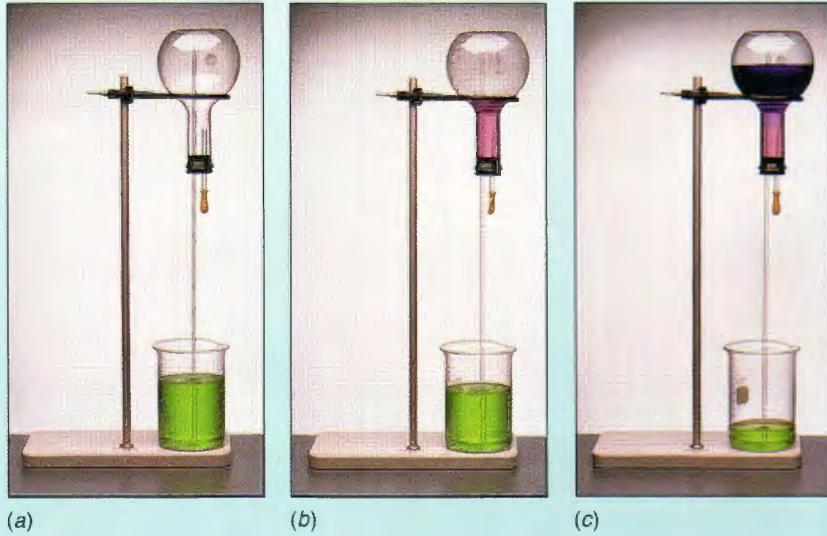
**COLOR PLATE 1 Fajans
Titration of Cl^- with AgNO_3 ,
Using Dichlorofluorescein
(Demonstration 6-1)**

(a) Indicator before beginning titration. (b) AgCl precipitate before end point. (c) Indicator adsorbed on precipitate after end point.

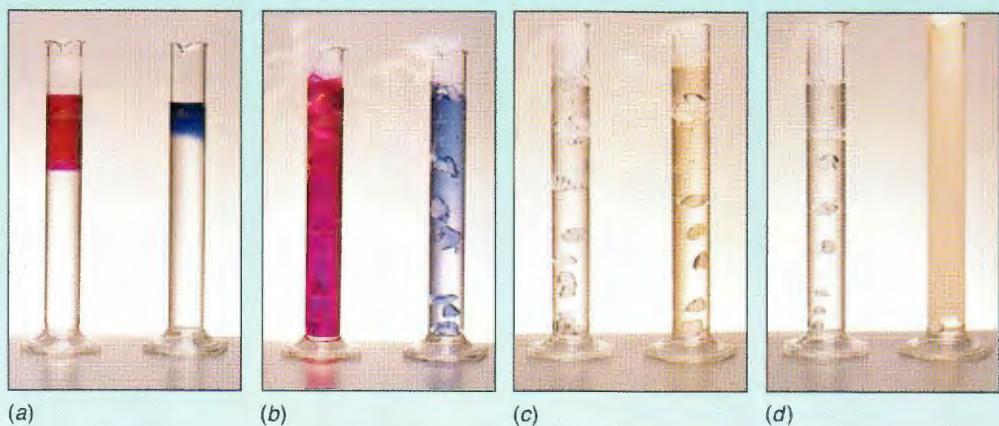


(a) (b) (c)

COLOR PLATE 2 Colloids and Dialysis (Demonstration 7-1) (a) Ordinary aqueous Fe(III) (right) and colloidal Fe(III) (left). (b) Dialysis bags containing colloidal Fe(III) (left) and a solution of Cu(II) (right) immediately after placement in flasks of water. (c) After 24 h of dialysis, the Cu(II) has diffused out and is dispersed uniformly between the bag and the flask, but the colloidal Fe(III) remains inside the bag.



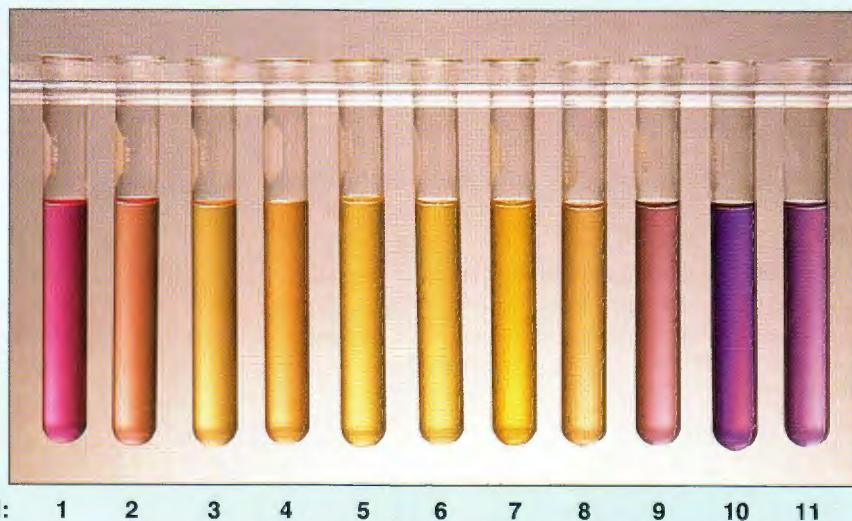
**COLOR PLATE 3 HCl Fountain
(Demonstration 8-1)** (a) Basic indicator solution in beaker.
(b) Indicator is drawn into flask and changes to acidic color. (c) Solution levels at end of experiment.



COLOR PLATE 4 Indicators and Acidity of CO₂
(Demonstration 9-2) (a) Cylinder before adding Dry Ice. Ethanol indicator solutions of phenolphthalein (*left*) and bromothymol blue (*right*) have not yet mixed with entire cylinder. (b) Adding Dry Ice causes bubbling and mixing. (c) Phenolphthalein changes to its colorless acidic form.

Color of bromothymol blue is due to mixture of acidic and basic forms. (d) After addition of HCl and stirring of right-hand cylinder, bubbles of CO₂ can be seen leaving solution, and indicator changes completely to its acidic yellow color.

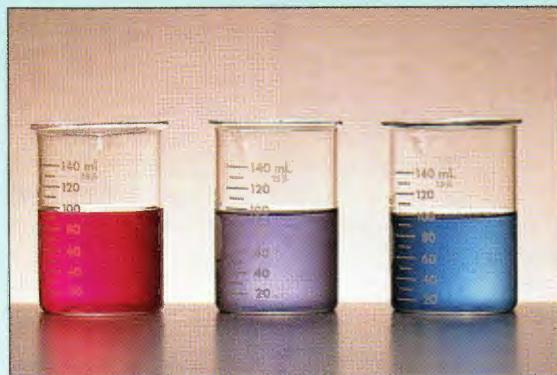
COLOR PLATE 5 Thymol Blue
(Section 9-6) Acid-base indicator thymol blue between pH 1 (*left*) and 11 (*right*). The pK values are 1.7 and 8.9.



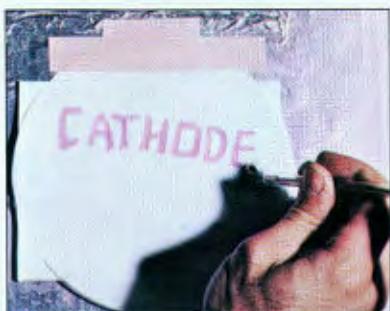
COLOR PLATE 6 Effect of Ionic Strength on Ionic Dissociation (Demonstration 12-1)
(b) Two beakers containing identical solutions with FeSCN²⁺, Fe³⁺ and SCN⁻. (b) Color change when KNO₃ is added to the right-hand beaker.



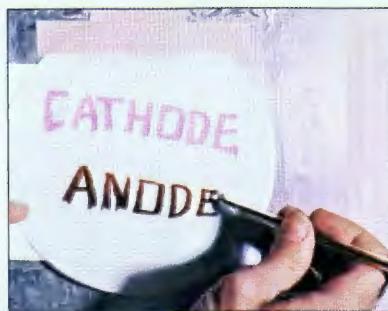
COLOR PLATE 7 Titration of Cu(II) with EDTA, Using Auxiliary Complexing Agent (Section 13-2)
Left: 0.02 M CuSO₄ before titration. Center: Color of Cu(II)-ammonia complex after addition of ammonia buffer, pH 10. Right: End-point color when all ammonia ligands have been displaced by EDTA. In this case, the auxiliary complexing agent accentuates the color change at the equivalence point.



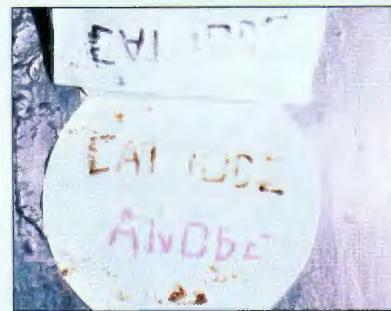
COLOR PLATE 8 Titration of Mg²⁺ by EDTA, Using Eriochrome Black T Indicator (Demonstration 13-1) Before (left), near (center), and after (right) equivalence point.



(a)

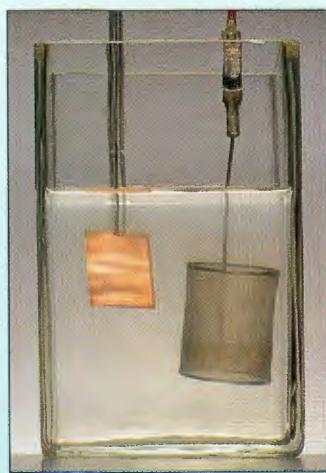


(b)

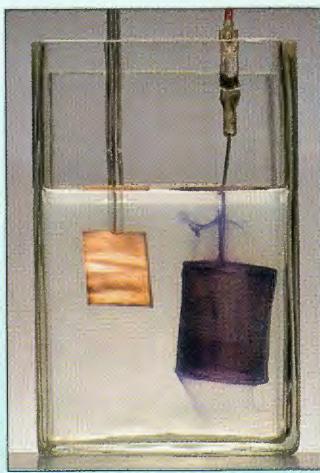


(c)

COLOR PLATE 9 Electrochemical Writing (Demonstration 14-1) (a) Stylus used as cathode. (b) Stylus used as anode. (c) Foil backing has a polarity opposite that of the stylus and produces reverse color on the bottom sheet of filter paper.



(a)



(b)

COLOR PLATE 10 Electrolysis of I⁻ in Solution to Make I₂ at the Anode (Demonstration 14-1)

(a) Cu electrode (flat plate, *left*) and Pt electrode (mesh basket, *right*) immersed in solution containing KI and starch, with no electric current.
(b) Starch-iodine complex forms at surface of Pt anode when current flows.

**COLOR PLATE 11 Photolytic Environmental Carbon Analyzer**

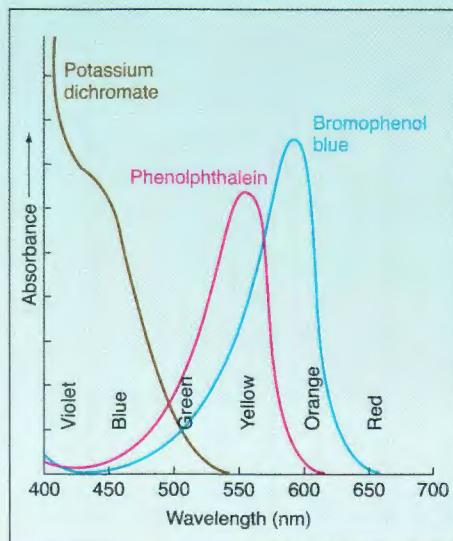
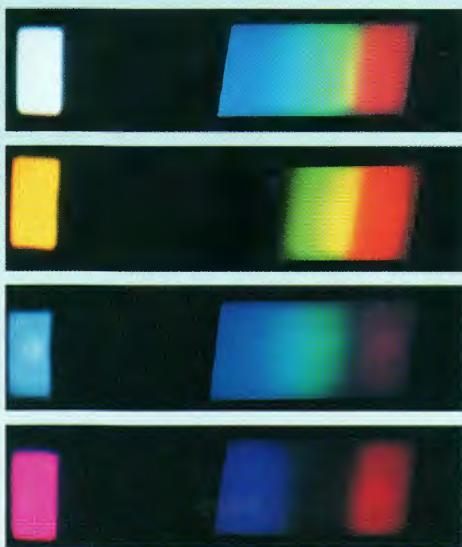
(Box 16-1) A measured water sample is injected into the chamber at the left, where it is acidified with H₃PO₄ and sparged (bubbled with Ar or N₂) to remove CO₂ derived from HCO₃⁻ and CO₃²⁻. The CO₂ is measured by its infrared absorbance. The sample is then forced into the digestion chamber, where S₂O₈²⁻ is added and the sample is exposed to ultraviolet radiation from an immersion lamp (the coil at the center of the photo). Sulfate radicals (SO₄²⁻) formed by irradiation oxidize most organic compounds to CO₂, which is measured by infrared absorbance. The U-tube at the right contains Sn and Cu granules to scavenge volatile acids such as HCl and HBr liberated in the digestion. [Courtesy Ed Urbansky, U.S. Environmental Protection Agency, Cincinnati, OH.]

**COLOR PLATE 12 Iodometric Titration (Section 16-3)** *Left:* Initial I₃⁻ solution.

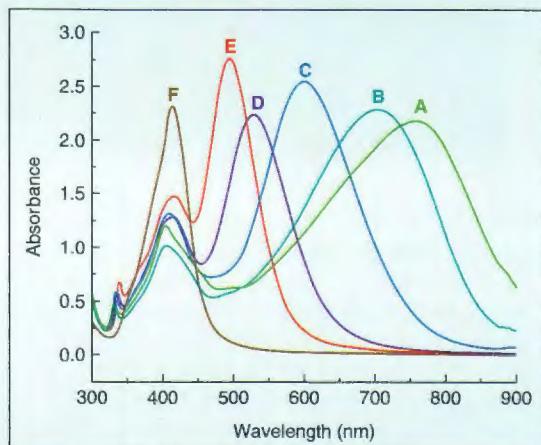
Left center: I₃⁻ solution before end point in titration with S₂O₈²⁻. *Right center:* I₃⁻ solution immediately before end point, with starch indicator present. *Right:* At the end point.

**COLOR PLATE 13 Fe(phenanthroline)₃²⁺ Standards for Spectrophotometric Analysis**

(Section 18-2) Volumetric flasks containing Fe(phenanthroline)₃²⁺ solutions with iron concentrations ranging from 1 mg/L (left) to 10 mg/L (right).

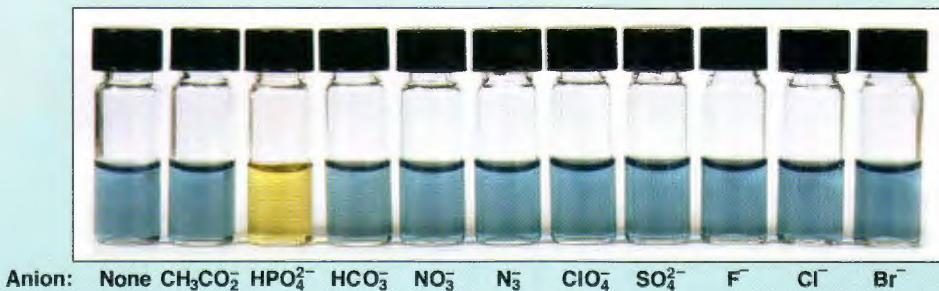


COLOR PLATE 14
Absorption Spectra
(Demonstration 18-1)
(a) Projected visible spectra of (from top to bottom) white light, potassium dichromate, bromophenol blue, and phenolphthalein.
(b) Absorption spectra recorded with a spectrophotometer.



COLOR PLATE 15 Absorption Spectra and Color

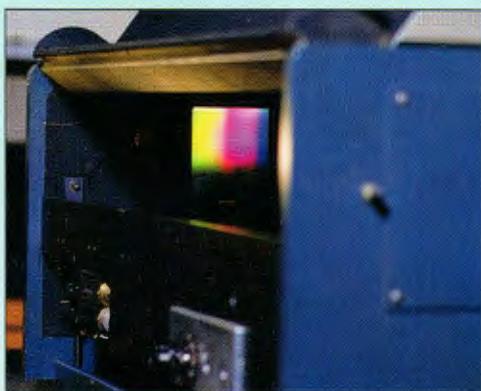
(Ask Yourself 18-B) (a) Flasks contain suspensions of silver nanoparticles whose color depends on the size and shape of the particles, which are approximately triangular plates with edge lengths of ~50–100 nm. (b) The visible absorption spectrum of each suspension is shown in the graph. Stable suspensions of nanoparticles are called *colloids* (Demonstration 7-1).
[From D. M. Ledwith, A. M. Whelan, and J. M. Kelly, *J. Mater. Chem.* **2007**, *17*, 2459. Courtesy J. M. Kelly and D. Ledwith, Trinity College, University of Dublin.]



COLOR PLATE 16 Colorimetric Reagent for Phosphate

(Box 18-2) The reagent in Box 18-2 was designed to turn yellow when phosphate is added, but not to respond to other common anions. Vials contain 50 μ M colorimetric

reagent plus 250 μ M anion. [From M. S. Han and D. H. Kim, *Angew. Chem. Int. Ed.* **2002**, *41*, 3809. Courtesy D. H. Kim, Pohang University of Science and Technology, Korea.]

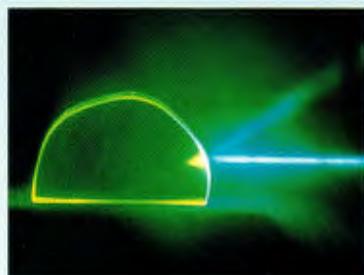


COLOR PLATE 17 Grating Dispersion
(Section 19-1) Visible spectrum produced by grating inside spectrophotometer.



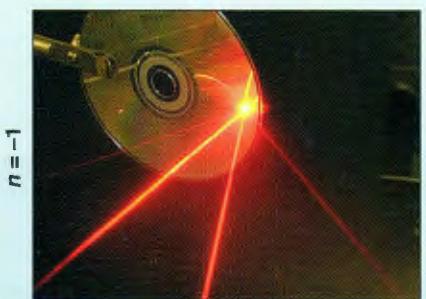
(a)

COLOR PLATE 18 Transmission, Reflection, Refraction, and Absorption of Light (Section 19-1) (a) Blue-green laser is directed into a semicircular crystal of yttrium aluminum garnet containing a small amount of Er^{3+} , which emits yellow light when it absorbs the laser light. Light entering the crystal from the right is refracted (bent) and partly reflected at the right-hand surface of the crystal. The laser beam appears yellow inside the crystal because

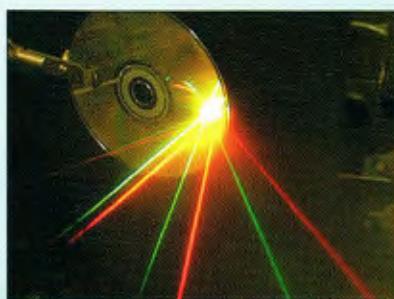


(b)

of luminescence from Er^{3+} . As it exits the crystal at the left side, the laser beam is refracted again, and partly reflected back into the crystal. (b) Same experiment, but with blue light instead of blue-green light. The blue light is absorbed by Er^{3+} and does not penetrate very far into the crystal.
 [Courtesy M. D. Seltzer, M. Johnson, and D. O'Connor, Michelson Laboratory, China Lake, CA.]



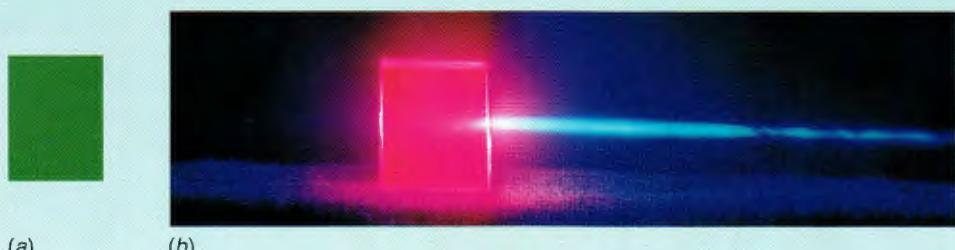
Incident beam:
 (a)



(b)

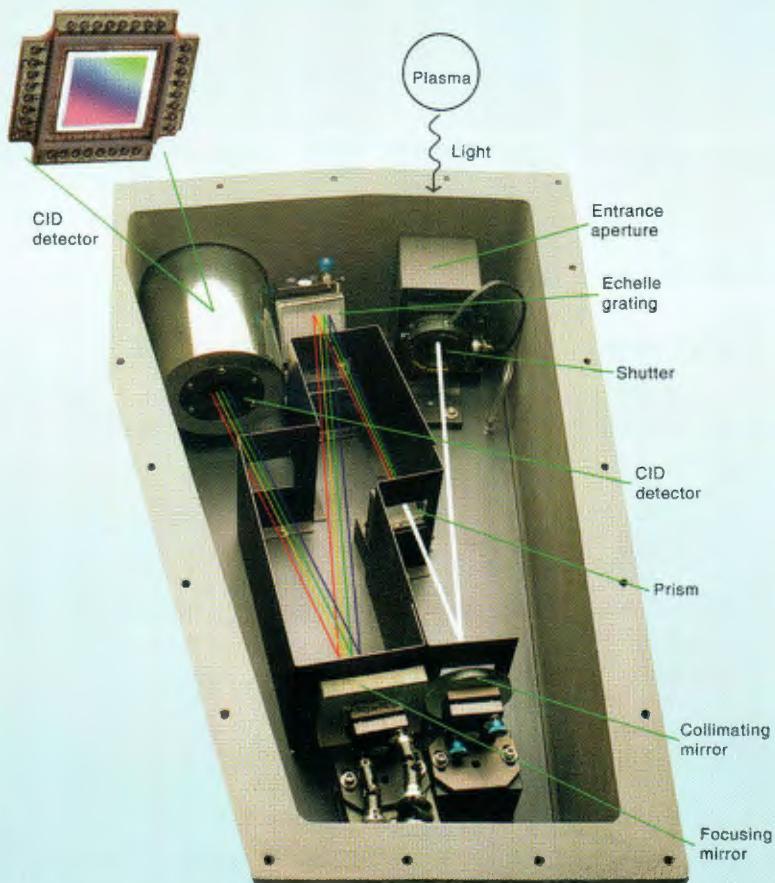
COLOR PLATE 19 Laser Diffraction from a Compact Disk (Section 19-1) The grooves in an audio compact disk or a computer compact disk have a spacing of $1.6 \mu\text{m}$. (a) When a red laser strikes disk at normal incidence ($\theta = 0$ in Figure 19-6 and Equation 19-2) three diffracted beams with orders $n = +1, +2$, and -1 are observed.

(b) Red and green lasers strike the disk at normal incidence. Green light has a shorter wavelength than red light, and so, according to Equation 19-2, green light is diffracted at smaller angle (ϕ). Beams have been made visible by "fog" from liquid nitrogen. [Courtesy J. Tellinghuisen, Vanderbilt University. See J. Tellinghuisen, *J. Chem. Ed.* **2002**, 79, 703.]

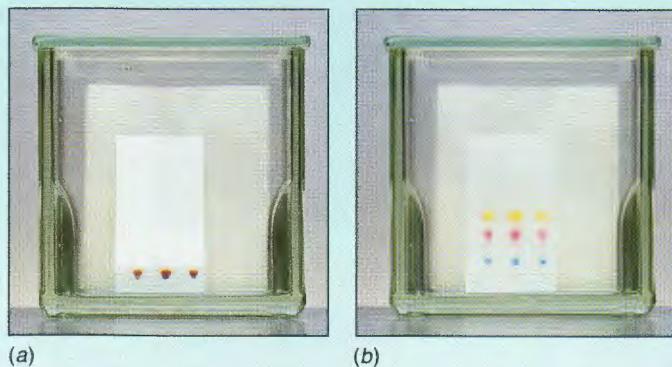


(a) (b)

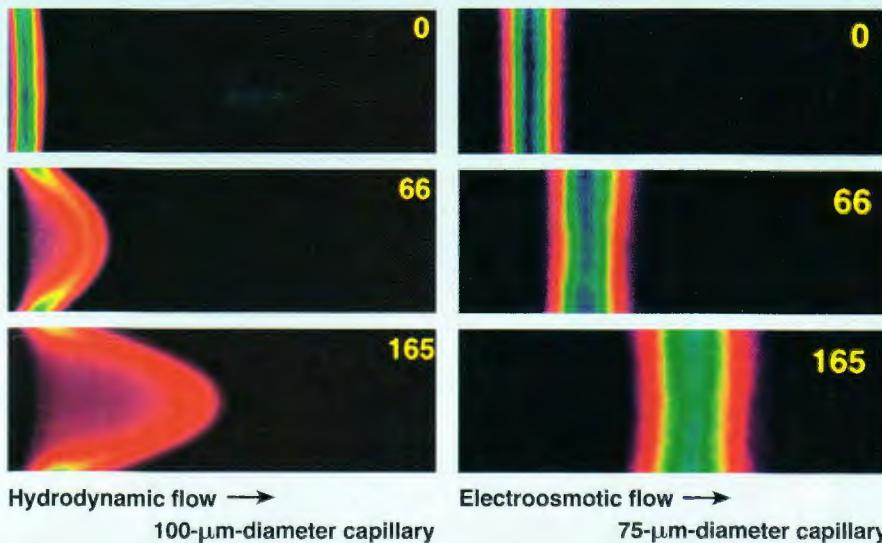
COLOR PLATE 20 Luminescence (Section 19-4) (a) Green crystal of yttrium aluminum garnet containing a small amount of Cr^{3+} . (b) When irradiated with high-intensity blue light from a laser at the right side, Cr^{3+} absorbs blue light and emits lower-energy red light. When the laser is removed, the crystal appears green again. [Courtesy M. D. Seltzer, M. Johnson, and D. O'Connor, Michelson Laboratory, China Lake, CA.]



COLOR PLATE 21 Multielement Detection in Inductively Coupled Plasma Atomic Emission Spectrometry (Section 20-4) Light emitted from the plasma enters the polychromator at the upper right and is dispersed vertically by a prism and then horizontally by a grating. The resulting two-dimensional pattern of wavelengths from 165 to 1 000 nm is detected by a charge injection device (CID), which is somewhat like the detector in a digital camera. Different emission wavelengths strike at different pixels of the detector. [Courtesy TJA Solutions, Franklin, MA.]



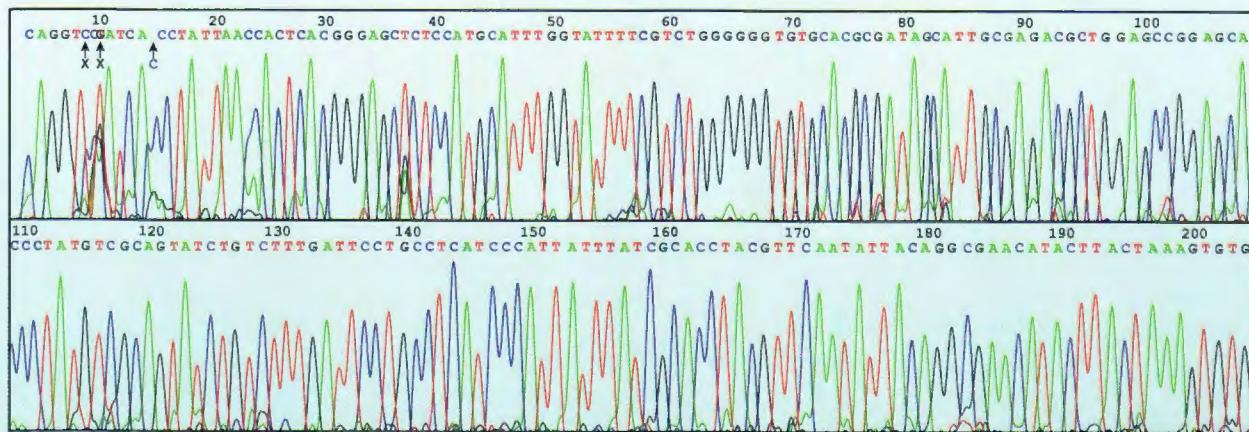
COLOR PLATE 22 Thin-Layer Chromatography
(Section 21-1) (a) Solvent ascends past mixture of dyes near bottom of flat plate coated with solid adsorbent. (b) Separation achieved after solvent has ascended most of the way up the plate.



COLOR PLATE 23 Velocity Profiles for Hydrodynamic and Electroosmotic Flow (Section 23-6) A fluorescent dye was imaged inside a capillary tube at times of 0, 66, and 165 ms after initiating flow. The highest concentration of dye is represented by blue and the lowest concentration by red in these images in which different colors are assigned to different fluorescence intensities.

[From P. H. Paul, M. G. Garguilo, and D. J. Rakestraw, *Anal. Chem.* **1998**,

[From P. H. Paul, M. G. Garguilo, and D. J. Rakestraw, *Anal. Chem.* **1998**, 70, 2459.]



COLOR PLATE 24 DNA Sequencing by Capillary Gel Electrophoresis with Fluorescence Detection
(Section 23-7) Portion of DNA nucleotide base sequence made with a “lab-on-a-chip” capable of reading a length of 365 bases with 99% accuracy. Successive peaks correspond to lengths of DNA with one more base. DNA strands terminated in each of the four different bases A, T, C, and G are tagged with a different fluorescent

label that identifies the terminal base when it passes through a fluorescence detector. Different lengths of DNA are separated by sieving through an 18-cm-long electrophoresis channel containing polyacrylamide gel with 6 M urea to stabilize single strands. Injected sample contains just 100 amol (60 million molecules) of DNA. [From R. G. Blazej, P. Kumaresan, S. A. Cronier, and R. A. Mathies, *Anal. Chem.* **2007**, *79*, 4499.]



Demonstration 14-1 Electrochemical Writing

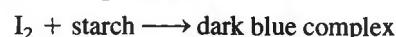
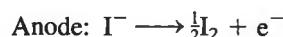
The electrolysis apparatus shown here consists of a $\sim 15 \times 15$ -cm sheet of aluminum foil taped to a wooden surface. On the metal is taped (at one edge only) a sandwich consisting of filter paper, printer paper, and another sheet of filter paper. A stylus is prepared from copper wire (18 gauge or thicker) looped at the end and passed through a length of glass tubing.

Prepare a fresh solution from 1.6 g of KI, 20 mL of water, 5 mL of 1 wt% starch solution, and 5 mL of phenolphthalein indicator solution. (If the solution darkens after standing for several days, decolorize it by adding a few drops of dilute $\text{Na}_2\text{S}_2\text{O}_3$.) Soak the three layers of paper with the KI-starch-phenolphthalein solution. Connect the stylus and foil to a ~ 12 -V DC power source and write on the paper with the stylus.

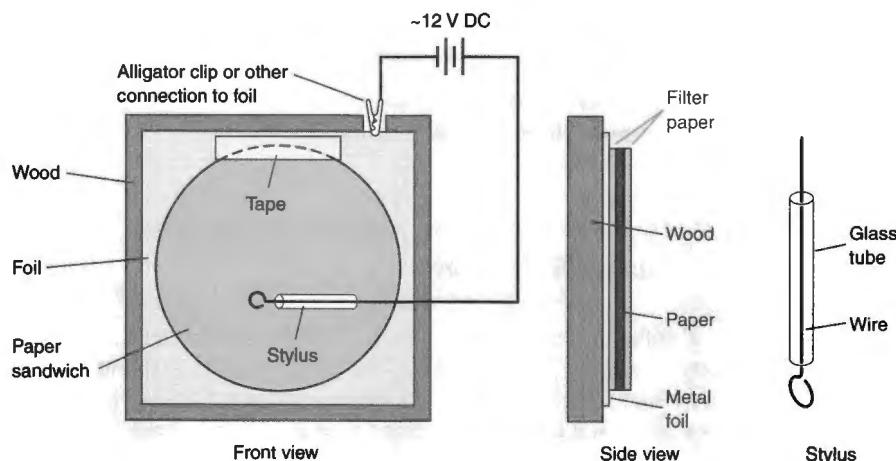
When the stylus is the cathode, pink color appears from the reaction of OH^- with phenolphthalein:



When the polarity is reversed and the stylus is the anode, a black (very dark blue) color appears from the reaction of the newly generated I_2 with starch:



Pick up the top sheet of filter paper and the printer paper, and you will discover that the writing appears in the opposite color on the bottom sheet of filter paper. This sequence is shown in Color Plate 9. Color Plate 10 shows the same oxidation reaction in a solution where you can clearly see the process in action.

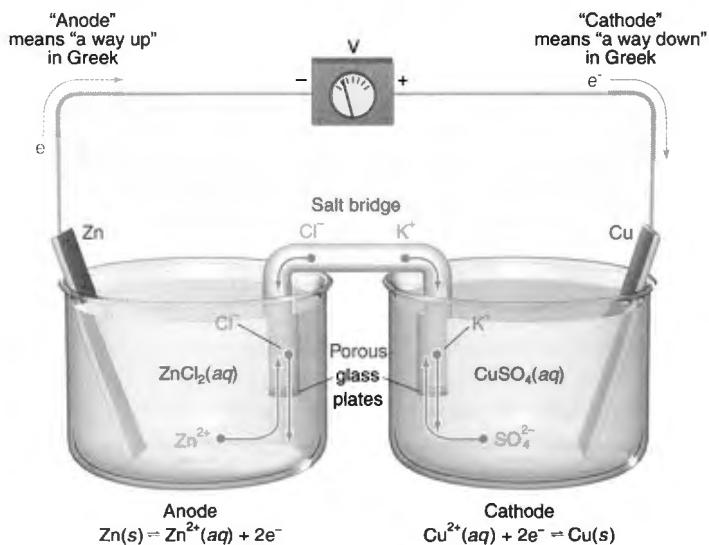


A Cell in Action

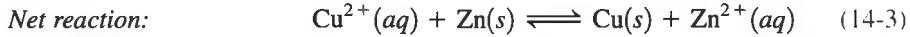
Figure 14-3 shows a galvanic cell consisting of two *half-cells connected by a salt bridge* through which ions migrate to maintain electroneutrality in each vessel.² The left half-cell has a zinc electrode dipped into aqueous ZnCl_2 . The right half-cell has a copper electrode immersed in aqueous CuSO_4 . The salt bridge is filled with a gel containing saturated aqueous KCl. The electrodes are connected by a *potentiometer* (a voltmeter that lets very little current flow) to measure the voltage difference between the two half-cells.

For more on salt bridges, see Demonstration 14-2.

Figure 14-3 A galvanic cell consisting of two half-cells and a salt bridge through which ions can diffuse to maintain electroneutrality on each side.



The spontaneous (energetically favorable) reactions taking place in this cell are



The two half-reactions are always written with equal numbers of electrons so that their sum includes no free electrons.

Demonstration 14-2 The Human Salt Bridge

A salt bridge is an ionic medium through which ions diffuse to maintain electroneutrality in each compartment of an electrochemical cell. One way to prepare a salt bridge is to heat 3 g of agar (the stuff used to grow bacteria in a Petri dish) with 30 g of KCl in 100 mL of water until a clear solution is obtained. Pour the solution into a U-tube and allow it to gel. Store the bridge in saturated aqueous KCl.

To conduct this demonstration, set up the galvanic cell in Figure 14-3 with 0.1 M ZnCl_2 on the left side and 0.1 M CuSO_4 on the right side. You can use a voltmeter or a pH meter to measure voltage. If you use a pH meter, the positive terminal is the connection for the glass electrode and the negative terminal is the reference electrode connection.

Write the two half-reactions for this cell and use the Nernst equations (14-7 and 14-8) to calculate the theoretical voltage. Measure the voltage with a conventional salt bridge. Then replace the salt bridge with one

made of filter paper freshly soaked in NaCl solution and measure the voltage again. Finally, replace the filter paper with two fingers of the same hand and measure the voltage again. Your body is really just a bag of salt housed in a *semipermeable membrane* (skin) through which ions can diffuse. Small differences in voltage observed when the salt bridge is replaced can be attributed to the junction potential discussed in Section 15-2.



Challenge One hundred eighty students at Virginia Polytechnic Institute and State University made a salt bridge by holding hands.³ (Their electric resistance was lowered by a factor of 100 by wetting everyone's hands.) Can your school beat this record?

Oxidation of $\text{Zn}(s)$ at the left side of Figure 14-3 produces $\text{Zn}^{2+}(aq)$. Electrons from Zn metal flow through the potentiometer into the Cu electrode, where $\text{Cu}^{2+}(aq)$ is reduced to $\text{Cu}(s)$. If there were no salt bridge, the left half-cell would soon build up positive charge (from excess Zn^{2+}) and the right half-cell would build up negative charge (by depletion of Cu^{2+}). In an instant, the charge buildup would oppose the otherwise energetically favorable chemical reaction and the process would cease.

To maintain electroneutrality, Zn^{2+} on the left side diffuses into the salt bridge and Cl^- from the salt bridge diffuses into the left half-cell. In the right half-cell, SO_4^{2-} diffuses into the salt bridge and K^+ diffuses out of the salt bridge. The result is that positive and negative charges in each half-cell remain exactly balanced.

We call the electrode at which *reduction* occurs the **cathode**. The **anode** is the electrode at which *oxidation* occurs. In Figure 14-3, Cu is the cathode because reduction takes place at its surface ($\text{Cu}^{2+} + 2\text{e}^- \rightarrow \text{Cu}$) and Zn is the anode because it is oxidized ($\text{Zn} \rightarrow \text{Zn}^{2+} + 2\text{e}^-$).

Electrons Move Toward More Positive Electric Potential

Being negatively charged, *electrons always move toward more positive electric potential*. In Figure 14-3, the Cu electrode is positive with respect to the Zn electrode. Therefore electrons move from Zn to Cu through the potentiometer. You will learn how to tell which electrode is more positive when we study the Nernst equation in Section 14-4.

In General, We Will Write All Half-Reactions as Reductions

In Reaction 14-3, we wrote an oxidation half-reaction for the anode and a reduction half-reaction for the cathode. I did not tell you how I knew which electrode was the anode and which was the cathode. *From now on, by convention, we will generally write all half-reactions as reductions*. We will introduce the Nernst equation to enable us to decide which electrode is positive and, therefore, in which direction electrons flow. Only then will we know where oxidation occurs and where reduction occurs.

Line Notation

We often use a notation employing two symbols to describe electrochemical cells:

| phase boundary || salt bridge

The cell in Figure 14-3 is represented by the *line diagram*

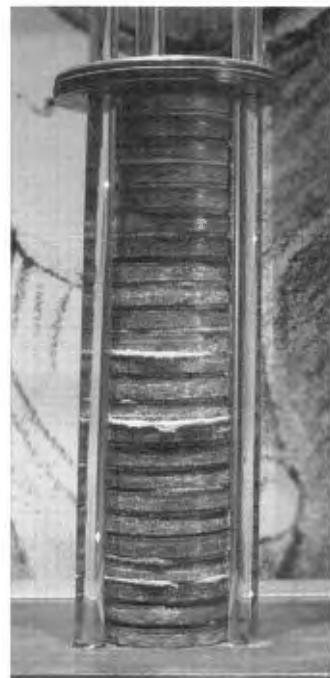


Each phase boundary is indicated by a vertical line. The electrodes are shown at the extreme left- and right-hand sides of the diagram. Only after we find the half-cell potentials will we know which electrode is the anode and which is the cathode. The contents of the salt bridge are not specified.

The symbol || for a salt bridge represents two phase boundaries. In Figure 14-3, the ZnCl_2 aqueous phase in the left half-cell is separated from the aqueous phase of the salt bridge by a porous glass plate that allows species to slowly pass through. The porous glass plate on the right side separates the aqueous phase of the salt bridge from the CuSO_4 aqueous phase in the right half-cell.

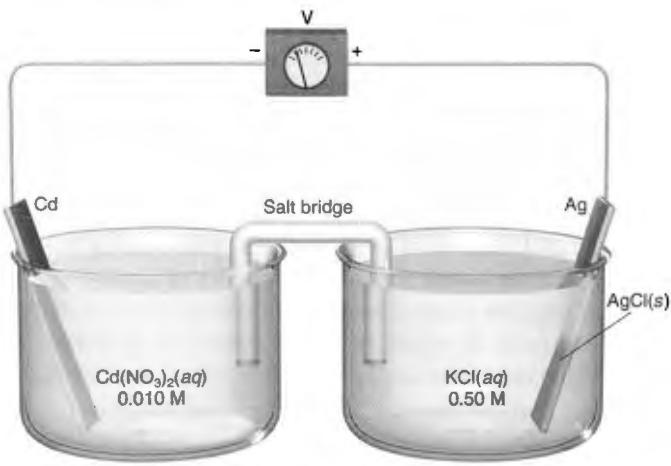
cathode \longleftrightarrow reduction
anode \longleftrightarrow oxidation

Michael Faraday wanted to describe his discoveries with terms that would “advance the general cause of science” and not “retard its progress.” He sought the aid of William Whewell in Cambridge, who coined words such as “anode” and “cathode.”⁴



The battery invented by Alessandro Volta (1745–1827) in 1799 consisted of layers of Zn and Ag separated by cardboard soaked in brine. This “voltaic pile” on display at the Royal Institution in London was given by Volta to Humphry Davy and Michael Faraday when they visited Italy in 1814. Using electrolysis, Davy was the first to isolate Na, K, Mg, Ca, Sr, and Ba. Faraday used piles to discover laws of electricity and magnetism.

Figure 14-4 Another galvanic cell.



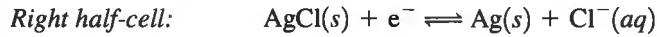
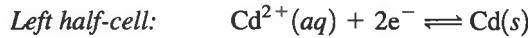
Example Interpreting Line Diagrams of Cells

Write a line diagram for the cell in Figure 14-4. Write a reduction half-reaction for each half-cell.

SOLUTION The right half-cell contains two solid phases (Ag and AgCl) and an aqueous phase. The left half-cell contains one solid phase and one aqueous phase. The line diagram is



Cd(NO₃)₂ dissociates to Cd²⁺ and NO₃⁻. KCl is dissociated to K⁺ and Cl⁻. In the left half-cell, we find cadmium in the oxidation states 0 and +2. In the right half-cell, we find silver in the 0 and +1 states. The Ag(I) is solid AgCl adhering to the Ag electrode. The electrode reactions are

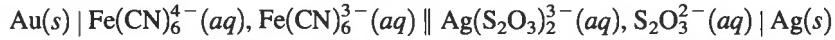


 **Test Yourself** Write a diagram for the cell in Figure 14-4 if the left half-cell is replaced by a Pt electrode dipped into a solution containing SnCl₄(aq) and SnCl₂(aq). What is the half-reaction for the left half-cell? (**Answer:** Pt(s) | SnCl₄(aq), SnCl₂(aq) || KCl(aq) | AgCl(s) | Ag(s). SnCl₄(aq) and SnCl₂(aq) are separated by a comma because they are in the same phase. Half-reaction: Sn⁴⁺(aq) + 2e⁻ \rightleftharpoons Sn²⁺(aq) or SnCl₄(aq) + 2e⁻ \rightleftharpoons SnCl₂(aq) + 2Cl⁻(aq))

 **Ask Yourself**

14-B. (a) Write the line notation for the cell in Figure 14-5.

(b) Draw a picture of the following cell and write a reduction half-reaction for each half-cell:



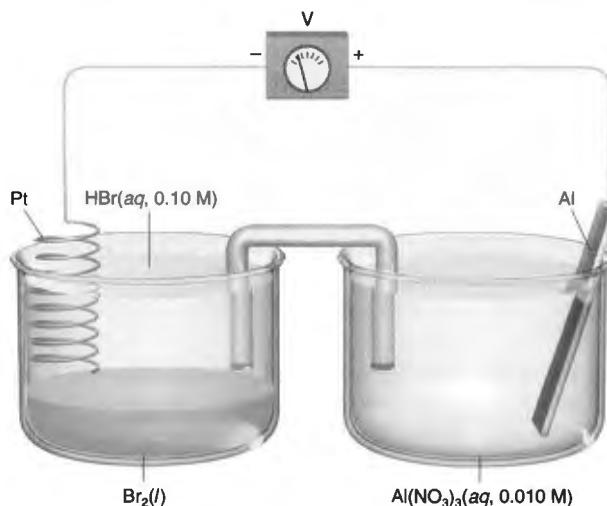


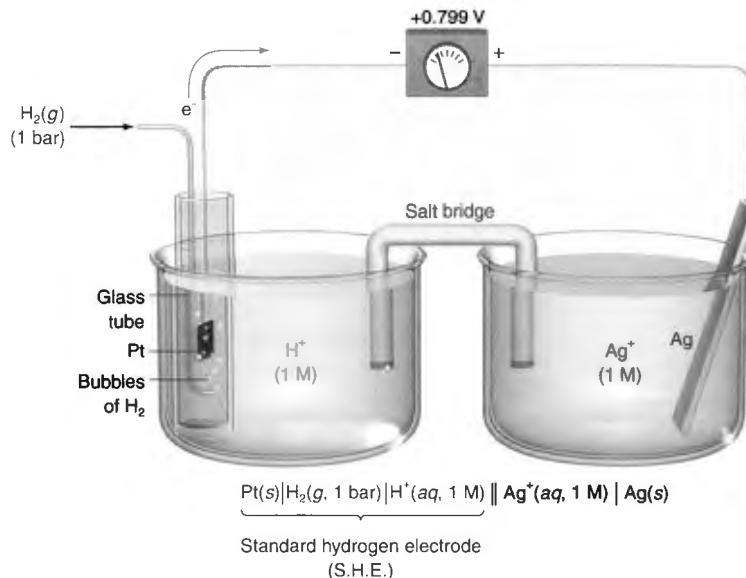
Figure 14-5 Cell for Ask Yourself 14-B.

14-3 Standard Potentials

The voltage measured in the experiment in Figure 14-3 is the difference in electric potential between the Cu electrode and the Zn electrode. The more energetically favorable the net cell reaction, the greater the voltage and the more work can be done by electrons flowing from one side to the other (Equation 14-2).

Potentiometer terminals are labeled + and -. The potentiometer displays the voltage difference $E_+ - E_-$, where E_+ is the potential of the electrode connected to the positive terminal of the meter and E_- is the potential of the electrode connected to the negative terminal. The difference can be positive or negative.

To predict the voltage when different half-cells are connected to each other, the **standard reduction potential** (E°) for each half-cell is measured by an experiment shown in idealized form in Figure 14-6. The half-reaction of interest in this diagram is

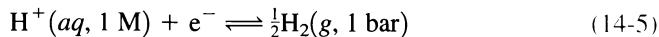


We oversimplify the standard half-cell in this text. The word *standard* really means that all *activities* are 1. Although activity (Section 12-2) is not the same as concentration, we will consider *standard* to mean that concentrations are 1 M or 1 bar for simplicity.

Figure 14-6 Setup used to measure the standard reduction potential (E°) for the half-reaction $\text{Ag}^+ + \text{e}^- \rightleftharpoons \text{Ag}(s)$. The left half-cell is called the standard hydrogen electrode (S.H.E.).

taking place in the right-hand half-cell, which is connected to the *positive* terminal of the potentiometer. The term *standard* means that species are solids or liquids or their concentrations are 1 M or their pressures are 1 bar. We call these conditions the *standard states* of the reactants and products.

The left half-cell is called the **standard hydrogen electrode (S.H.E.)**. It consists of a catalytic Pt surface in contact with an acidic solution in which $[H^+] = 1\text{ M}$. A stream of $H_2(g, 1\text{ bar})$ is bubbled past the electrode. The half-reaction of interest at the surface of the Pt electrode, *written as a reduction*, is

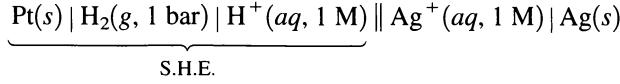


We assign a potential of 0 to the standard hydrogen electrode at all temperatures. The voltage measured in Figure 14-6 can therefore be assigned to Reaction 14-4, which occurs in the right half-cell. The measured value $E^\circ = +0.799\text{ V}$ is the *standard reduction potential* for Reaction 14-4. The sign tells us that Ag is more positive than Pt and electrons flow from Pt to Ag through the meter.

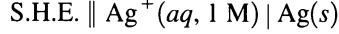
The reaction at the Pt electrode goes in the reverse direction from what is written in Reaction 14-5. The direction of the reaction is not a problem. We write a reduction for each half-reaction and only later find out in which direction electrons flow.

We can arbitrarily assign a potential to Reaction 14-5 because it serves as a reference from which we can measure other half-cell potentials. An analogy is the arbitrary assignment of 0°C to the freezing point of water. Relative to the freezing point, hexane boils at 69°C and benzene boils at 80°C . The difference between the boiling points of benzene and hexane is $80^\circ - 69^\circ = 11^\circ$. If we had assigned the freezing point of water to be 200°C instead of 0°C , we would say that hexane boils at 269°C and benzene boils at 280°C . The difference between their boiling points is still 11° . When we measure half-cell potentials relative to that of the standard hydrogen electrode, we are simply putting the potentials on a scale that allows us to measure differences. Regardless of where we set zero on the scale, differences between points remain constant.

Line notation for the cell in Figure 14-6 is

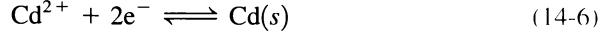


which is abbreviated

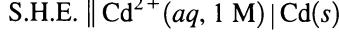


The *standard reduction potential* is the *difference* between the standard potential of the reaction of interest and the potential of S.H.E., which we have set to 0.

If we wanted to measure the standard reduction potential of the half-reaction



we would construct the cell



with cadmium connected to the positive terminal of the potentiometer. In this case, we observe a *negative* voltage of -0.402 V . The negative sign means that Cd is more

Question What is the pH of the standard hydrogen electrode? (*Answer:* 0. Be sure this makes sense to you.)

We will write all half-reactions as *reductions*. By convention, $E^\circ = 0$ for S.H.E.

Challenge Draw a picture of the cell $\text{S.H.E.} \parallel Cd^{2+}(aq, 1\text{ M}) | Cd(s)$ and show the direction of electron flow.

Table 14-1 Ordered standard reduction potentials

Oxidizing agent	Reducing agent	E° (V)
↑ Oxidizing power increases	$\text{F}_2(g) + 2\text{e}^- \rightleftharpoons 2\text{F}^-$	2.890
	$\text{O}_3(g) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{O}_2(g) + \text{H}_2\text{O}$	2.075
	$\text{MnO}_4^- + 8\text{H}^+ + 5\text{e}^- \rightleftharpoons \text{Mn}^{2+} + 4\text{H}_2\text{O}$	1.507
	$\text{Ag}^+ + \text{e}^- \rightleftharpoons \text{Ag}(s)$	0.799
	$\text{Cu}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cu}(s)$	0.339
	$2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2(g)$	0.000
	$\text{Cd}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cd}(s)$	-0.402
	$\text{K}^+ + \text{e}^- \rightleftharpoons \text{K}(s)$	-2.936
	$\text{Li}^+ + \text{e}^- \rightleftharpoons \text{Li}(s)$	-3.040
	↓ Reducing power increases	

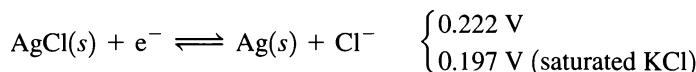
negative than Pt. Electrons flow from Cd to Pt, a direction opposite that of the cell in Figure 14-6.

Strengths of Oxidizing and Reducing Agents

Table 14-1 lists a few reduction half-reactions in order of decreasing E° value. The more positive E° , the more energetically favorable is the half-reaction. The strongest oxidizing agents are the reactants at the upper-left side of the table because they have the strongest tendency to accept electrons. $\text{F}_2(g)$ is the strongest oxidizing agent in the table. Conversely, F^- is the weakest reducing agent because it has the least tendency to give up electrons to make F_2 . The strongest reducing agents in Table 14-1 are at the lower-right side. $\text{Li}(s)$ and $\text{K}(s)$ are very strong reducing agents.

Formal Potential

Appendix C lists some standard reduction potentials. Sometimes, multiple potentials are given for one reaction, as for the $\text{AgCl}(s) \mid \text{Ag}(s)$ half-reaction:



Standard potential: 0.222 V

Formal potential for saturated KCl: 0.197 V

The value 0.222 V is the standard potential that would be measured in the cell



The value 0.197 V is measured in a cell containing saturated KCl solution instead of 1 M Cl^- :



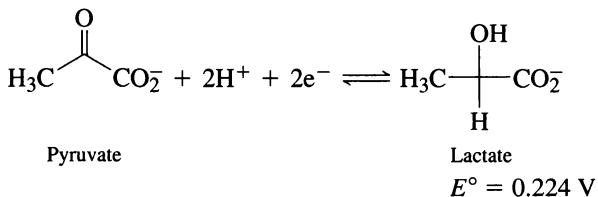
The potential for a cell containing a specified concentration of reagent other than 1 M is called the **formal potential**. Biochemists use a different formal potential, called E°' , which is described in Box 14-1.

Ask Yourself

- 14-C. Draw a line diagram and a picture of the cell used to measure the standard potential for $\text{Fe}^{3+} + \text{e}^- \rightleftharpoons \text{Fe}^{2+}$ at the surface of a Pt electrode. Use Appendix C to find the cell voltage. Show the direction of electron flow in your picture.

Box 14-1 Why Biochemists Use E°

Redox reactions are essential for life. For example, the enzyme-catalyzed reduction of pyruvate to lactate is a step in the anaerobic fermentation of sugar by bacteria:



The same reaction causes lactate to build up in your muscles and make you feel fatigued during intense exercise, when the flow of oxygen cannot keep pace with your requirement for energy.

E° applies when the concentrations of reactants and products are 1 M. For a reaction involving H^+ , E° applies when the pH is 0 (because $[\text{H}^+] = 1 \text{ M}$ and $\log 1 = 0$). Biochemists studying the energetics of fermentation or respiration are more interested in reduction potentials that apply near physiologic pH, not at pH 0. Therefore biochemists use a formal potential designated E°' , which applies at pH 7.

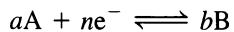
For the conversion of pyruvate into lactate, the reactant and product are carboxylic acids at pH 0 and carboxylate anions at pH 7. The value of E°' at pH 7 is -0.190 V , which is quite different from $E^\circ = +0.224 \text{ V}$ at pH 0.

14-4 The Nernst Equation

Le Chatelier's principle tells us that, if we increase reactant concentrations, we drive a reaction to the right. Increasing the product concentrations drives a reaction to the left. The net driving force for a redox reaction is expressed by the **Nernst equation**, whose two terms include the driving force under standard conditions (E° , which applies when concentrations are 1 M or 1 bar) and a term that shows the dependence on concentrations.

Nernst Equation for a Half-Reaction

For the half-reaction



the Nernst equation giving the half-cell potential, E , is

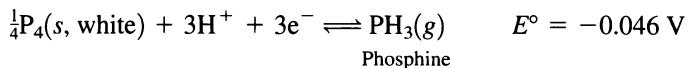
$$\text{Nernst equation: } E = E^\circ - \frac{0.05916}{n} \log\left(\frac{[\text{B}]^b}{[\text{A}]^a}\right) \quad (\text{at } 25^\circ\text{C}) \quad (14-7)$$

where E° is the standard reduction potential that applies when $[\text{A}] = [\text{B}] = 1 \text{ M}$, n is the number of electrons in the half-reaction, and a and b are stoichiometry coefficients.

The logarithmic term in the Nernst equation is the *reaction quotient*, Q ($= [\text{B}]^b / [\text{A}]^a$). Q has the same form as the equilibrium constant, but the concentrations need not be at their equilibrium values. Concentrations of solutes are expressed as moles per liter and concentrations of gases are expressed as pressures in bars. Pure solids, pure liquids, and solvents are omitted from Q . When all concentrations are 1 M and all pressures are 1 bar, $Q = 1$ and $\log Q = 0$, and the Nernst equation reduces to $E = E^\circ$.

Example Writing the Nernst Equation for a Half-Reaction

Let's write the Nernst equation for the reduction of white phosphorus to phosphine gas:



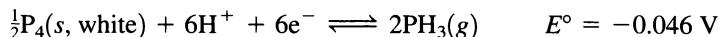
SOLUTION We omit solids from the reaction quotient, and the concentration of phosphine gas is expressed as its pressure in bars (P_{PH_3}):

$$E = -0.046 - \frac{0.059 \ 16}{3} \log \left(\frac{P_{\text{PH}_3}}{[\text{H}^+]^3} \right)$$

 **Test Yourself** Write the Nernst equation for a hydrogen electrode (Reaction 14-5). (Answer: $E = 0 - (0.059 \ 16/1) \log(P_{\text{H}_2}^{1/2}/[\text{H}^+])$)

Example Multiplication of a Half-Reaction

If you multiply a half-reaction by any factor, E° does not change. However, the factor n before the log term and the exponents in the reaction quotient do change. Write the Nernst equation for the reaction in the preceding example, multiplied by 2:



SOLUTION

$$E = -0.046 - \frac{0.059 \ 16}{6} \log \left(\frac{P_{\text{PH}_3}^2}{[\text{H}^+]^6} \right)$$

E° remains at -0.046 V . However, the factor in front of the log term and the exponents in the log term have changed.

 **Test Yourself** Write the Nernst equation for Reaction 14-5 multiplied by 2. (Answer: $E = 0 - (0.059 \ 16/2) \log(P_{\text{H}_2}/[\text{H}^+]^2)$)

Species reacting at the surface of an electrode immersed in a solution are generally dissolved in solution. We can write gas pressure in the Nernst equation because dissolved molecules are in equilibrium with the gas. Specifying P_{H_2} specifies $[\text{H}_2(\text{aq})]$ at equilibrium.

Nernst Equation for a Complete Reaction

Consider the cell in Figure 14-4, which measures the difference in potential between the Ag and Cd electrodes:

$$\text{Nernst equation for a complete cell:} \quad E = E_+ - E_- \quad (14-8)$$

where E_+ is the potential of the half-cell attached to the positive terminal of the potentiometer and E_- is the potential of the half-cell attached to the negative terminal. The potential of each half-cell (written as a reduction) is governed by the Nernst equation 14-7.

Here is a procedure for finding the voltage difference for a complete cell:

Both half-reactions are written as reductions.

Step 1. Write *reduction* half-reactions for both half-cells and find E° for each in Appendix C. Multiply the half-reactions as necessary so that they each contain the same number of electrons. When you multiply a reaction by any number, *do not* multiply E° .

Step 2. Write a Nernst equation for the right half-cell, which is attached to the positive terminal of the potentiometer. This is E_+ .

Step 3. Write a Nernst equation for the left half-cell, which is attached to the negative terminal of the potentiometer. This is E_- .

Step 4. Find the voltage difference: $E = E_+ - E_-$.

Step 5. To write a balanced net cell reaction, subtract the left half-reaction from the right half-reaction. (*This operation is equivalent to reversing the left half-reaction and adding.*)

Which way does the reaction go? *Electrons flow toward more positive potential.* If the right-hand electrode is more positive, electrons flow from left to right through the wire and reduction occurs in the right half-cell. In this case, the right-hand electrode is the cathode (Figure 14-7). If the left-hand electrode is more positive, then electrons flow from right to left through the wire and reduction occurs in the left half-cell. In this case, the left-hand electrode is the cathode.

If the cell voltage in step 5 above is positive, then we wrote the reaction in the spontaneous direction. If the cell voltage in step 5 is negative, then the reaction goes in the opposite direction from what we wrote.

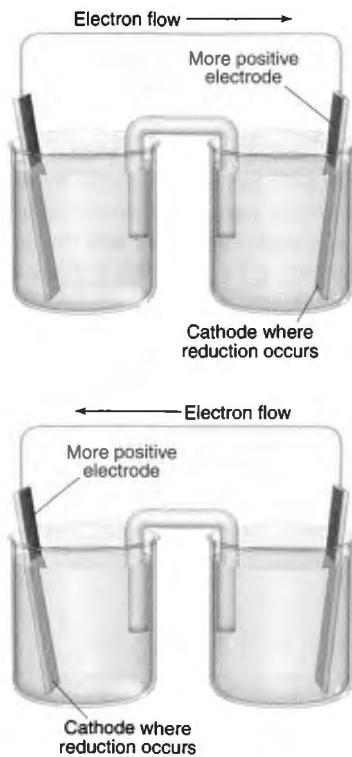


Figure 14-7 Electrons flow toward the more positive electrode. Therefore reduction occurs at the more positive electrode, which chemists call the cathode.

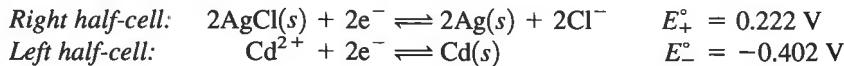
Pure solids, pure liquids, and solvents are omitted from Q .

Example Nernst Equation for a Complete Reaction

Find the voltage of the cell in Figure 14-4 if the right half-cell contains 0.50 M KCl(aq) and the left half-cell contains 0.010 M Cd(NO₃)₂(aq). Write the net cell reaction and state which direction is spontaneous.

SOLUTION

Step 1. Write reduction half-reactions:



Step 2. Nernst equation for right half-cell:

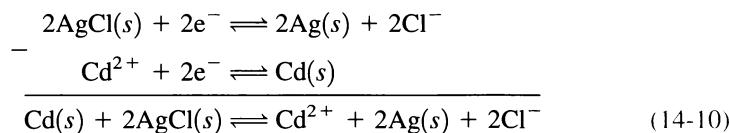
$$\begin{aligned} E_+ &= E_+^\circ - \frac{0.05916}{2} \log([Cl^-]^2) & (14-9) \\ &= 0.222 - \frac{0.05916}{2} \log([0.50]^2) = 0.240 \text{ V} \end{aligned}$$

Step 3. Nernst equation for left half-cell:

$$\begin{aligned} E_- &= E_-^\circ - \left(\frac{0.05916}{2} \right) \log \left(\frac{1}{[Cd^{2+}]} \right) = -0.402 - \frac{0.05916}{2} \log \left(\frac{1}{[0.010]} \right) \\ &= -0.461 \text{ V} \end{aligned}$$

Step 4. Cell voltage: $E = E_+ - E_- = 0.240 - (-0.461) = 0.701 \text{ V}$

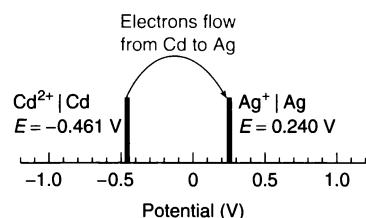
Step 5. Net cell reaction:



Subtracting a reaction is the same as reversing the reaction and adding.

The Ag electrode is more positive than the Cd electrode, so electrons flow from Cd to Ag. Reaction 14-10 is written in the spontaneous direction.

 **Test Yourself** Write the Nernst equation for the cell Pt(s) | H₂(g, 1.0 × 10⁻⁶ bar) | H⁺(aq, 0.50 M) || Ag⁺(aq, 1.0 × 10⁻¹⁰ M) | Ag(s) and find the cell voltage. In what direction will electrons flow through the circuit? (**Answer:** 0.048 V, electrons flow from Pt to Ag)



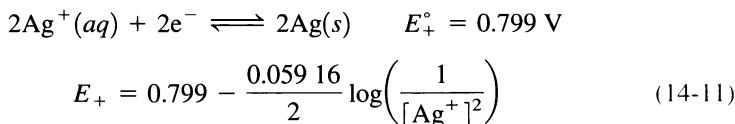
In the example above, we found that E for the silver half-cell was 0.240 V and E for the cadmium half-cell was -0.461 V. Place these values on the number line in Figure 14-8 and note that *electrons flow toward more positive potential*. Therefore electrons in the circuit flow from Cd (-0.461 V) to Ag (0.240 V). Electrons always flow from less positive to more positive in Figure 14-8, even if both half-cell potentials are positive or both are negative.

Figure 14-8 Electrons flow toward more positive potential. Electrons always flow to the right in this diagram.⁵

Different Descriptions of the Same Reaction

What if you had written Nernst equation 14-9 for a half-reaction with just one electron instead of two: $\text{AgCl}(s) + e^- \rightleftharpoons \text{Ag}(s) + \text{Cl}^-$? Try this and you will discover that the half-cell potential is unchanged. *Neither E° nor E changes when you multiply a reaction.*

We know that the silver half-cell in Figure 14-4 must contain some $\text{Ag}^+(aq)$ in equilibrium with $\text{AgCl}(s)$. Suppose that, instead of writing the reaction $2\text{AgCl}(s) + 2e^- \rightleftharpoons 2\text{Ag}(s) + 2\text{Cl}^-$, a different, less handsome, author wrote the reaction



Both descriptions of the right half-cell are valid. In both cases, Ag(I) is reduced to Ag(0).

If two cell descriptions are both valid, then they should predict the same voltage. To use Equation 14-11, you must know the concentration of Ag^+ in the right half-cell, which is not obvious. But you are clever and realize that you can find $[\text{Ag}^+]$ from the solubility product for AgCl and the concentration of Cl^- , which is 0.50 M.

$$K_{\text{sp}} = [\text{Ag}^+][\text{Cl}^-] \implies [\text{Ag}^+] = \frac{K_{\text{sp}}}{[\text{Cl}^-]} = \frac{1.8 \times 10^{-10}}{0.50} = 3.6 \times 10^{-10} \text{ M}$$

Putting this concentration into Equation 14-11 gives

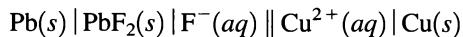
$$E_+ = 0.799 - \frac{0.059\ 16}{2} \log\left(\frac{1}{(3.6 \times 10^{-10})^2}\right) = 0.240\ \text{V}$$

Cell voltage is an experimental quantity that cannot depend on how we write the reaction!

which is the same potential computed in Equation 14-9! The two choices of half-reaction describe the same cell and must give the same potential.

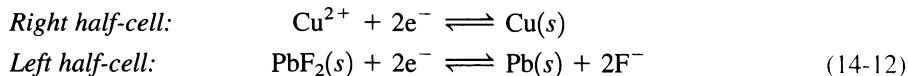
Advice for Finding Relevant Half-Reactions

When faced with a cell drawing or line diagram, the first step is to write reduction reactions for each half-cell. To do this, *look for elements in two oxidation states*. For the cell

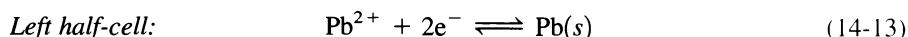


we see lead in the oxidation states 0 in $\text{Pb}(s)$ and +2 in $\text{PbF}_2(s)$, and we see copper in the oxidation states 0 in $\text{Cu}(s)$ and +2 in Cu^{2+} . Thus, the half-reactions are

Don't write a reaction such as $\text{F}_2(g) + 2\text{e}^- \rightleftharpoons 2\text{F}^-$, because $\text{F}_2(g)$ is not shown in the line diagram of the cell. $\text{F}_2(g)$ is neither a reactant nor a product.



You might have chosen to write the lead half-reaction as



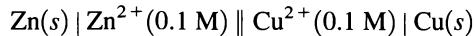
because you know that if $\text{PbF}_2(s)$ is present, there must be some Pb^{2+} in the solution. Reactions 14-12 and 14-13 are both valid descriptions of the cell, and each should predict the same cell voltage. Your choice of reaction depends on whether the F^- or Pb^{2+} concentration is more easily known to you.



Ask Yourself

14-D. (a) Arsine (AsH_3) is a poisonous gas used to make gallium arsenide for diode lasers. Find E for the half-reaction $\text{As}(s) + 3\text{H}^+ + 3\text{e}^- \rightleftharpoons \text{AsH}_3(g)$ if pH = 3.00 and $P_{\text{AsH}_3} = 0.010\ 0\text{ bar}$.

(b) The cell in Demonstration 14-2 (and Figure 14-3) can be written



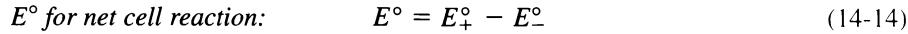
Write a reduction half-reaction for each half-cell and use the Nernst equation to predict the cell voltage. Draw a diagram like Figure 14-8 and show the direction of electron flow.

14-5 E° and the Equilibrium Constant

At equilibrium, E (not E°) = 0. E° is the potential when all reactants and products are present in their standard state (1 M, 1 bar, pure solid, pure liquid).

A galvanic cell produces electricity because the cell reaction is not at equilibrium. If the cell runs long enough, reactants are consumed and products are created until the reaction comes to equilibrium and the cell voltage, E , reaches 0. This is what happens to a battery when it runs down.⁶

If E_+° is the standard reduction potential for the right half-cell and E_-° is the standard reduction potential for the left half-cell, E° for the net cell reaction is



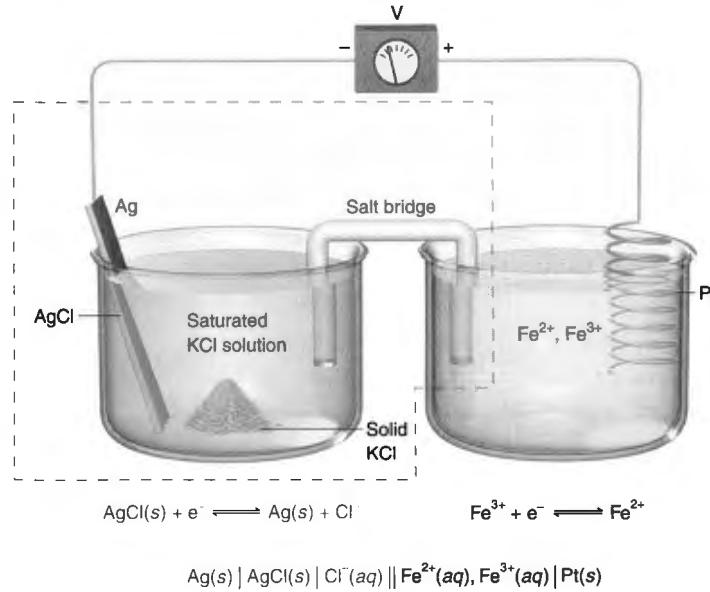


Figure 14-9 Cell used to illustrate the relation of E° to the equilibrium constant. The dashed line encloses the part of the cell that we will call the *reference electrode* in Section 14-6.

Now let's relate E° to the equilibrium constant for the net cell reaction.

Consider Figure 14-9 in which the left half-cell contains a silver electrode coated with solid AgCl and dipped into saturated aqueous KCl . The right half-cell has a platinum wire dipped into a solution containing Fe^{2+} and Fe^{3+} .

The two half-reactions, written as reductions, are

$$\text{Right: } \text{Fe}^{3+} + \text{e}^- \rightleftharpoons \text{Fe}^{2+} \quad E_+^\circ = 0.771 \text{ V} \quad (14-15)$$

$$\text{Left: } \text{AgCl}(s) + \text{e}^- \rightleftharpoons \text{Ag}(s) + \text{Cl}^- \quad E_-^\circ = 0.222 \text{ V} \quad (14-16)$$

Subtract the left half-reaction from the right half-reaction to get a net reaction:

$$\begin{aligned} \text{Net reaction: } & \text{Fe}^{3+} + \text{Ag}(s) + \text{Cl}^- \rightleftharpoons \text{Fe}^{2+} + \text{AgCl}(s) \\ & E^\circ = E_+^\circ - E_-^\circ = 0.771 - 0.222 = 0.549 \text{ V} \end{aligned} \quad (14-17)$$

The two electrode potentials are given by the Nernst equation 14-7:

$$E_+ = E_+^\circ - \frac{0.05916}{n} \log\left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]}\right) \quad (14-18)$$

$$E_- = E_-^\circ - \frac{0.05916}{n} \log([\text{Cl}^-]) \quad (14-19)$$

where $n = 1$ for Reactions 14-15 and 14-16. The cell voltage is the difference $E_+ - E_-$:

$$\begin{aligned} E = E_+ - E_- &= \left\{ E_+^\circ - \frac{0.05916}{n} \log\left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]}\right) \right\} - \left\{ E_-^\circ - \frac{0.05916}{n} \log([\text{Cl}^-]) \right\} \\ &= (E_+^\circ - E_-^\circ) - \left\{ \frac{0.05916}{n} \log\left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]}\right) \right. \\ &\quad \left. - \frac{0.05916}{n} \log([\text{Cl}^-]) \right\} \end{aligned} \quad (14-20)$$

E_+ and E_+ are for half-cell connected to positive terminal of potentiometer.
 E_-° and E_- are for half-cell connected to negative terminal.

Algebra of logarithms:

$$\log x + \log y = \log(xy)$$

$$\log x - \log y = \log(x/y)$$

To go from Equation 14-22 to 14-23:

$$\frac{0.059\ 16}{n} \log K = E^\circ$$

$$\log K = \frac{nE^\circ}{0.059\ 16}$$

$$10^{\log K} = 10^{nE^\circ/0.059\ 16}$$

$$K = 10^{nE^\circ/0.059\ 16}$$

To combine logarithms, we use the equality $\log x - \log y = \log(x/y)$:

$$E = (E_+^\circ - E_-^\circ) - \underbrace{\frac{0.059\ 16}{n} \log \left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}][\text{Cl}^-]} \right)}_{\substack{E^\circ \text{ for net reaction} \\ \text{Reaction quotient} \\ (Q) \text{ for net reaction}}} = E^\circ - \frac{0.059\ 16}{n} \log Q \quad (14-21)$$

Equation 14-21 is true at any time. In the special case when the cell is at equilibrium, $E = 0$ and $Q = K$, the equilibrium constant. At equilibrium,

$$0 = E^\circ - \frac{0.059\ 16}{n} \log K \implies E^\circ = \frac{0.059\ 16}{n} \log K \quad (14-22)$$

Finding K from E° :

$$K = 10^{nE^\circ/0.059\ 16} \quad (\text{at } 25^\circ\text{C}) \quad (14-23)$$

Equation 14-23 gives the equilibrium constant for a net cell reaction from E° .

A positive value of E° means that $K > 1$ in Equation 14-23, and a negative value means that $K < 1$. A reaction is spontaneous under standard conditions (that is, when all concentrations of reactants and products are 1 M or 1 bar) if E° is positive.

Example Using E° to Find the Equilibrium Constant

Find the equilibrium constant for the net cell reaction $\text{Fe}^{3+} + \text{Ag}(s) + \text{Cl}^- \rightleftharpoons \text{Fe}^{2+} + \text{AgCl}(s)$ in Figure 14-9.

SOLUTION Equation 14-17 states that $E^\circ = 0.549$ V. The equilibrium constant is computed with Equation 14-23, using $n = 1$, because one electron is transferred in each half-reaction:

$$K = 10^{nE^\circ/0.059\ 16} = 10^{(1)(0.549)/(0.059\ 16)} = 1.9 \times 10^9$$

K has two significant figures because E° has three digits. One digit of E° is used for the exponent (9), and the other two are left for the multiplier (1.9).

 **Test Yourself** Write the net cell reaction for Figure 14-6 with two electrons in each half-reaction. Find E° and K . If you had written half-reactions with one electron, what would be the equilibrium constant? (Answer: $2\text{Ag}^+ + \text{H}_2(g) \rightleftharpoons 2\text{Ag}(s) + 2\text{H}^+$, $E^\circ = 0.799$ V, $K = 1.0 \times 10^{27}$, 3×10^{13})

Ask Yourself

14-E. (a) Write half-reactions for Figure 14-3. Calculate E° and the equilibrium constant for the net cell reaction.

(b) The solubility product reaction of AgBr is $\text{AgBr}(s) \rightleftharpoons \text{Ag}^+ + \text{Br}^-$. Use the reactions $\text{Ag}^+ + e^- \rightleftharpoons \text{Ag}(s)$ and $\text{AgBr}(s) + e^- \rightleftharpoons \text{Ag}(s) + \text{Br}^-$ to compute the solubility product of AgBr . Compare your answer with that in Appendix A.

14-6 Reference Electrodes

Imagine a solution containing an electroactive species whose concentration we wish to measure. We construct a half-cell by inserting an electrode (such as a Pt wire) into the solution to transfer electrons to or from the species of interest. Because this electrode responds directly to the analyte, it is called the **indicator electrode**. The potential of the indicator electrode is E_+ . We then connect this half-cell to a second half-cell by a salt bridge. The second half-cell has a fixed composition that provides a known, constant potential, E_- . Because the second half-cell has a constant potential, it is called a **reference electrode**. The cell voltage ($E = E_+ - E_-$) is the difference between the variable potential that reflects changes in the analyte concentration and the constant reference potential.

Suppose you have a solution containing Fe^{2+} and Fe^{3+} . If you are clever, you can make this solution part of a cell whose voltage tells you the quotient $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$. Figure 14-9 shows one way to do this. A Pt wire acts as an indicator electrode through which Fe^{3+} can receive electrons or Fe^{2+} can lose electrons. The left half-cell completes the galvanic cell and has a known, constant potential.

The two half-reactions were 14-15 and 14-16, and the two electrode potentials were given in Equations 14-18 and 14-19. The cell voltage is the difference $E_+ - E_-$:

$$E = E_+ - E_- = \underbrace{\left\{ E_+^\circ - 0.05916 \log \left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \right) \right\}}_{\text{Constant}} - \underbrace{\left\{ E_-^\circ - 0.05916 \log ([\text{Cl}^-]) \right\}}_{\text{Constant}} \quad (14-24)$$

Constant The variable of interest Constant Constant concentration in left half-cell

Because $[\text{Cl}^-]$ in the left half-cell is constant (fixed by the solubility of saturated KCl), the cell voltage changes only when the quotient $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$ changes.

The half-cell on the left in Figure 14-9 is a **reference electrode**. We can picture the cell and salt bridge enclosed by the dashed line as a single unit dipped into the analyte solution, as in Figure 14-10. The Pt wire is the indicator electrode, whose potential responds to changes in the quotient $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$. The reference electrode completes the redox reaction and provides a *constant potential* to the left side of the potentiometer. Changes in the cell voltage can be assigned to changes in the quotient $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$.

Silver-Silver Chloride Reference Electrode

The half-cell enclosed by the dashed line in Figure 14-9 is called a **silver-silver chloride electrode**. Figure 14-11 shows how the half-cell is reconstructed as a thin, glass-enclosed electrode that can be dipped into the analyte solution in Figure 14-10. The porous plug at the base of the electrode functions as a salt bridge. It allows ions to diffuse between solutions inside and outside the electrode with minimal physical mixing. We use silver-silver chloride or other reference electrodes because they are more convenient than a hydrogen electrode, which requires bubbling gas over a freshly prepared catalytic Pt surface.

The standard reduction potential for $\text{AgCl} | \text{Ag}$ is +0.222 V at 25°C. If the cell is saturated with KCl, the potential is +0.197 V. We will use this value for all problems

Indicator electrode: responds to analyte concentration

Reference electrode: maintains a fixed (reference) potential

Voltage in Figure 14-9 responds only to changes in the quotient $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$. Everything else is constant.

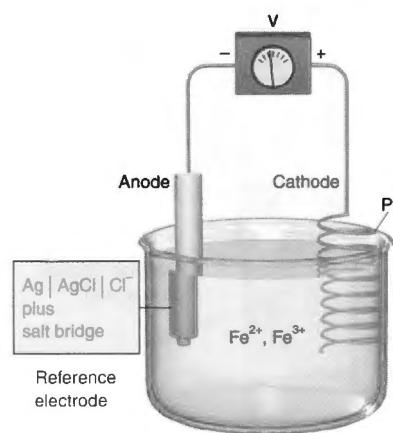


Figure 14-10 Another view of Figure 14-9. The contents of the dashed box in Figure 14-9 are now considered to be a reference electrode dipped into the analyte solution.

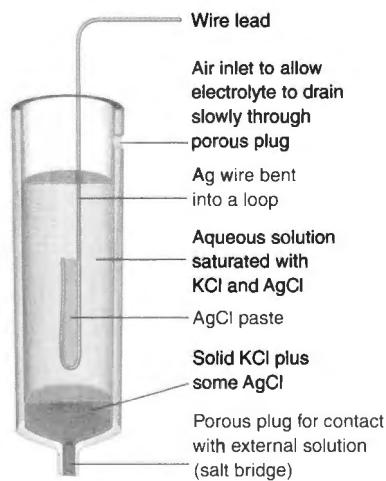
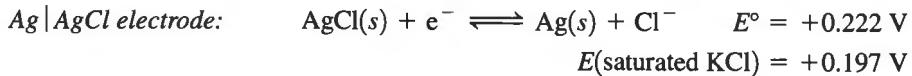


Figure 14-11 Silver-silver chloride reference electrode.

involving the $\text{Ag}|\text{AgCl}$ reference electrode. The advantage of saturated KCl is that the concentration of Cl^- does not change if some of the liquid evaporates.



Example Using a Reference Electrode

Calculate the cell voltage in Figure 14-10 if the reference electrode is a saturated silver-silver chloride electrode and $[\text{Fe}^{2+}]/[\text{Fe}^{3+}] = 10$.

SOLUTION We use Equation 14-24, noting that $E_- = 0.197 \text{ V}$ for a saturated silver-silver chloride electrode:

$$E = E_+ - E_- = \underbrace{\left\{ E_+^\circ - 0.05916 \log \left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \right) \right\}}_{0.771 \text{ V}} - \underbrace{0.197}_{10} \quad \text{Reference electrode voltage}$$

$$E = \{0.712\} - 0.197 = 0.515 \text{ V}$$

 **Test Yourself** Find the voltage if $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$ increases to 100. (**Answer:** 0.456 V)

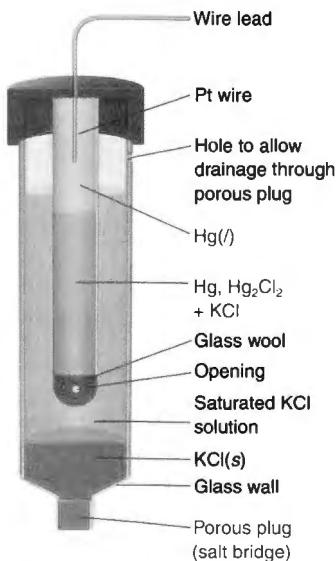
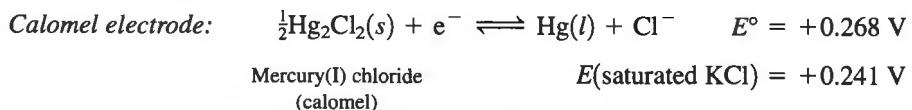


Figure 14-12 Saturated calomel electrode (S.C.E.).

Calomel Reference Electrode

The *calomel electrode* in Figure 14-12 is based on the reaction



If the cell is saturated with KCl, it is called a **saturated calomel electrode** and the cell potential is $+0.241 \text{ V}$ at 25°C . This electrode is encountered so frequently that it is abbreviated **S.C.E.**

Voltage Conversions Between Different Reference Scales

It is sometimes necessary to convert potentials between different reference scales. If an electrode has a potential of -0.461 V with respect to a calomel electrode, what is the potential with respect to a silver-silver chloride electrode? What would be the potential with respect to the standard hydrogen electrode?

To answer these questions, Figure 14-13 shows the positions of the calomel and silver-silver chloride electrodes with respect to the standard hydrogen electrode. Point A, which is -0.461 V from S.C.E., is -0.417 V from the silver-silver chloride electrode and -0.220 V from S.H.E. What about point B, whose potential is $+0.033 \text{ V}$ with respect to silver-silver chloride? It lies -0.011 V from S.C.E. and $+0.230 \text{ V}$ from S.H.E. By keeping this diagram in mind, you can convert potentials from one scale to another.

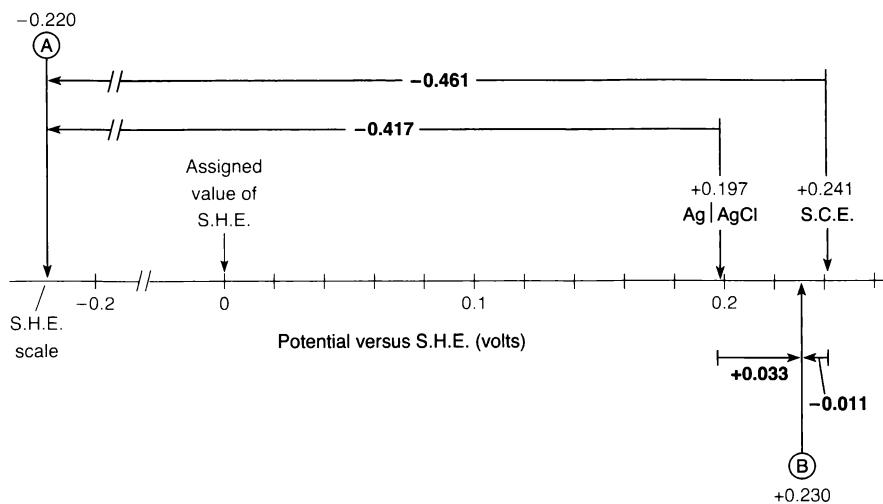


Figure 14-13 Converting potentials from one reference scale into another.



Ask Yourself

- 14-F. (a) Find the concentration ratio $[Fe^{2+}]/[Fe^{3+}]$ for the cell in Figure 14-10 if the measured voltage is 0.703 V.
 (b) Convert the potentials listed below. The $Ag | AgCl$ and calomel reference electrodes are saturated with KCl.
 (i) 0.523 V versus S.H.E. = ? versus $Ag | AgCl$
 (ii) 0.222 V versus S.C.E. = ? versus S.H.E.

Key Equations

Definitions	Oxidizing agent—takes electrons Reducing agent—gives electrons Anode—where oxidation occurs Cathode—where reduction occurs
Relation between electric charge and moles	$q = nNF$ q = electric charge (coulombs) n = number of unit charges per molecule N = moles F = Faraday constant
Relation between work and voltage	$work = Eq$ E = voltage difference through which charge q is moved
Standard potential	Measured by the cell S.H.E. half-reaction of interest, where S.H.E. is the standard hydrogen electrode and all reagents in the right half-cell are in their standard state (= 1 M, 1 bar, pure solid, or pure liquid)
Nernst equation	For the half-reaction $aA + ne^- \rightleftharpoons bB$
	$E = E^\circ - \frac{0.05916}{n} \log\left(\frac{[B]^b}{[A]^a}\right) \quad (\text{at } 25^\circ\text{C})$

Voltage of complete cell	$E = E_+ - E_-$
	E_+ = voltage of electrode connected to + terminal of meter
	E_- = voltage of electrode connected to – terminal of meter
E° for net cell reaction	$E^\circ = E_+^\circ - E_-^\circ$
	E_+° = standard potential for half-reaction in cell connected to + terminal of meter
	E_-° = standard potential for half-reaction in cell connected to – terminal of meter
Direction of electron travel through a circuit	Electrons move from less positive electrode to more positive electrode
Finding K from E°	$K = 10^{nE^\circ/0.05916}$
	n = number of electrons in half-reaction

Important Terms

ampere	Faraday constant	redox reaction	silver-silver chloride
anode	formal potential	reducing agent	electrode
cathode	galvanic cell	reductant	standard hydrogen
coulomb	indicator electrode	reduction	electrode (S.H.E.)
current	Nernst equation	reference electrode	standard reduction
electric potential	oxidant	salt bridge	potential
electroactive species	oxidation	saturated calomel	volt
electrode	oxidizing agent	electrode (S.C.E.)	
electrolysis			

Problems

14-1. (a) Explain the difference between electric charge (q , coulombs), electric current (I , amperes), and electric potential (E , volts).

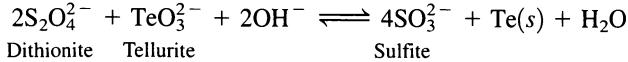
(b) How many electrons are in 1 coulomb?

(c) How many coulombs are in 1 mole of charge?

14-2. Identify the oxidizing and reducing agents in the *thermite reaction* $\text{Fe}_2\text{O}_3 + 2\text{Al} \longrightarrow 2\text{Fe} + \text{Al}_2\text{O}_3$, which generates enough heat to melt iron.

14-3. Identify oxidizing and reducing agents among the following reactants and write a balanced half-reaction for each: $\text{Na}(s) + \text{H}_2\text{O} \rightleftharpoons \text{Na}^+ + \text{OH}^- + \frac{1}{2}\text{H}_2(g)$

14-4. (a) Identify the oxidizing and reducing agents among the following reactants and write a balanced half-reaction for each.



(b) How many coulombs of charge are passed from reductant to oxidant when 1.00 g of Te is deposited?

(c) If Te is created at a rate of 1.00 g/h, how much current is flowing?

14-5. Fine particles of metallic iron can be injected underground to remediate pollution of underground aquifers by the industrial solvent trichloroethene. In one experiment, 2 400 L of an aqueous emulsion containing ~480 kg of $\text{Fe}(0)$ consumed 17 kg of trichloroethene in 5 months.

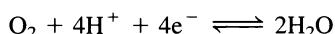
(a) Identify oxidizing and reducing agents for the (unbalanced) reaction $\text{Fe} + \text{C}_2\text{HCl}_3 \longrightarrow \text{Fe}^{2+} + \text{C}_2\text{H}_4 + \text{Cl}^-$. Write a balanced half-reaction for each, using H_2O and H^+ to complete the balancing. Write a balanced net reaction.

(b) What percentage of injected Fe was used by this reaction in 5 months?

(c) How much current is flowing between the reactants if trichloroethene reacts at a constant rate of 17 kg/150 days?

14-6. Hydrogen ions can carry out useful work in a living cell (such as the synthesis of the molecule ATP that provides energy for chemical synthesis) when they pass from a region of high potential to a region of lower potential. How many joules of work can be done when 1.00 μmol of H^+ crosses a membrane and goes from a potential of +0.075 V to a potential of -0.090 V (that is, through a potential difference of 0.165 V)?

14-7. The basal rate of consumption of O_2 by a 70-kg human is 16 mol O_2 /day. This O_2 oxidizes food and is reduced to H_2O , thereby providing energy for the organism:



(a) To what current (in amperes = C/s) does this respiration rate correspond? (Current is defined by the flow of electrons from food to O_2 .)

(b) If the electrons flow from reduced nicotinamide adenine dinucleotide (NADH) to O_2 , they experience a potential drop of 1.1 V. How many joules of work can be done by 16 mol O_2 ?

14-8. (a) Draw a picture of the following cell.



(b) Write a reduction half-reaction for each half-cell.

(c) For particular conditions, the zinc electrode has a potential of -0.75 V and the Pt electrode has a potential of $+0.25$ V. In which direction will electrons flow when the Zn and Hg are connected by a wire? Which electrode is the anode and which is the cathode in this cell? Write a net cell reaction combining the correct oxidation and reduction.

14-9. Redraw the cell in Figure 14-6, showing KNO_3 in the salt bridge. Noting the direction of electron flow through the circuit, show what happens to each reactant and product in each half-cell and show the direction of motion of each ion in each half-cell and in the salt bridge. Show the reaction at each electrode. When you finish this, you should understand what is happening in the cell.

14-10. Suppose that the concentrations of NaF and KCl were each 0.10 M in the cell



(a) Using the half-reactions $2AgCl(s) + 2e^- \rightleftharpoons 2Ag(s) + 2Cl^-$ and $PbF_2(s) + 2e^- \rightleftharpoons Pb(s) + 2F^-$, calculate the cell voltage.

(b) Now calculate the cell voltage by using the reactions $2Ag^+ + 2e^- \rightleftharpoons 2Ag(s)$ and $Pb^{2+} + 2e^- \rightleftharpoons Pb(s)$ and K_{sp} for $AgCl$ and PbF_2 (see Appendix A).

14-11. Consider a circuit in which the left half-cell is prepared by dipping a Pt wire in a beaker containing an equimolar mixture of Cr^{2+} and Cr^{3+} . The right half-cell contains a Tl rod immersed in 1.00 M $TlClO_4$.

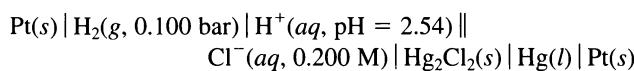
(a) Use line notation to describe this cell.

(b) Find the potential of each half-cell and calculate the cell voltage.

(c) Draw a diagram like Figure 14-8 for this cell. When the electrodes are connected by a salt bridge and a wire, which terminal (Pt or Tl) is the anode?

(d) Write the spontaneous net cell reaction.

14-12. Consider the cell



(a) Write a reduction reaction and Nernst equation for each half-cell and find each half-cell potential. For the Hg_2Cl_2 half-reaction, $E^\circ = 0.268$ V.

(b) Draw a diagram like Figure 14-8 for this cell. Which half-cell is the anode?

(c) Find E for the net cell reaction.

14-13. (a) Write reduction reactions for each half-cell in Figure 14-5. Calculate each half-cell potential and the net cell voltage.

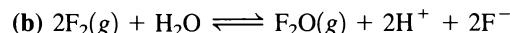
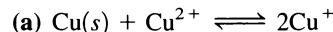
(b) Draw a diagram like Figure 14-8 for this cell. Which way do electrons flow? Write the net cell reaction in the spontaneous direction.

(c) The left half-cell was loaded with 14.3 mL of $Br_2(l)$ (density = 3.12 g/mL). The aluminum electrode contains 12.0 g of Al. Which element, Br_2 or Al, is the limiting reagent in this cell? (That is, which reagent will be used up first?)

(d) If the cell is somehow operated under conditions in which it produces a constant voltage of 1.50 V, how much electrical work will have been done when 0.231 mL of $Br_2(l)$ has been consumed?

(e) If the current is 2.89×10^{-4} A, at what rate (grams per second) is Al(s) dissolving?

14-14. Combine suitable half-reactions from Appendix C to calculate E° and K for each of the following reactions:



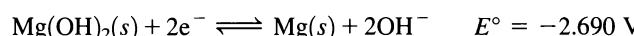
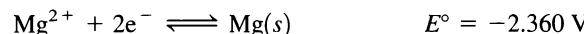
14-15. In fuel cells⁷ used in *Apollo* flights to the moon, $H_2(g)$ is oxidized to $H_2O(l)$ at a catalytic cathode and $O_2(g)$ is reduced to $H_2O(g)$ at a catalytic anode.

(a) Write the half-reactions and the net reaction. Find the cell voltage if H_2 and O_2 are each present at 1 bar, the cathode compartment pH is 0, and the anode compartment pH is 14.

(b) Find the equilibrium constant for the net cell reaction and write the equilibrium expression in terms of concentrations of reactants and products.

(c) If the cell produces a constant current of 10.0 A, how many days will it take to consume 1.00 kg of H_2 ? How many kilograms of O_2 will be consumed in the same time?

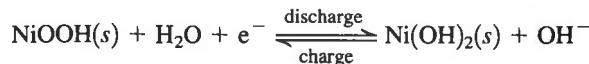
14-16. From the following half-reactions, calculate the solubility product of $Mg(OH)_2$.



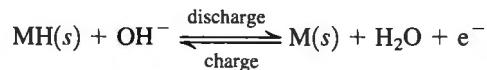
14-17. Select half-reactions from Appendix C to compute the formation constant for the reaction $Ca^{2+} + acetate^- \rightleftharpoons Ca(acetate)^+$. Find the value of K_f .

14-18. The nickel-metal hydride rechargeable battery used in early laptop computers is based on the following chemistry:

Cathode:



Anode:



The anode material, MH, is a metal hydride in which the metal is one of several transition metal or rare earth alloys. Explain why the voltage of this cell remains nearly constant during its entire discharge cycle.

14-19. The cell in Ask Yourself 14-B(b) contains 1.3 mM $\text{Fe}(\text{CN})_6^{4-}$, 4.9 mM $\text{Fe}(\text{CN})_6^{3-}$, 1.8 mM $\text{Ag}(\text{S}_2\text{O}_3)_2^{3-}$, and 55 mM $\text{S}_2\text{O}_3^{2-}$.

(a) Find E° and K for the net cell reaction.

(b) Find the cell voltage.

(c) In which direction do electrons flow through the circuit? Is $\text{Ag}(s)$ oxidized or reduced in the spontaneous cell reaction?

14-20. From the standard potentials for reduction of $\text{Br}_2(aq)$ and $\text{Br}_2(l)$ in Appendix C, calculate the solubility of Br_2 in water at 25°C. Express your answer as g/L.

14-21. A solution contains 0.010 0 M IO_3^- , 0.010 0 M I^- , 1.00×10^{-4} M I_3^- , and pH 6.00 buffer. Consider the reactions



(a) Write a balanced net reaction that can take place in this solution.

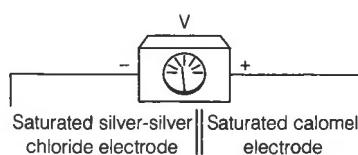
(b) Calculate E° and K for the reaction.

(c) Find the two half-cell potentials for the given conditions and calculate E for the net cell reaction. In which direction does the reaction proceed?

(d) At what pH would the given concentrations of IO_3^- , I^- , and I_3^- listed above be in equilibrium?

14-22. (a) Write the half-reactions for the silver-silver chloride and calomel reference electrodes.

(b) Predict the voltage for the following cell:



14-23. (a) Find the potential (versus S.H.E.) of the half cell $\text{Pt}|\text{VO}^{2+}$ (0.050 M), VO_2^+ (0.025 M), pH 2.00.

(b) What would be the voltage of the cell S.C.E. || VO^{2+} (0.050 M), VO_2^+ (0.025 M), pH 2.00 | Pt?

14-24. Convert the following potentials. The $\text{Ag}|\text{AgCl}$ and calomel reference electrodes are saturated with KCl.

(a) -0.111 V versus $\text{Ag}|\text{AgCl} = ?$ versus S.H.E.

(b) 0.023 V versus $\text{Ag}|\text{AgCl} = ?$ versus S.C.E.

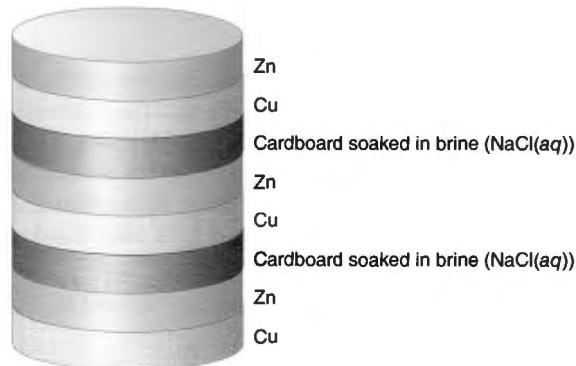
(c) -0.023 V versus S.C.E. = ? versus $\text{Ag}|\text{AgCl}$

14-25. Suppose that the silver-silver chloride electrode in Figure 14-10 is replaced by a saturated calomel electrode. Calculate the cell voltage if $[\text{Fe}^{2+}]/[\text{Fe}^{3+}] = 2.5 \times 10^{-3}$.

14-26. The formal reduction potential for $\text{Fe}^{3+} + \text{e}^- \rightleftharpoons \text{Fe}^{2+}$ in 1 M HClO_4 is 0.73 V. The formal reduction potential for the complex $\text{LFe(III)} + \text{e}^- \rightleftharpoons \text{LFe(II)}$ (where L is the chelate desferrioxamine B at the opening of Chapter 13) is -0.48 V .⁸ What do these potentials tell you about the relative stability of LFe(III) and LFe(II) ?

How Would You Do It?

14-27. The voltaic pile invented by Alessandro Volta in 1799 is made of many repeating layers that could have the following structure:



Possible reactants for the cell $\text{Zn}|\text{brine}|\text{Cu}$ are $\text{Zn}(s)$, $\text{Cu}(s)$, $\text{H}_2\text{O}(l)$, $\text{Na}^+(aq)$, and $\text{Cl}^-(aq)$. List possible half-reactions and propose which ones could give a galvanic cell. Predict the direction of electron flow through a wire connecting the top layer to the bottom layer in the diagram.

14-28. One measure of the capability of a battery is how much electricity it can produce per kilogram of reactants. The quantity of electricity could be measured in coulombs, but it is customarily measured in ampere · hours, where 1 A · h provides 1 A for 1 h. Thus, if 0.5 kg of reactants can produce 3 A · h, the storage capacity would be $3 \text{ A} \cdot \text{h}/0.5 \text{ kg} = 6 \text{ A} \cdot \text{h/kg}$. Compare the capabilities of a conventional lead-acid car battery with that of a hydrogen-oxygen fuel cell in terms of $\text{A} \cdot \text{h/kg}$.

Lead-acid battery: $\text{Pb} + \text{PbO}_2 + 2\text{H}_2\text{SO}_4 \longrightarrow 2\text{PbSO}_4 + 2\text{H}_2\text{O}$

FM of reactants = $207.2 + 239.2 + (2 \times 98.079) = 642.6$

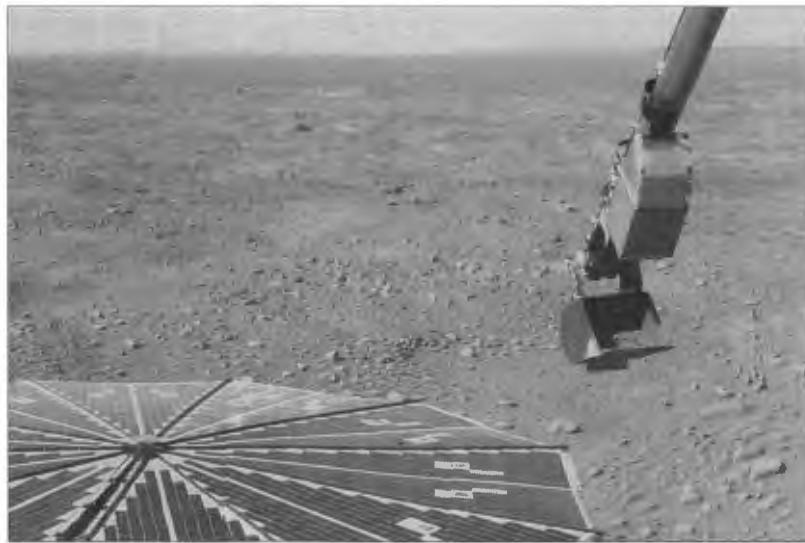
Hydrogen-oxygen fuel cell: $2\text{H}_2 + \text{O}_2 \longrightarrow 2\text{H}_2\text{O}$

FM of reactants = 36.031

Notes and References

1. Quotation from Lady Pollock cited in J. Kendall, *Great Discoveries by Young Chemists* (New York: Thomas Y. Crowell Co., 1953, p. 63).
2. For demonstrations of half-cells, see J. D. Ciparick, *J. Chem. Ed.* **1991**, *68*, 247 and P.-O. Eggen, T. Grønneberg, and L. Kvittengen, *J. Chem. Ed.* **2006**, *83*, 1201.
3. L. P. Silverman and B. B. Bunn, *J. Chem. Ed.* **1992**, *69*, 309.
4. J. Hamilton, *A Life of Discovery: Michael Faraday, Giant of the Scientific Revolution* (New York: Random House, 2004, pp. 258–260).
5. K. Rajeshwar and J. G. Ibanez, *Environmental Electrochemistry* (San Diego: Academic Press, 1997).
6. For an experiment on measuring battery lifetime, see M. J. Smith and C. A. Vincent, *J. Chem. Ed.* **2002**, *79*, 851.
7. Fuel cell demonstrations: O. Zerbinati, *J. Chem. Ed.* **2002**, *79*, 829; M. Shirkhanzadeh, *J. Chem. Ed.* **2009**, *86*, 324.
8. I. Spasojević, S. K. Armstrong, T. J. Brickman, and A. L. Crumbliss, *Inorg. Chem.* **1999**, *38*, 449.

How Perchlorate Was Discovered on Mars



Robotic arm of *Phoenix Mars Lander* scoops up soil for chemical analysis on Mars. [NASA photograph courtesy S. Kounaves, Tufts University.]

Each of four Wet Chemistry Laboratories on the *Phoenix Mars Lander* shown at the opening of Chapter 6 was equipped with 23 electrochemical sensors, of which 15 were ion-selective electrodes similar to those discussed in this chapter. The robotic arm delivered soil through the sieve into the “beaker” compartment. Then aqueous solution was added to leach soluble salts from the soil. Sensors measured ions appearing in the liquid.

Nobody expected perchlorate (ClO_4^-) to be abundant on Mars, so the Wet Chemistry Lab was not designed to look for ClO_4^- . However, the nitrate ion-selective electrode sent to Mars was *1 000 times more sensitive* to ClO_4^- than to NO_3^- .

When salts were leached from the soil, the NO_3^- electrode potential changed by 200 mV, corresponding to an apparent NO_3^- concentration above 1 M, which would have required more NO_3^- than the mass of soil that was analyzed.¹ However, 4 to 6 mg of ClO_4^- in 1 g of soil would have produced the observed response. Also, heating the soil to 400°–600°C released a product with a molecular mass of 32, consistent with thermal decomposition of ClO_4^- to give O_2 . Perchlorate occurs at similar levels on Earth in arid regions, including the Atacama Desert. On Earth, ClO_4^- is thought to arise from photochemical reactions of ozone (O_3) with chlorine species in the atmosphere.

Electrode Measurements

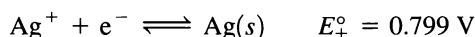
A critically ill patient is wheeled into the emergency room, and the doctor needs blood chemistry information quickly to help her make a diagnosis and begin treatment. Every analyte in Table 15-1, which is part of the critical care profile of blood chemistry, can be measured by electrochemical means. Ion-selective electrodes described in this chapter are the method of choice for Na^+ , K^+ , Cl^- , pH, and P_{CO_2} . Analytes produce a voltage difference between the outside and inside of an ion-selective electrode. The use of voltage measurements to extract chemical information is called **potentiometry**.

15-1 The Silver Indicator Electrode

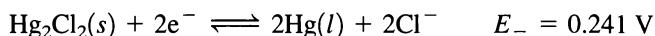
In Chapter 14, we learned that the voltage of an electrochemical cell is related to the concentrations of species in the cell. We saw that some cells could be divided into a *reference electrode* that provides a constant electric potential and an *indicator electrode* whose potential varies in response to analyte concentration.

Chemically inert platinum, gold, and carbon indicator electrodes are frequently used to conduct electrons to or from species in solution. In contrast with chemically inert elements, silver participates in the reaction $\text{Ag}^+ + \text{e}^- \rightleftharpoons \text{Ag}(s)$.

Figure 15-1 shows how a silver electrode can be used in conjunction with a saturated calomel reference electrode to measure $[\text{Ag}^+]$ during the titration of halide ions by Ag^+ (as shown in Figures 6-4 and 6-5). The reaction at the silver indicator electrode is



and the reference half-cell reaction is



The reference half-cell potential (E_- , not E_f°) is constant at 0.241 V because $[\text{Cl}^-]$ is fixed by the concentration of saturated KCl. The Nernst equation for the entire cell is therefore

Table 15-1 Critical care profile^a

Function	Analyte
Conduction	K^+ , Ca^{2+}
Contraction	Ca^{2+} , Mg^{2+}
Energy level	Glucose, P_{O_2} , lactate, hematocrit
Ventilation	P_{O_2} , P_{CO_2}
Perfusion	Lactate, $\text{SO}_2\%$, hematocrit
Acid-base	pH, P_{CO_2} , HCO_3^-
Osmolality	Na^+ , glucose
Electrolyte balance	Na^+ , K^+ , Ca^{2+} , Mg^{2+}
Renal function	Blood urea nitrogen, creatinine

^a C. C. Young, *J. Chem. Ed.* 1997, 74, 177.

E_- = reference electrode potential with actual concentrations in the reference cell

E_f° = standard potential of reference half-reaction when all species are in their standard states (pure solid, pure liquid, 1 M, or 1 bar)

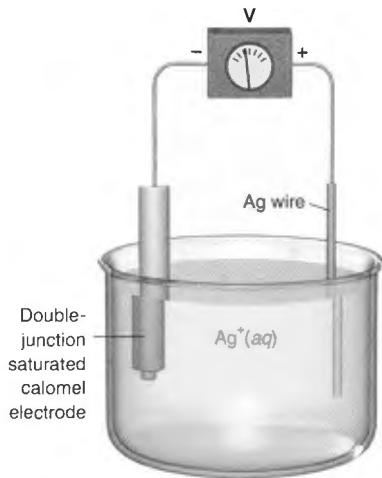


Figure 15-1 Use of silver and calomel electrodes to measure the concentration of Ag^+ in a solution. The calomel electrode has a double junction, like that in Figure 15-3. The outer compartment of the electrode is filled with KNO_3 , so there is no direct contact between the KCl solution in the inner compartment and Ag^+ in the beaker.

When Ag^+ is added to I^- :

- Before V_e , there is a known excess of I^- : $[\text{Ag}^+] = K_{\text{sp}}/[\text{I}^-]$
- At V_e , $[\text{Ag}^+] = [\text{I}^-] = \sqrt{K_{\text{sp}}}$
- After V_e , there is a known excess of Ag^+

$$E = E_+ - E_- = \underbrace{\left\{ 0.799 - 0.05916 \log\left(\frac{1}{[\text{Ag}^+]}\right) \right\}}_{\text{Potential of } \text{Ag}^+ | \text{Ag}^+ \text{ indicator electrode}} - \underbrace{\{0.241\}}_{\text{Constant potential of S.C.E. reference electrode}}$$

Noting that $\log(1/[\text{Ag}^+]) = -\log[\text{Ag}^+]$, we rewrite the preceding expression as

$$E = 0.558 + 0.05916 \log[\text{Ag}^+] \quad (15-1)$$

The voltage changes by 0.05916 V (at 25°C) for each factor-of-10 change in $[\text{Ag}^+]$.

The experiment in Figure 6-4 used a silver indicator electrode and a glass reference electrode. The glass electrode responds to the pH of the solution, which is held constant by a buffer. Therefore the glass electrode remains at a constant potential.

Titration of a Halide Ion with Ag^+

Let's consider how the concentration of Ag^+ varies during the titration of I^- with Ag^+ , as shown in Figure 6-5b. We derive the theoretical shape of the titration curve for a known concentration of Ag^+ titrant added to a known solution of I^- . The purpose of an actual titration is to measure the concentration of an unknown solution of I^- . The titration reaction is



If you were monitoring the reaction with silver and calomel electrodes, you could use Equation 15-1 to compute the expected voltage at each point in the titration.

At any point prior to the equivalence point (V_e), there is a known excess of I^- from which we can calculate $[\text{Ag}^+]$:

$$\text{Before } V_e: \quad K_{\text{sp}} = [\text{Ag}^+][\text{I}^-] \Rightarrow [\text{Ag}^+] = K_{\text{sp}}/[\text{I}^-] \quad (15-2)$$

At the equivalence point, the quantity of Ag^+ added is exactly equal to the I^- that was originally present. We can imagine that $\text{AgI}(s)$ is made stoichiometrically and a little bit redissolves:

$$\text{At } V_e: \quad K_{\text{sp}} = [\text{Ag}^+][\text{I}^-] \Rightarrow [\text{Ag}^+] = [\text{I}^-] = \sqrt{K_{\text{sp}}} \quad (15-3)$$

Beyond the equivalence point, the quantity of excess Ag^+ added from the buret is known, and the concentration is just

$$\text{After } V_e: \quad [\text{Ag}^+] = \frac{\text{moles of excess } \text{Ag}^+}{\text{total volume of solution}} \quad (15-4)$$

Example Potentiometric Precipitation Titration

A 20.00-mL solution containing 0.1004 M KI was titrated with 0.0845 M AgNO_3 , using the cell in Figure 15-1. Calculate the voltage at volumes $V_{\text{Ag}^+} = 15.00$, V_e , and 25.00 mL.

SOLUTION The titration reaction is $\text{Ag}^+ + \text{I}^- \rightarrow \text{AgI}(s)$, and the equivalence volume is

$$\underbrace{(V_e \text{ (mL)})(0.084 \text{ M})}_{\text{mmol Ag}^+} = \underbrace{(20.00 \text{ mL})(0.100 \text{ M})}_{\text{mmol I}^-} \Rightarrow V_e = 23.76 \text{ mL}$$

15.00 mL: We began with $(20.00 \text{ mL})(0.100 \text{ M}) = 2.008 \text{ mmol I}^-$ and added $(15.00 \text{ mL})(0.084 \text{ M}) = 1.268 \text{ mmol Ag}^+$. The concentration of unreacted I^- is

$$[\text{I}^-] = \frac{(2.008 - 1.268) \text{ mmol}}{(20.00 + 15.00) \text{ mL}} = 0.021 \text{ M}$$

The concentration of Ag^+ in equilibrium with the solid AgI is therefore

$$[\text{Ag}^+] = \frac{K_{\text{sp}}}{[\text{I}^-]} = \frac{8.3 \times 10^{-17}}{0.021 \text{ M}} = 3.9 \times 10^{-15} \text{ M}$$

The cell voltage is computed with Equation 15-1:

$$E = 0.558 + 0.059 \text{ } 16 \log(3.9 \times 10^{-15}) = -0.294 \text{ V}$$

At V_e : Equation 15-3 tells us that $[\text{Ag}^+] = \sqrt{K_{\text{sp}}} = 9.1 \times 10^{-9} \text{ M}$, so

$$E = 0.558 + 0.059 \text{ } 16 \log(9.1 \times 10^{-9}) = 0.082 \text{ V}$$

25.00 mL: Now there is an excess of $25.00 - 23.76 = 1.24 \text{ mL}$ of 0.084 M AgNO_3 in a total volume of 45.00 mL.

$$[\text{Ag}^+] = \frac{(1.24 \text{ mL})(0.084 \text{ M})}{45.00 \text{ mL}} = 2.33 \times 10^{-3} \text{ M}$$

and the cell voltage is

$$E = 0.558 + 0.059 \text{ } 16 \log(2.33 \times 10^{-3}) = 0.402 \text{ V}$$

 **Test Yourself** Find the voltage at $V_{\text{Ag}^+} = 20.00$ and 30.00 mL. (Answer: -0.294 V, 0.441 V)

The voltage in Figure 15-2 barely changes prior to the equivalence point because the concentration of Ag^+ is very low and relatively constant until I^- is used up. When I^- has been consumed, $[\text{Ag}^+]$ suddenly increases and so does the voltage. Figure 15-2 is upside down relative to curve b in Figure 6-5. The reason is that, in Figure 15-1, the indicator electrode is connected to the *positive* terminal of the potentiometer. In Figure 6-4, the indicator electrode is connected to the *negative* terminal because the glass pH electrode only fits into the positive terminal of the meter. In addition to their opposite polarities, the voltages in Figures 15-2 and 6-5 are different because each experiment uses a different reference electrode.

Double-Junction Reference Electrode

If you tried to titrate I^- with Ag^+ by using the cell in Figure 15-1, KCl solution would slowly leak into the titration beaker from the porous plug at the base of the

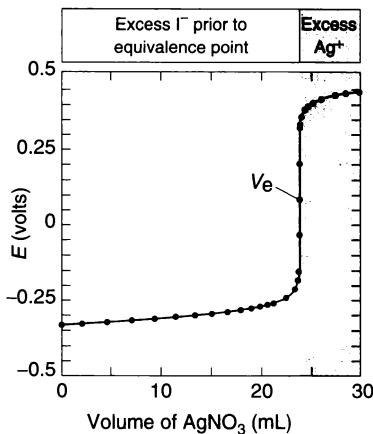


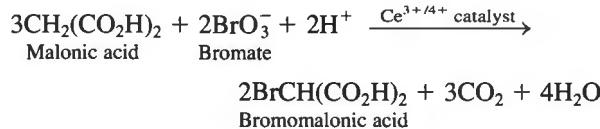
Figure 15-2 Calculated titration curve for the addition of 0.084 M Ag^+ to 20.00 mL of 0.100 M I^- in the cell in Figure 15-1.

Demonstration 15-1 uses a pair of electrodes to monitor a pretty amazing chemical reaction.



Demonstration 15-1 Potentiometry with an Oscillating Reaction

Principles of potentiometry are illustrated in a fascinating manner by *oscillating reactions* in which chemical concentrations oscillate between high and low values. An example is the Belousov-Zhabotinskii reaction:



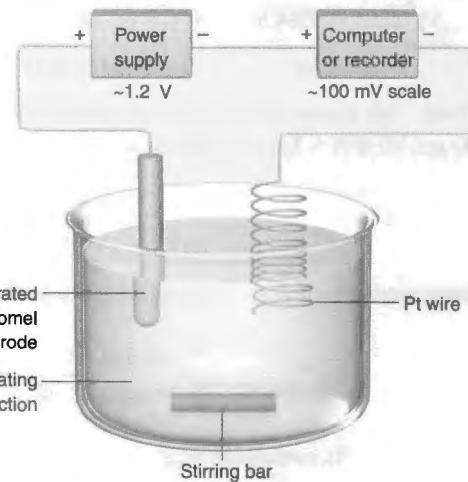
During this reaction, the quotient $[\text{Ce}^{3+}]/[\text{Ce}^{4+}]$ oscillates by a factor of 10 to 100. When the Ce^{4+} concentration is high, the solution is yellow. When Ce^{3+} predominates, the solution is colorless.

To start the show, combine the following solutions in a 300-mL beaker:

- 160 mL of 1.5 M H_2SO_4
- 40 mL of 2 M malonic acid
- 30 mL of 0.5 M NaBrO_3 (or saturated KBrO_3)
- 4 mL of saturated ceric ammonium sulfate, $\text{Ce}(\text{SO}_4)_2 \cdot 2(\text{NH}_4)_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$

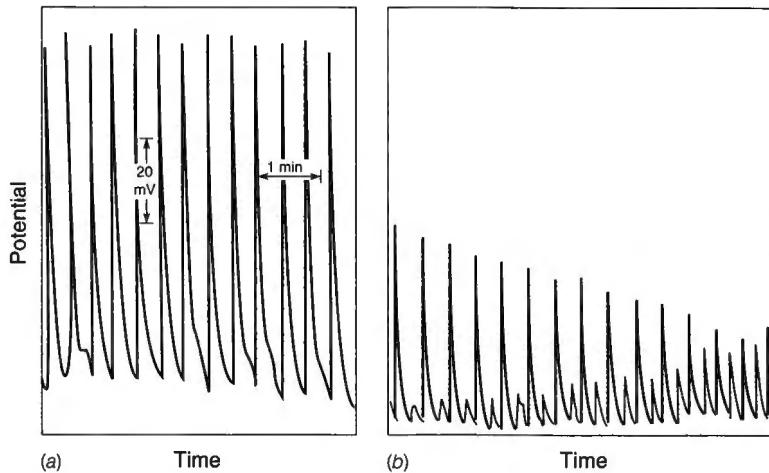
After an induction period of 5 to 10 min with magnetic stirring, you can initiate oscillations by adding 1 mL of ceric ammonium sulfate solution. The reaction is somewhat temperamental and may need more Ce^{4+} over a 5-min period to initiate oscillations.

Monitor the $[\text{Ce}^{3+}]/[\text{Ce}^{4+}]$ ratio with Pt and calomel electrodes. You should be able to write the cell reactions and a Nernst equation for this experiment.



Apparatus used to monitor relative concentrations of Ce^{3+} and Ce^{4+} in an oscillating reaction. [George Rossman, California Institute of Technology.]

In place of a potentiometer (a pH meter), we use a computer to obtain a permanent record of the oscillations. The potential oscillates over a range of ~ 100 mV centered near ~ 1.2 V, so we offset the cell voltage by ~ 1.2 V with any available power supply. Trace *a* shows what is usually observed. The potential changes rapidly during the abrupt colorless-to-yellow transition and more gradually during the gentle yellow-to-colorless transition. Trace *b* shows two different cycles superimposed in the same solution.



reference electrode (Figure 14-12). Cl^- introduces a titration error because it consumes Ag^+ . The *double-junction reference electrode* in Figure 15-3 prevents the inner electrolyte solution from leaking directly into the titration vessel.

Ask Yourself

- 15-A. Consider the titration of 40.0 mL of 0.050 0 M NaCl with 0.200 M AgNO_3 , using the cell in Figure 15-1. The equivalence volume is $V_e = 10.0 \text{ mL}$.
- Prior to V_e , there is a known excess of Cl^- . Find $[\text{Cl}^-]$ at the following volumes of added silver: $V_{\text{Ag}^+} = 0.10, 2.50, 5.00, 7.50,$ and 9.90 mL . From $[\text{Cl}^-]$, use K_{sp} for AgCl to find $[\text{Ag}^+]$ at each volume.
 - Find $[\text{Cl}^-]$ and $[\text{Ag}^+]$ at $V_{\text{Ag}^+} = V_e = 10.00 \text{ mL}$.
 - After V_e , there is a known excess of Ag^+ . Find $[\text{Ag}^+]$ at $V_{\text{Ag}^+} = 10.10$ and 12.00 mL .
 - Find the cell voltage at each volume in (a)–(c) and make a graph of the titration curve.

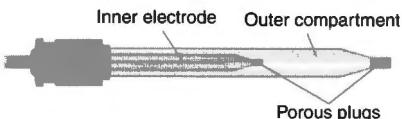


Figure 15-3 Double-junction reference electrode has an inner electrode identical to those in Figures 14-11 and 14-12. The outer compartment is filled with an electrolyte such as KNO_3 that is compatible with the titration solution. KCl electrolyte from the inner electrode slowly leaks into the outer electrode, so the outer electrolyte should be changed periodically. [Fisher Scientific, Pittsburgh, PA.]

15-2 | What Is a Junction Potential?

When two dissimilar electrolyte solutions are placed in contact, a voltage difference called the **junction potential** develops at the interface. This small, unknown voltage (usually a few millivolts) exists at each end of a salt bridge connecting two half-cells. *The junction potential puts a fundamental limitation on the accuracy of direct potentiometric measurements*, because we usually do not know the contribution of the junction to the measured voltage.

To see why a junction potential occurs, consider a solution containing NaCl in contact with pure water (Figure 15-4). The Na^+ and Cl^- ions diffuse from the NaCl solution into the water phase. However, Cl^- ion has a greater *mobility* than Na^+ . That is, Cl^- diffuses faster than Na^+ . As a result, a region rich in Cl^- , with excess negative charge, develops at the front. Behind it is a positively charged region depleted of Cl^- . The result is an electric potential difference at the junction of the NaCl and H_2O phases.

Mobilities of ions are shown in Table 15-2, and several junction potentials are listed in Table 15-3. Because K^+ and Cl^- have similar mobilities, junction potentials of a KCl salt bridge are slight. This is why saturated KCl is used in salt bridges.

$$E_{\text{observed}} = E_{\text{cell}} + E_{\text{junction}}$$

Because the junction potential is usually unknown, E_{cell} is uncertain.

Direct Versus Relative Potentiometric Measurements

In a *direct potentiometric measurement*, we use an electrode such as a silver wire to measure $[\text{Ag}^+]$ or a pH electrode to measure $[\text{H}^+]$ or a calcium ion-selective electrode to measure $[\text{Ca}^{2+}]$. There is inherent inaccuracy in most direct potentiometric measurements because there is usually a liquid-liquid junction with an unknown voltage difference making the intended indicator electrode potential uncertain. For example, Figure 15-5 shows a 4% standard deviation among 14 measurements by direct potentiometry. Part of the variation could be attributed to differences in the indicator (ion-selective) electrodes and part could be from varying liquid junction potentials.

By contrast, in *relative potentiometric measurements*, changes in the potential observed during a titration, such as that in Figure 6-5, are relatively precise and

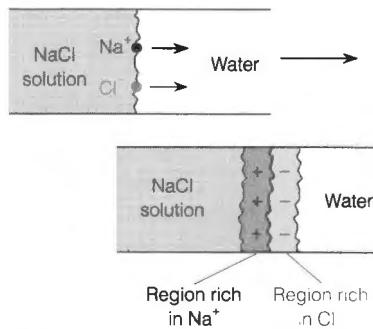


Figure 15-4 Development of the junction potential caused by unequal mobilities of Na^+ and Cl^- .

Table 15-2 Mobilities of ions in water at 25°C

Ion	Mobility [$\text{m}^2/(\text{s} \cdot \text{V})$] ^a	Ion	Mobility [$\text{m}^2/(\text{s} \cdot \text{V})$] ^a
H^-	36.30×10^{-8}	OH^-	20.50×10^{-8}
K^+	7.62×10^{-8}	SO_4^{2-}	8.27×10^{-8}
NH_4^+	7.61×10^{-8}	Br^-	8.13×10^{-8}
La^{3+}	7.21×10^{-8}	I^-	7.96×10^{-8}
Ba^{2+}	6.59×10^{-8}	Cl^-	7.91×10^{-8}
Ag^+	6.42×10^{-8}	NO_3^-	7.40×10^{-8}
Ca^{2+}	6.12×10^{-8}	ClO_4^-	7.05×10^{-8}
Cu^{2+}	5.56×10^{-8}	F^-	5.70×10^{-8}
Na^+	5.19×10^{-8}	CH_3CO_2^-	4.24×10^{-8}
Li^+	4.01×10^{-8}		

a. The mobility of an ion is the velocity achieved in an electric field of 1 V/m.
Mobility = velocity/field. The units of mobility are therefore (m/s)/(V/m) = $\text{m}^2/(\text{s} \cdot \text{V})$.

Table 15-3 Liquid junction potentials at 25°C

Junction	Potential (mV) ^a
0.1 M NaCl 0.1 M KCl	-6.4
0.1 M NaCl 3.5 M KCl	-0.2
1 M NaCl 3.5 M KCl	-1.9
0.1 M HCl 0.1 M KCl	+27
0.1 M HCl 3.5 M KCl	+3.1

a. A positive sign means that the right side of the junction becomes positive with respect to the left side.

permit an end point to be identified with little uncertainty. Measuring the *absolute* Ag^+ concentration by direct potentiometry is inherently inaccurate, but measuring *changes* in Ag^+ can be accurate and precise.

Ask Yourself

15-B. A 0.1 M NaCl solution is placed in contact with a 0.1 M NaNO_3 solution. The concentration of Na^+ is the same on both sides of the junction, so there is no net diffusion of Na^+ from one side to the other. The mobility of Cl^- is greater than that of NO_3^- , so Cl^- diffuses away from the NaCl side faster than NO_3^- diffuses away from NaNO_3 . Which side of the junction will become positive and which will become negative? Explain your reasoning.

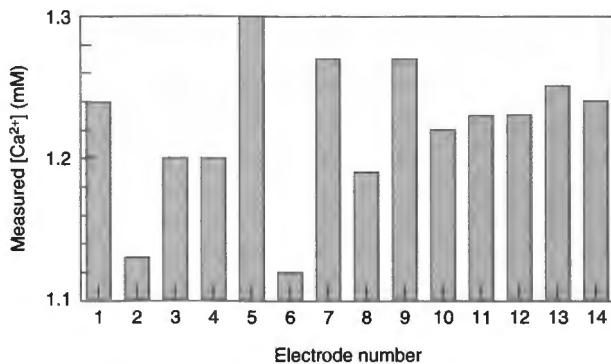
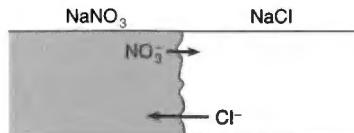


Figure 15-5 Response of 14 different Ca^{2+} ion-selective electrodes to identical human blood serum samples. The mean value is 1.22 ± 0.05 mM. [From M. Umemoto, W. Tani, K. Kuwa, and Y. Ujihira, *Anal. Chem.* **1994**, 66, 352A.]

15-3 How Ion-Selective Electrodes Work

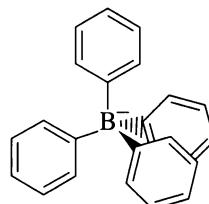
An **ion-selective electrode** responds preferentially to one species in a solution. Differences in concentration of the selected ion inside and outside the electrode produce a voltage difference across the membrane.²

Consider the *liquid-based ion-selective electrode* shown schematically in Figure 15-6a. This electrode develops a voltage that is related to the concentration of analyte cation C^+ in an unknown solution. The electrode is “liquid based” because the ion-selective membrane is a hydrophobic organic polymer impregnated with an organic liquid containing the hydrophobic anion, R^- , and a ligand, L, that selectively binds the analyte cation. R^- is an “ion exchanger” that reversibly associates with cations by electrostatic attraction. R^- is soluble in the organic phase, but not in water, so it is confined to the membrane.

The aqueous filling solution inside the electrode contains the ions $C^+(aq)$ and $B^-(aq)$. The outside of the electrode is immersed in unknown aqueous solution containing analyte $C^+(aq)$ and anion $A^-(aq)$. Ideally, it does not matter what A^- and B^- are. The electric potential difference (the voltage) across the ion-selective membrane is measured by two reference electrodes, which might be $\text{Ag} \mid \text{AgCl}$. If the concentration of C^+ in the unknown solution changes, the voltage changes. With the use of a calibration curve, the voltage tells us the concentration of C^+ in the analyte solution.

Hydrophobic: “water hating” (does not mix with water)

Example of hydrophobic anion, R^- :



Tetraphenylborate, $(\text{C}_6\text{H}_5)_4\text{B}^-$

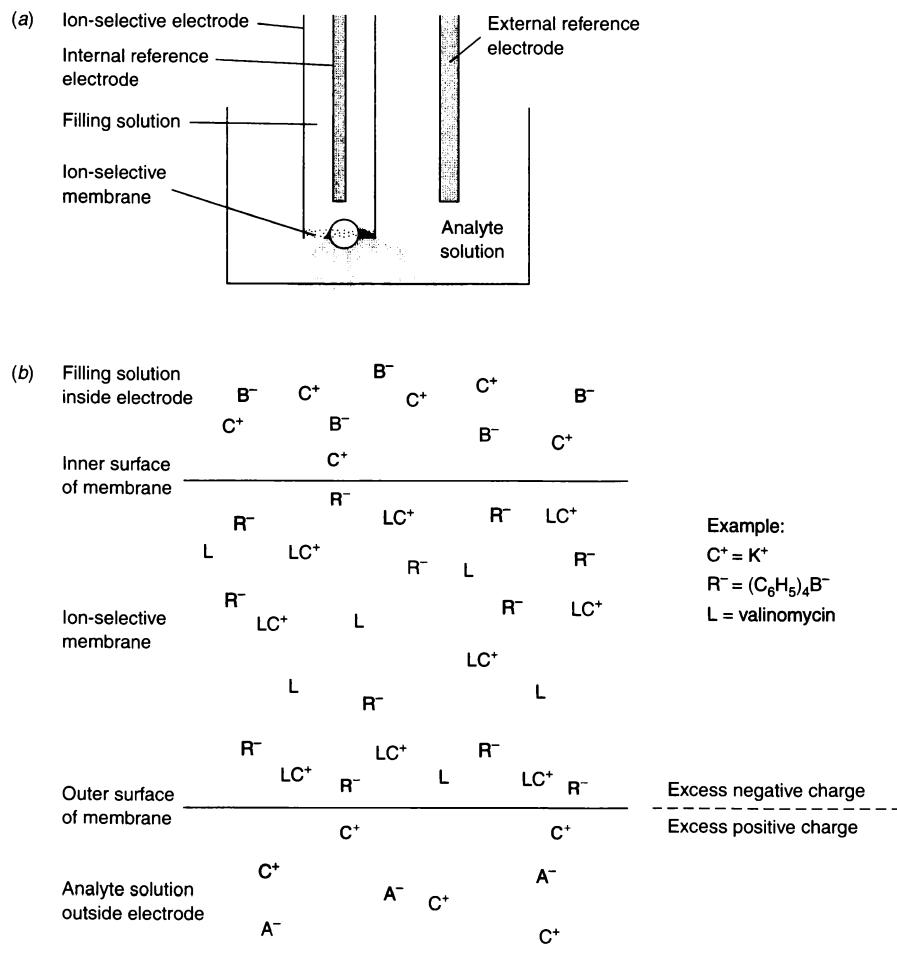
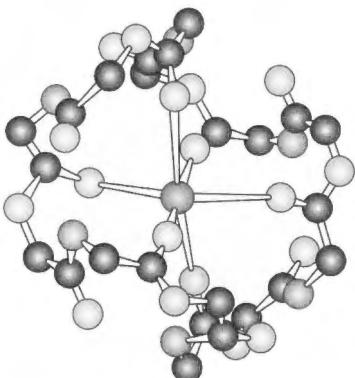


Figure 15-6 (a) Ion-selective electrode immersed in aqueous solution containing analyte cation C^+ . Typically, the membrane is made of poly(vinyl chloride) impregnated with a nonpolar liquid containing the ionophore L, the complex LC^- , and a hydrophobic anion R^- . (b) Close-up of membrane. Ellipses encircling pairs of ions are a guide for the eye to count the charge in each phase. Colored ions represent excess charge in each phase.



K^+ -valinomycin complex

Key \bullet K^+ \circ O \circ N \bullet C

Valinomycin has a cyclic structure containing six amino acids and six carboxylic acids. Isopropyl and methyl substituents are not shown in this diagram. [From L. Stryer, *Biochemistry*, 4th ed. (New York: W. H. Freeman and Company, 1995).]

The electrode really responds to the *activity* of analyte (Section 12-2), not the concentration. In this book, we will write concentrations instead of activities.

Chemists use molecular modeling calculations to design synthetic ligands with high selectivity for a specific ion.

Figure 15-6b shows how the electrode works. The key in this example is the ligand, L (called an *ionophore*), which is soluble inside the membrane and selectively binds analyte ion. In a potassium ion-selective electrode, for example, L could be valinomycin, a natural antibiotic secreted by certain microorganisms to carry K^+ ion across cell membranes. The ligand L is chosen to have a high affinity for analyte cation C^+ and low affinity for other ions.

Almost all the analyte ion inside the membrane in Figure 15-6b is bound in the complex LC^+ , which is in equilibrium with a small amount of free C^+ . The membrane also contains excess free L. C^+ can diffuse across the interface. In an ideal electrode, R^- cannot leave the membrane because it is not soluble in water; the aqueous anion A^- cannot enter the membrane because it is not soluble in the organic phase. As soon as a tiny number of C^+ ions diffuse from the membrane into the aqueous phase, there is excess positive charge in the first few nanometers of the aqueous phase and excess negative charge in the outer few nanometers of the membrane. This imbalance creates an electric potential difference that opposes diffusion of more C^+ into the aqueous phase.

The excess positive charge (C^+) in the outer (unknown) aqueous solution depends on the concentration of C^+ in the unknown. The excess positive charge in the inner aqueous solution is constant because the inner solution has a constant composition. Thermodynamics predicts that the potential difference between the outer and the inner solutions is

Electric potential difference for ion-selective electrode:

$$E = \frac{0.05916}{n} \log \left(\frac{[\text{C}^+]_{\text{outer}}}{[\text{C}^+]_{\text{inner}}} \right) \quad (\text{volts at } 25^\circ\text{C}) \quad (15-5)$$

where n is the charge of the analyte ion, $[\text{C}^+]_{\text{outer}}$ is its concentration in the outer (unknown) solution, and $[\text{C}^+]_{\text{inner}}$ is its concentration in the inner solution (which is constant). Equation 15-5 applies to any ion-selective electrode, including a glass pH electrode. If analyte is an anion, the sign of n is negative. Later, we will modify the equation to account for interfering ions.

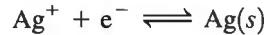
If C^+ were K^+ , then $n = +1$ and there would be a potential increase of $+0.059\ 16\ \text{V}$ for every factor-of-10 increase of $[\text{K}^+]$ in the analyte (outer) solution. If C^+ were Ca^{2+} , then $n = +2$ and there would be a potential increase of $+0.059\ 16/2 = +0.029\ 58\ \text{V}$ for every factor-of-10 increase in $[\text{Ca}^{2+}]$ in the unknown. For a carbonate electrode, $n = -2$ and there is a potential decrease of $-0.059\ 16/2\ \text{V}$ for every factor-of-10 increase in $[\text{CO}_3^{2-}]$.

The key feature of an ion-selective electrode is a membrane that selectively binds the analyte of interest. No membrane is perfectly selective, so there is always some interference from unintended species.

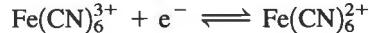
Two Classes of Indicator Electrodes

Metal electrodes such as silver or platinum function as surfaces on which redox reactions can take place:

Equilibrium on a silver electrode:



Equilibrium on a platinum electrode:



Ion-selective electrodes such as a calcium electrode or a glass pH electrode selectively bind analyte ion. *There is no redox chemistry in the ion-selective electrode.* The potential across the electrode membrane depends on the concentration of analyte ion in the unknown.

Ask Yourself

- 15-C. (a) Predict the change in voltage across the membranes of ion-selective electrodes for NH_4^+ , F^- , and S^{2-} for a 10-fold increase in analyte concentration.
 (b) The ion-selective membrane in Figure 15-6 contains the hydrophobic anion R^- = tetraphenylborate and the neutral ligand L = valinomycin to bind K^+ . A CO_3^{2-} ion-selective electrode contains a neutral ligand L that forms $\text{L}(\text{CO}_3^{2-})(\text{H}_2\text{O})$. Which hydrophobic ion, $\text{R}^- = (\text{C}_6\text{H}_5)_4\text{B}^-$ or $\text{R}^+ = (\text{C}_{12}\text{H}_{25})_3\text{NCH}_3^+$ (tridodecylmethylammonium) is required for the ion-selective membrane? Why?

15-4 pH Measurement with a Glass Electrode

The most widely employed ion-selective electrode is the **glass electrode** for measuring pH. A pH electrode responds selectively to H^+ , with a potential difference of 0.059 16 V for every factor-of-10 change in $[\text{H}^+]$. A factor-of-10 difference in $[\text{H}^+]$ is one pH unit, so a change of, say, 4.00 pH units leads to a change in electrode potential of $4.00 \times 0.059 16 \text{ V} = 0.237 \text{ V}$.

A **combination electrode**, incorporating both the glass and reference electrodes in one body, is shown in Figure 15-7. The line diagram of this cell is

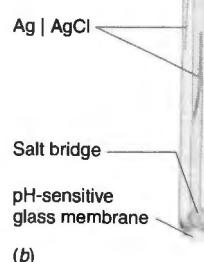
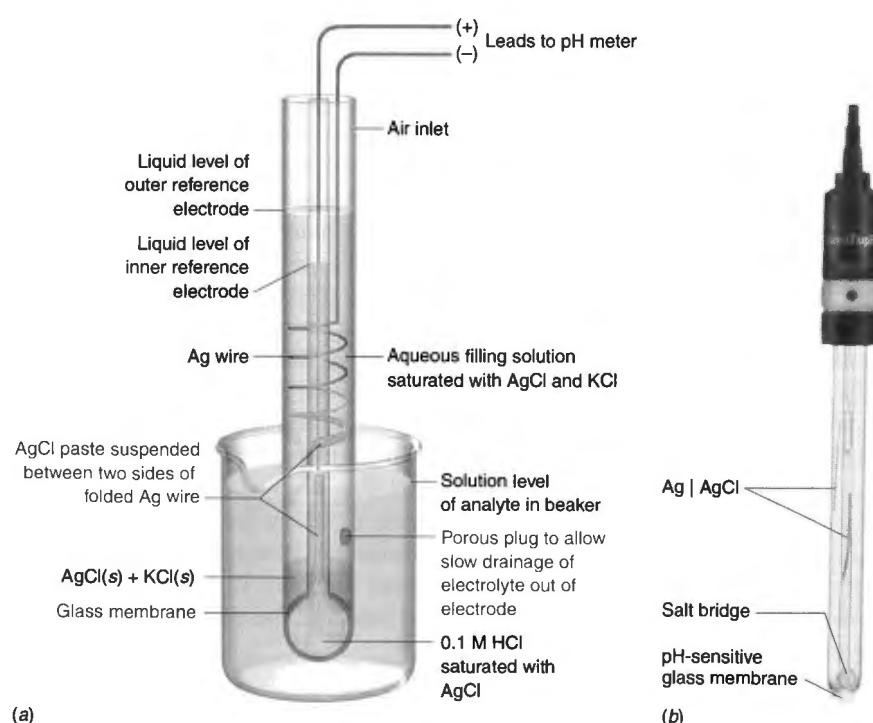
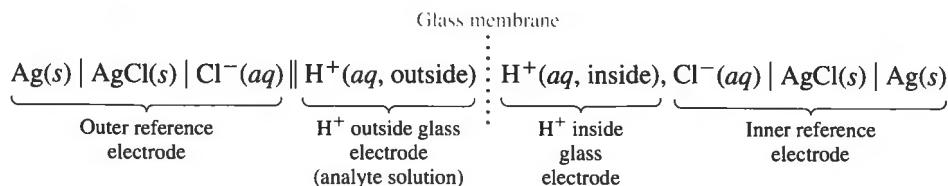


Figure 15-7 (a) Glass combination electrode with a silver-silver chloride reference electrode. The glass electrode is immersed in a solution of unknown pH so that the porous plug on the lower right is below the surface of the liquid. The two $\text{Ag} | \text{AgCl}$ electrodes measure the voltage across the glass membrane. (b) Photograph of combination electrode with pH-sensitive glass bulb at the bottom. The salt bridge is the porous junction to the reference electrode compartment. [Part (b): Fisher Scientific, Pittsburgh, PA.]

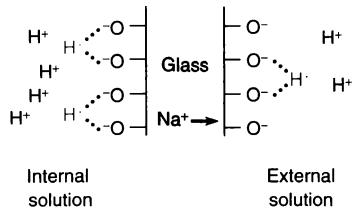


Figure 15-8 Ion-exchange equilibria on the inner and outer surfaces of the glass membrane. The pH of the internal solution is fixed. As the pH of the external solution (the sample) changes, the electric potential difference across the glass membrane changes.

The pH-sensitive part of the electrode is the thin glass membrane in the shape of a bulb at the bottom of the electrode.³

The glass membrane at the bottom of the pH electrode consists of an irregular network of SiO_4 tetrahedra through which Na^+ ions move sluggishly. Studies with tritium (the radioactive isotope ${}^3\text{H}$) show that H^+ does *not* diffuse through the membrane. The glass surface contains exposed $-\text{O}^-$ that can bind H^+ from the solutions on either side of the membrane (Figure 15-8). H^+ equilibrates with the glass surface, giving the side of the membrane exposed to the higher concentration of H^+ a more positive charge. To measure a potential difference, at least some tiny amount of electric current must flow through the complete circuit. Na^+ ions in the glass carry electric current by migrating across the membrane. The resistance of the glass membrane is high, so little current flows across it.

The potential difference between the inner and outer silver-silver chloride electrodes in Figure 15-7 depends on $[\text{Cl}^-]$ in each electrode compartment and on the potential difference across the glass membrane. Because $[\text{Cl}^-]$ is fixed and because $[\text{H}^+]$ is constant inside the glass electrode, the only variable is the pH of the analyte solution outside the glass membrane.

Real glass electrodes are described by the equation

$$\text{Response of glass electrode: } E = \text{constant} + \beta(0.059\ 16)\Delta\text{pH} \quad (\text{at } 25^\circ\text{C}) \quad (15-6)$$

where ΔpH is the difference in pH between analyte and the solution inside the glass bulb. The factor β , which is ideally 1, is typically 0.98–1.00. The constant term, called the *asymmetry potential*, arises because no two sides of a real object are identical, so a small voltage exists even if the pH is the same on both sides of the membrane. We correct for asymmetry, and we measure β by calibrating the electrode in solutions of known pH.

Calibrating a Glass Electrode

A pH electrode *must* be calibrated before use. It should be calibrated every 2 h in sustained use. Ideally, calibration standards should bracket the pH of the unknown.

Don't leave a glass electrode out of water (or in a nonaqueous solvent) longer than necessary.

Before using a pH electrode, be sure that the air inlet near the upper end of the electrode in Figure 15-7a is not capped. (This hole is capped during storage to prevent evaporation of the reference electrode filling solution.) Wash the electrode with distilled water and gently *blot* it dry with a tissue. Do not *wipe* it because this action might produce a static charge on the glass. Dip the electrode in a standard buffer whose pH is near 7 and allow the electrode to equilibrate with stirring for at least a minute. Following the manufacturer's instructions, press a key that might say "calibrate" or "read" on a microprocessor-controlled meter or adjust the reading of an analog meter to indicate the pH of the standard buffer. Wash the electrode with water, blot it dry, and immerse it in a second standard whose pH is further from 7 than the pH of the first standard. Enter the second buffer on the meter. If the electrode were ideal, the voltage would change by 0.059 16 V per pH unit at 25°C ; the actual change may be slightly less. These two measurements establish the values of β and the constant in Equation 15-6. Finally, dip the electrode in unknown, stir the liquid, allow the reading to stabilize, and read the pH.

Store the glass electrode in aqueous solution to prevent dehydration of the glass. Ideally, the solution should be similar to that inside the reference compartment of the electrode. Distilled water is *not* a good storage medium. If the electrode is dry, recondition it in aqueous solution for several hours. If the electrode is to be used above pH 9, soak it in a high-pH buffer.

If electrode response becomes sluggish or if the electrode cannot be calibrated properly, try soaking it in 6 M HCl, followed by water. As a last resort, dip the

electrode in 20 wt% aqueous ammonium bifluoride, NH_4HF_2 in a plastic beaker for 1 min. This reagent dissolves glass, exposing fresh surface. Wash the electrode with water and try calibrating it again. **CAUTION** *Ammonium bifluoride must not contact your skin, because it produces HF burns.* (See page 289 for precautions with HF.)

Errors in pH Measurement

To use a glass electrode intelligently, you should understand its limitations:

- 1. Standards.** A pH measurement cannot be more accurate than our pH standards, typically ± 0.01 – 0.02 pH units.
- 2. Junction potential.** A junction potential exists at the porous plug near the bottom of the electrode in Figure 15-7. If the ionic composition of analyte is different from that of the standard buffer, the junction potential will change *even if the pH of the two solutions is the same*. This factor gives an uncertainty of at least 0.01 pH unit. Box 15-1 describes how junction potentials affect the measurement of the pH of rainwater.

Box 15-1 Systematic Error in Rainwater pH Measurement: The Effect of Junction Potential

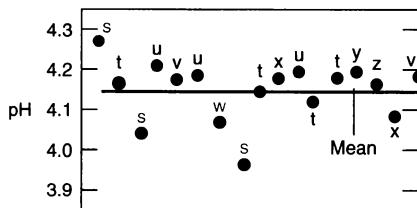
The opening of Chapter 8 shows the pH of rainfall over the U.S. and Europe. Acidity in rainfall is partly a result of human activities and is slowly changing the nature of many ecosystems. Monitoring the pH of rainwater is a critical component of programs to reduce the production of acid rain.

To identify and correct systematic errors in the measurement of pH of rainwater, 8 samples were provided to each of 17 laboratories, along with explicit instructions for how to conduct the measurements. Each lab used two standard buffers to calibrate its pH meter.

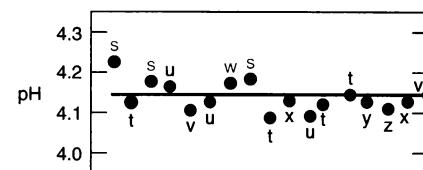
Panel *a* shows typical results for the pH of rainwater. The average of the 17 measurements is given by the horizontal line at pH 4.14, and the letters *s*, *t*, *u*, *v*, *w*, *x*, *y*, and *z* identify the types of pH electrodes used for the measurements. Laboratories using electrode types *s* and *w* had relatively large systematic errors. The type *s* electrode was a combination electrode (Figure 15-7) containing a reference electrode liquid junction with an

exceptionally large area. Electrode type *w* had a reference electrode filled with a gel.

Variability in the liquid junction potential (Section 15-2) was hypothesized to lead to the variability in the pH measurements. Standard buffers used for pH meter calibration typically have ion concentrations of ~ 0.05 M, whereas rainwater samples have ion concentrations two or more orders of magnitude lower. To test the hypothesis that junction potential caused systematic errors, a pure HCl solution with a concentration near 2×10^{-4} M was used as a pH calibration standard in place of high ionic strength buffers. The data in panel *b* were obtained, with good results from all but the first lab. The standard deviation of all 17 measurements was reduced from 0.077 pH unit with the standard buffer to 0.029 pH unit with the HCl standard. It was concluded that junction potential is the cause of most of the variability between labs and that a low ionic strength standard is appropriate for rainwater pH measurements.



(a) pH of rainwater from identical samples measured at 17 different labs using standard calibration buffers. Letters designate different types of pH electrodes.



(b) Rainwater pH measured after using low-ionic-strength HCl for calibration. [W. F. Koch, G. Marinenko, and R. C. Paule, *J. Res. Natl. Bur. Stand.* **1986**, 91, 23.]

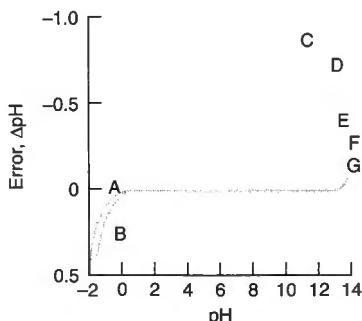


Figure 15-9 Acid and alkaline errors of some glass electrodes. A: Corning 015, H_2SO_4 . B: Corning 015, HCl. C: Corning 015, 1 M Na^+ . D: Beckman-GP, 1 M Na^+ . E: L & N Black Dot, 1 M Na^+ . F: Beckman Type E, 1 M Na^+ . G: Ross electrode.⁴ [From R. G. Bates, *Determination of pH: Theory and Practice*, 2nd ed. (New York: Wiley, 1973). Ross electrode data is from Orion, *Ross pH Electrode Instruction Manual*.]

Challenge Show that the potential of the glass electrode changes by 1.3 mV when the analyte H^+ concentration changes by 5.0%. Because $59 \text{ mV} \approx 1 \text{ pH unit}$, $1.3 \text{ mV} = 0.02 \text{ pH unit}$.

Moral: A small uncertainty in voltage (1.3 mV) or pH (0.02 units) corresponds to a large uncertainty (5%) in H^+ concentration. Similar uncertainties arise in other potentiometric measurements.

3. Junction potential drift. Most combination electrodes have a silver-silver chloride reference electrode containing saturated KCl solution. More than 350 mg of silver per liter dissolve in the KCl (mainly as AgCl_4^{3-} and AgCl_3^{2-}). In the porous plug salt bridge in Figure 15-7, KCl becomes dilute and AgCl precipitates in the plug. If analyte contains a reducing agent, $\text{Ag}(s)$ also can precipitate in the plug. Both effects change the junction potential, causing slow drift of the pH reading. Compensate for this error by recalibrating the electrode every 2 h.

4. Sodium error. When $[\text{H}^+]$ is very low and $[\text{Na}^+]$ is high, the electrode responds to Na^+ as if Na^+ were H^+ , and the apparent pH is lower than the true pH. This response is called the *alkaline error or sodium error* (Figure 15-9).

5. Acid error. In strong acid, the measured pH is higher than the actual pH, for reasons that are not well understood (Figure 15-9).

6. Equilibration time. In a well-buffered solution with adequate stirring, equilibration of the glass with analyte solution takes seconds. In a poorly buffered solution near the equivalence point of a titration, it could take minutes.

7. Hydration. A dry electrode takes several hours in aqueous solution before it responds to H^+ correctly.

8. Temperature. A pH meter should be calibrated at the same temperature at which the measurement will be made. You cannot calibrate your equipment at one temperature and make accurate measurements at a second temperature.

Errors 1 and 2 limit the accuracy of pH measurements with the glass electrode to $\pm 0.02 \text{ pH unit}$, at best. Measurement of pH differences can be accurate to $\pm 0.002 \text{ pH unit}$, but knowledge of the true pH will still be at least an order of magnitude more uncertain. An uncertainty of $\pm 0.02 \text{ pH unit}$ corresponds to an uncertainty of $\pm 5\%$ in $[\text{H}^+]$.

Solid-State pH Sensors

Some pH sensors do not depend on a fragile glass membrane. The *field effect transistor* in Figure 15-10 is a tiny semiconductor device whose surface binds H^+ from the medium in which the transistor is immersed. The higher the concentration of H^+ in the external medium, the more positively charged is the transistor's surface. The surface charge regulates the flow of current through the transistor, which therefore behaves as a pH sensor.

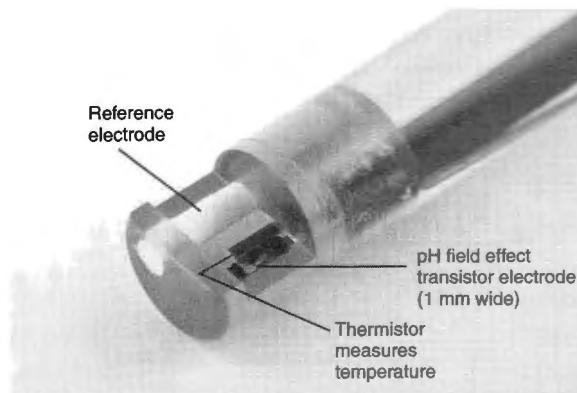


Figure 15-10 Combination pH electrode based on field effect transistor. The thermistor senses temperature and is used for automatic temperature compensation. [Courtesy Sentron, Europe BV.]

Ask Yourself

- 15-D. (a) List the sources of error associated with pH measurements made with the glass electrode.
- (b) When the difference in pH across the membrane of a glass electrode at 25°C is 4.63 pH units, how much voltage is generated by the pH gradient? Assume that the constant β in Equation 15-6 is 1.00.
- (c) Why do glass electrodes indicate a pH lower than the actual pH in 0.1 M NaOH?

15-5 Ion-Selective Electrodes

The glass pH electrode is an example of a *solid-state ion-selective electrode* whose operation depends on (1) an ion-exchange reaction of H^+ between the glass surface and analyte solution and (2) transport of Na^+ across the glass membrane. We now examine several ion-selective electrodes.

Solid-State Electrodes

The ion-sensitive component of a fluoride **solid-state ion-selective electrode** is a crystal of LaF_3 doped with EuF_2 (Figure 15-11a). *Doping* is the addition of an impurity (EuF_2 in this case) to the solid crystal (LaF_3). The inner surface of the crystal is exposed to filling solution with a constant concentration of F^- . The outer surface is exposed to a variable concentration of F^- in the unknown. Fluoride on each surface of the crystal equilibrates with F^- in the solution contacting that surface. Anion vacancies in doped LaF_3 permit F^- to jump from one site to the next, thereby transporting electric charge across the crystal (Figure 15-11b). So few ions cross the crystal that there is a negligible effect on concentrations in solution.

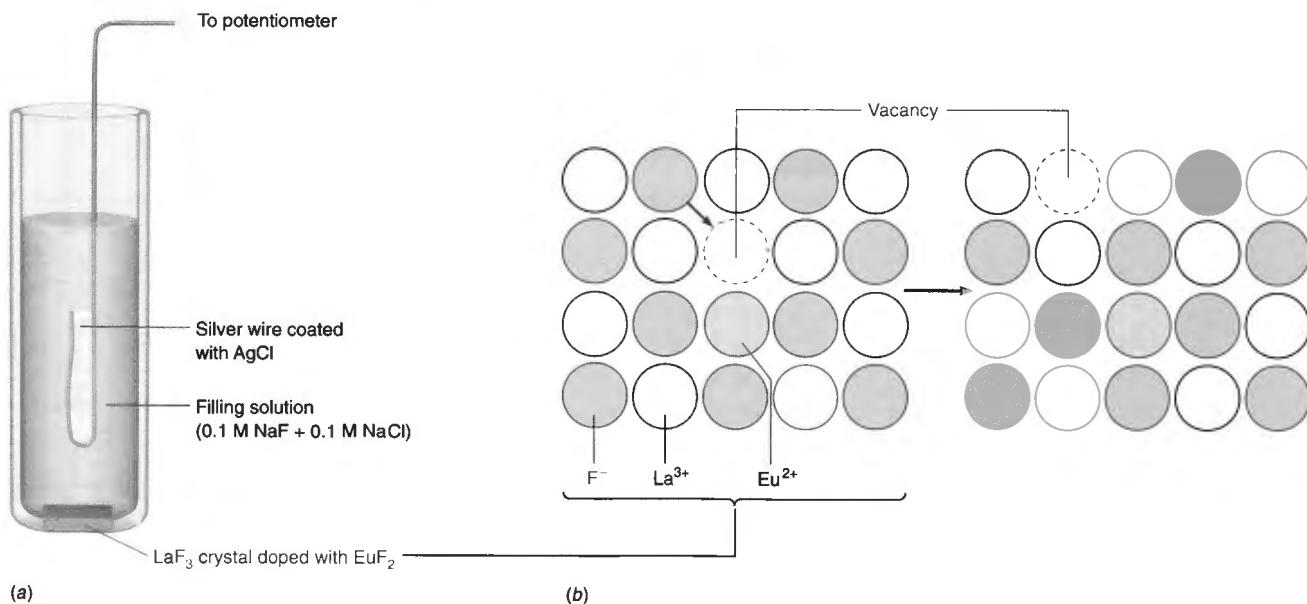


Figure 15-11 (a) Fluoride ion-selective electrode employs a crystal of LaF_3 doped with EuF_2 as the ion-selective membrane. (b) Migration of F^- through the doped crystal: For charge conservation, every Eu^{2+} is accompanied by an anion vacancy in the crystal. When a neighboring F^- jumps into the vacancy, another site becomes vacant. Repetition of this process moves F^- through the lattice.

Table 15-4 Solid-state ion-selective electrodes

Ion	Concentration range (M)	Membrane crystal ^a	pH range	Interfering species
F ⁻	10 ⁻⁶ –1	LaF ₃	5–8	OH ⁻
Cl ⁻	10 ⁻⁴ –1	AgCl	2–11	CN ⁻ , S ²⁻ , I ⁻ , S ₂ O ₃ ²⁻ , Br ⁻
Br ⁻	10 ⁻⁵ –1	AgBr	2–12	CN ⁻ , S ²⁻ , I ⁻
I ⁻	10 ⁻⁶ –1	AgI	3–12	S ²⁻
CN ⁻	10 ⁻⁶ –10 ⁻²	AgI	11–13	S ²⁻ , I ⁻
S ²⁻	10 ⁻⁵ –1	Ag ₂ S	13–14	

a. Electrodes containing silver-based crystals such as Ag₂S should be stored in the dark and protected from light during use to prevent light-induced chemical degradation.

The response of the electrode to F⁻ is

Electrode response depends on

$$\log\left(\frac{[F^-]_{\text{outside}}}{[F^-]_{\text{inside}}}\right)$$

The constant value of [F⁻]_{inside} is incorporated into the constant term in Equation 15-7.

$$\text{Response of } F^- \text{ electrode: } E = \text{constant} - \beta(0.059 16) \log[F^-]_{\text{outside}} \quad (15-7)$$

where [F⁻]_{outside} is the concentration of F⁻ in analyte solution and β is close to 1.00. The electrode response is close to 59 mV per decade (factor of 10) over a F⁻ concentration range from about 10⁻⁶ M to 1 M. The electrode is >10³ times more responsive to F⁻ than to most other ions. However, response to OH⁻ is one-tenth as great as the response to F⁻, so OH⁻ causes serious interference. At low pH, F⁻ is converted into HF (pK_a = 3.17), to which the electrode is insensitive. Fluoride is added to drinking water to help prevent tooth decay. The F⁻ electrode is used to monitor and control the fluoridation of municipal water supplies. Several other solid-state ion-selective electrodes are listed in Table 15-4.

Example Calibration Curve for an Ion-Selective Electrode

A fluoride electrode immersed in standard solutions gave the following potentials:

[F ⁻] (M)	log[F ⁻]	E (mV vs S.C.E.)
1.00 × 10 ⁻⁵	5.00	100.0
1.00 × 10 ⁻⁴	4.00	41.4
1.00 × 10 ⁻³	3.00	-17.0
1.00 × 10 ⁻²	2.00	-75.4

- (a) What potential is expected if [F⁻] = 5.00 × 10⁻⁵ M? (b) What concentration of F⁻ will give a potential of 0.0 mV?

SOLUTION (a) Our strategy is to fit the calibration data with Equation 15-7 and then to substitute the concentration of F⁻ into the equation to find the potential:

$$E = \text{constant} - m \cdot \log[F^-]$$

y $\underbrace{\text{Intercept}}$ $\underbrace{\text{Slope}}$ x

Using the method of least squares from Chapter 4, we plot E versus $\log[F^-]$ to find a straight line with a slope of -58.46 mV and an intercept of -192.4 mV (Figure 15-12). Setting $[F^-] = 5.00 \times 10^{-5} \text{ M}$ gives

$$E = -192.4 - 58.46 \log[5.00 \times 10^{-5}] = 59.0 \text{ mV}$$

(b) If $E = 0.0 \text{ mV}$, we can solve for the concentration of $[F^-]$:

$$0.0 = -192.4 - 58.46 \log[F^-] \Rightarrow [F^-] = 5.1 \times 10^{-4} \text{ M}$$

 **Test Yourself** Find $[F^-]$ if $E = -22.3 \text{ mV}$. (Answer: 1.23 mM)

Liquid-Based Ion-Selective Electrodes

The principle of a **liquid-based ion-selective electrode** was described in Figure 15-6. Figure 15-13 shows a Ca^{2+} ion-selective electrode, featuring a hydrophobic poly(vinyl chloride) membrane saturated with a neutral Ca^{2+} -binding ligand (L) and a salt of a hydrophobic anion (Na^+R^-) dissolved in a hydrophobic liquid (Figure 15-14). The response is

$$\begin{array}{l} \text{Response of } \text{Ca}^{2+} \\ \text{electrode:} \end{array} E = \text{constant} + \beta \left(\frac{0.059 16}{2} \right) \log[\text{Ca}^{2+}]_{\text{outside}} \quad (15-8)$$

where β is close to 1.00. Equations 15-8 and 15-7 have different signs before the log term because one involves an anion and the other a cation. The charge of Ca^{2+} requires a factor of 2 in the denominator before the logarithm. The liquid-based NH_4^+ ion-selective electrode used to measure ammonia in marine sediments in Box 6-1 is described in Box 15-2.

Selectivity Coefficient

No electrode responds exclusively to one kind of ion, but the glass electrode is among the most selective. A high-pH glass electrode responds to Na^+ only when $[\text{H}^+] \leq 10^{-12} \text{ M}$ and $[\text{Na}^+] \geq 10^{-2} \text{ M}$ (Figure 15-9 electrodes E and F).

If an electrode designed to measure ion A also responds to interfering ion X, the **selectivity coefficient** is defined as

$$\text{Selectivity coefficient: } K_{A,X}^{\text{Pot}} = \frac{\text{response to X}}{\text{response to A}} \quad (15-9)$$

The smaller the selectivity coefficient, the less the interference by X. A K^+ ion-selective electrode that uses valinomycin as the liquid ion exchanger has selectivity coefficients $K_{\text{K}^+, \text{Na}^+}^{\text{Pot}} = 1 \times 10^{-5}$, $K_{\text{K}^+, \text{Cs}^+}^{\text{Pot}} = 0.44$, and $K_{\text{K}^+, \text{Rb}^+}^{\text{Pot}} = 2.8$. These coefficients tell us that Na^+ hardly interferes with the measurement of K^+ , but Cs^+ and Rb^+ strongly interfere.

For interfering ions, X, with the same charge as the primary ion, A, the response of ion-selective electrodes is described by the equation

$$\begin{array}{l} \text{Response of ion-} \\ \text{selective electrode:} \end{array} E = \text{constant} + \beta \left(\frac{0.059 16}{n} \right) \log \left[[A] + \sum_X K_{A,X}^{\text{Pot}} [X] \right] \quad (15-10)$$

where n is the charge of A. β is near 1 for most electrodes.

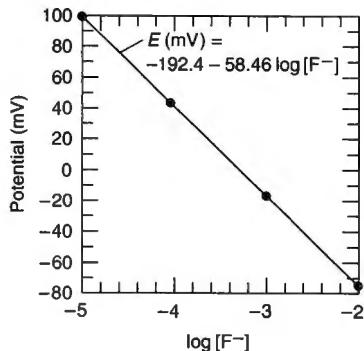


Figure 15-12 Calibration curve for fluoride ion-selective electrode.

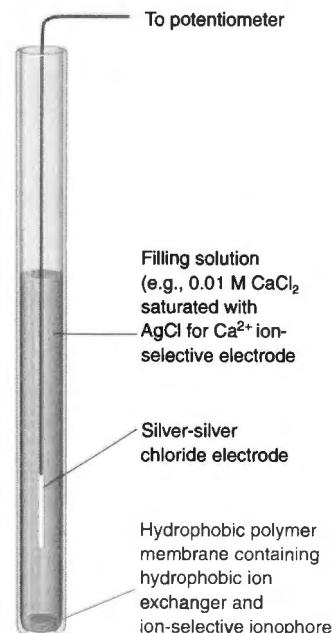
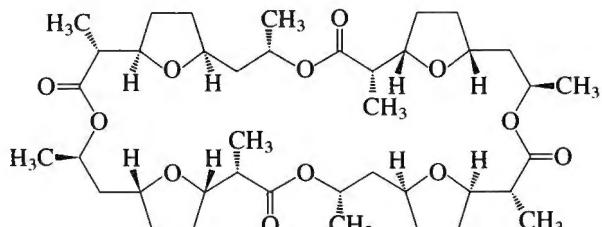


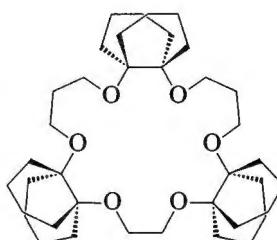
Figure 15-13 Calcium ion-selective electrode with a liquid ion exchanger. Figure 15-5 shows the variability in response from different electrodes.

Equation 15-10 describes the response of an electrode to its primary ion, A, and to interfering ions, X, of the same charge.

Box 15-2 Ammonium Ion-Selective Microelectrode



Nonactin—a natural antibiotic isolated from fermentation

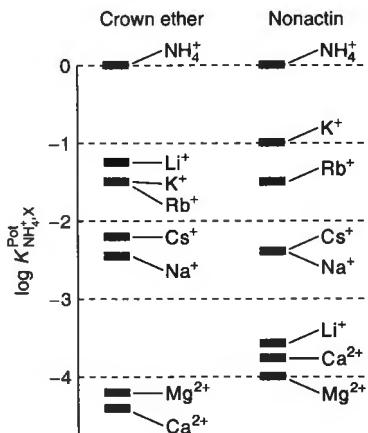
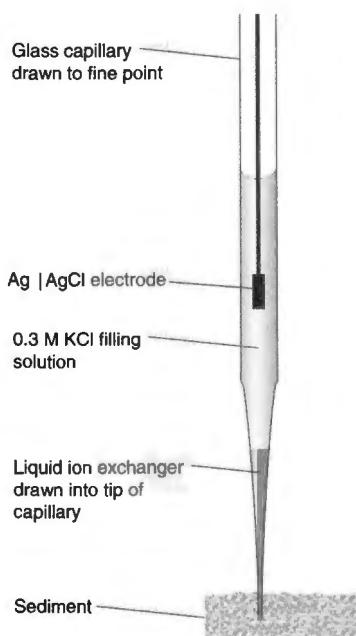


Synthetic crown ether
with improved selectivity for
 NH_4^+ over K^+

Box 6-1 describes a marine ecosystem in which NH_3 is oxidized to NO_2^- (nitrite) and then to NO_3^- (nitrate). Ammonium ion in the top millimeter of sediment was measured with a microelectrode made by drawing a glass capillary tube to a fine point with an opening diameter of $1 \mu\text{m}$. Liquid ion exchanger drawn into the tip of the capillary serves as the ion-selective membrane in Figure 15-6. The natural antibiotic nonactin is used as the ligand L in Figure 15-6. It selectively binds

ammonia in a cage of oxygen atoms. Other components of the ion exchanger are sodium tetraphenylborate to provide the hydrophobic anion R^- and *o*-nitrophenyl octyl ether as the hydrophobic solvent.

Current research is aimed at finding ligands that discriminate better between NH_4^+ and K^+ . The synthetic crown ether shown above has a selectivity coefficient $K_{\text{NH}_4^+, \text{K}^+}^{\text{Pot}} = 0.03$, whereas the selectivity of nonactin is only $K_{\text{NH}_4^+, \text{K}^+}^{\text{Pot}} = 0.1$. Note that the smaller the selectivity coefficient in Equation 15-9, the more selective is the ligand. The diagram compares selectivity coefficients for the two ligands with various interfering ions.



Selectivities of nonactin and synthetic crown ether.
[Data from S. Sasaki, T. Amano, G. Monma, T. Otsuka, N. Iwasawa, D. Citterio, H. Hisamoto, and K. Suzuki, *Anal. Chem.* **2002**, 74, 4845.]

The most serious interference for the Ca^{2+} electrode containing the liquids in Figure 15-14 comes from Sr^{2+} , with a selectivity coefficient $K_{\text{Ca}^{2+}, \text{Sr}^{2+}}^{\text{Pot}} = 0.13$. That is, the response to Sr^{2+} is 13% as great as the response to an equal concentration of Ca^{2+} . For most cations, $K^{\text{Pot}} < 10^{-3}$. It is a good idea to hold pH and ionic strength of standards and unknowns constant when using ion-selective electrodes.

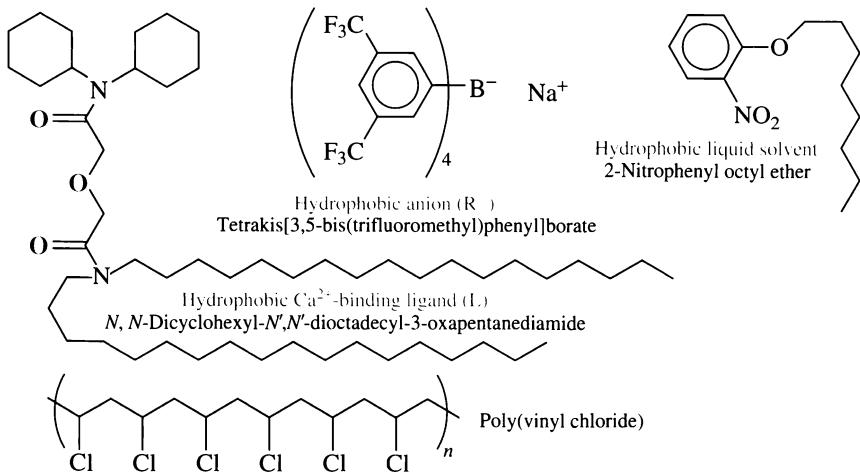


Figure 15-14 Components of the liquid phase in the membrane at the base of the Ca^{2+} -ion selective electrode in Figure 15-13.

Example Using the Selectivity Coefficient

A fluoride ion-selective electrode has a selectivity coefficient $K_{\text{F}^-, \text{OH}^-}^{\text{pot}} = 0.1$. What will be the change in electrode potential when $1.0 \times 10^{-4} \text{ M F}^-$ at pH 5.5 is raised to pH 10.5?

SOLUTION If $n = -1$ and $\beta = 1$ in Equation 15-10, the potential with negligible OH^- at pH 5.5 is

$$E = \text{constant} - 0.05916 \log[1.0 \times 10^{-4}] = \text{constant} + 236.6 \text{ mV}$$

At pH 10.50, $[\text{OH}^-] = 3.2 \times 10^{-4} \text{ M}$, so the electrode potential is

$$\begin{aligned} E &= \text{constant} - 0.05916 \log[(1.0 \times 10^{-4}) + (0.1)(3.2 \times 10^{-4})] \\ &= \text{constant} + 229.5 \text{ mV} \end{aligned}$$

The change is $229.5 - 236.6 = -7.1 \text{ mV}$, which is quite significant. If you didn't know about the pH change, you would think that the concentration of F^- had increased by 32%.

Test Yourself Show that a change of -7.1 mV is equivalent to an increase in $[\text{F}^-]$ of 32%.

Ion-Selective Electrode Detection Limits⁵

The black curve in Figure 15-15 was typical of liquid-based ion-selective electrodes until recently. The response of this Pb^{2+} electrode levels off at an analyte concentration around 10^{-6} M . The electrode detects changes in concentration above 10^{-6} M but not below 10^{-6} M . The filling solution inside the electrode contains 0.5 mM PbCl_2 .

The colored curve in Figure 15-15 was obtained with the same electrode components, but the internal filling solution was replaced by a *metal ion buffer* that fixes $[\text{Pb}^{2+}]$ at 10^{-12} M . Now the electrode responds to changes in analyte Pb^{2+} concentration down to $\sim 10^{-11} \text{ M}$.

The sensitivity of liquid-based ion-selective electrodes has been limited by leakage of the primary ion (Pb^{2+} in this case) from the internal filling solution through the ion-exchange membrane. By lowering the concentration of primary ion inside the electrode, the concentration of leaking ion outside the membrane is reduced by orders of magnitude and the detection limit is correspondingly reduced. Not only is

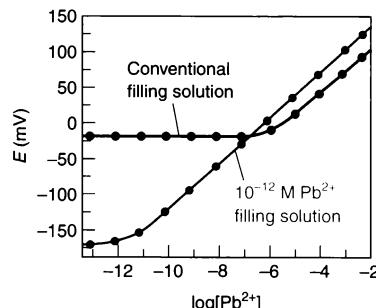


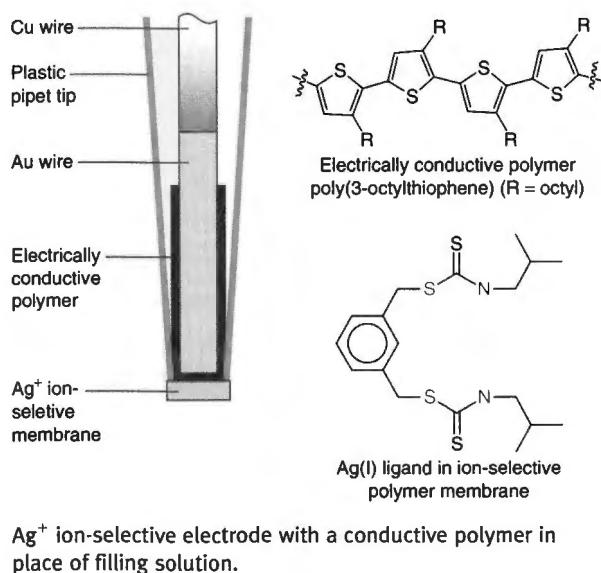
Figure 15-15 Response of Pb^{2+} liquid-based ion-selective electrode with (black curve) conventional filling solution containing 0.5 mM Pb^{2+} or (colored curve) metal ion buffer filling solution with $[\text{Pb}^{2+}] = 10^{-12} \text{ M}$. [T. Sokalski, A. Ceresa, T. Zwickl, and E. Pretsch, *J. Am. Chem. Soc.* **1997**, *119*, 11347.]

Problem 15-27 describes the metal ion buffer.

Box 15-3 Protein Immunosensing by Ion-Selective Electrodes with Electrically Conductive Polymers

It is possible to reduce interference by ions from the filling solution of a liquid-based ion-selective electrode (Figures 15-13 and 15-15) by replacing the filling solution with an electrically conductive polymer. The filling solution or the conductive polymer translates an electric potential difference at the Ag^+ ion-exchange membrane into an electric potential at the metallic inner electrode.

The electrode shown here has a gold wire coated with a thin layer of electrically conductive poly(3-octylthiophene). When the polymer is oxidized, electrons can move along the conjugated backbone of the molecule.



Ag^+ ion-selective electrode with a conductive polymer in place of filling solution.

(*Conjugation* means that the molecule contains alternating single and double bonds.) The conductivity of the oxidized polymer can be up to $\sim 0.1\%$ that of copper metal. The coated wire fills a plastic 10- μL pipet tip whose opening is overcoated with an ion-exchange membrane containing a ligand (L in Figure 15-6) that is highly selective for Ag^+ . When conditioned in 1 nM AgNO_3 solution, this electrode exhibits linear response down to 10 nM Ag^+ and a detection limit of ~ 2 nM.

An application of the Ag^+ -electrode is in the sensitive detection of a protein by a “sandwich immunoassay” using *antibodies*. An **antibody** is a protein produced by the immune system of an animal in response to a foreign molecule, which is called an **antigen**. An antibody specifically recognizes and binds to the antigen that stimulated its synthesis.

In the sandwich immunoassay, analyte protein is the antigen. An antibody is bound to a gold surface. When analyte is introduced, it binds to the antibody. Then a second antibody that binds to the analyte is introduced. The second antibody contains covalently attached gold particles with a diameter of ~ 13 nm containing $\sim 10^5$ gold atoms. After washing away unbound antibody, Ag metal is catalytically deposited on the surface of the gold nanoparticles. Approximately 100 atoms of Ag are deposited for every atom of Au in the original particle. Therefore there are $\sim 10^7$ Ag atoms per antibody molecule.

To complete the analysis, Ag metal is oxidized to Ag^+ with hydrogen peroxide (H_2O_2) and the liberated Ag^+ is measured by the ion-selective electrode. For

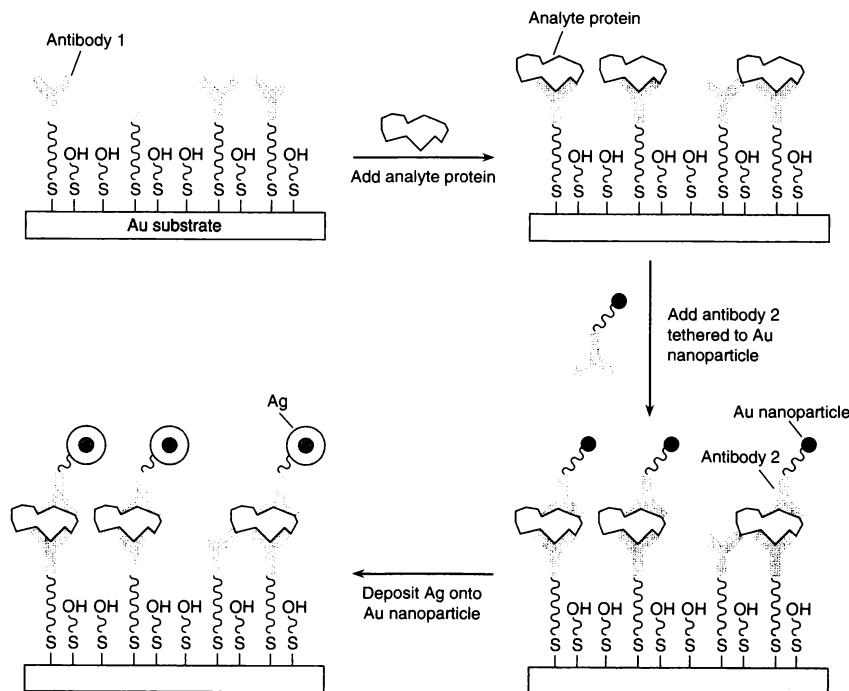
the detection limit for Pb^{2+} improved by 10^5 , but the observed selectivity for Pb^{2+} over other cations increases by several orders of magnitude. The sensitivity of a solid-state electrode cannot be lowered by changing the filling solution because analyte concentration is governed by the solubility of the inorganic salt crystal forming the ion-sensitive membrane. Box 15-3 describes an ion-selective electrode in which an electrically conductive polymer replaces the inner filling solution.

Compound Electrodes

A **compound electrode** is a conventional electrode surrounded by a membrane that isolates (or generates) the analyte to which the electrode responds. The CO_2 gas-sensing electrode in Figure 15-16 is an ordinary glass pH electrode surrounded by electrolyte solution enclosed in a semipermeable membrane made of rubber, Teflon, or polyethylene.⁶ A silver-silver chloride reference electrode is immersed in the electrolyte solution. When CO_2 diffuses through the semipermeable membrane, it lowers the pH in the electrolyte compartment and changes the potential difference across the glass membrane, which is measured by the two silver-silver chloride electrodes.

each molecule of protein analyte, roughly 10^7 ions of Ag^+ are produced. We say that the assay *amplifies* the analyte signal by a factor of 10^7 . The assay detects $\sim 12 \text{ pmol}$ ($12 \times 10^{-12} \text{ mol}$) of protein analyte in a

$50\text{-}\mu\text{L}$ sample. An analogous assay of ribonucleic acid (RNA) with the Ag^+ ion-selective electrode detects 0.2 amol ($2 \times 10^{-19} \text{ mol}$, 120 000 molecules) in a $4\text{-}\mu\text{L}$ sample.



Sandwich immunoassay with deposition of silver metal on Au nanoparticles. [From K. Y. Chumbimuni-Torres, Z. Dai, N. Rubinova, Y. Xiang, E. Prötsch, J. Wang, and E. Bakker, *J. Am. Chem. Soc.* **2006**, 128, 13676.]

Other acidic or basic gases, including NH_3 , SO_2 , H_2S , NO_x (nitrogen oxides), and HN_3 (hydrazoic acid), can be detected in the same manner. These electrodes can be used to measure gases *in the gas phase* or dissolved in solution. In Demonstration 8-2, a thin film of water on the glass pH electrodes serves as a detector for $\text{SO}_2(g)$ and gaseous $\text{NH}_3(g)$ without the need for a semipermeable membrane.

Some ingenious compound electrodes contain a conventional electrode coated with an enzyme that catalyzes a reaction of the analyte. The product of the reaction is detected by the electrode. Compound electrodes based on enzymes are among the most selective because enzymes tend to be extremely specific in their reactivity with just the species of interest.

Ask Yourself

15-E. Box 6-1 discussed nitrogen species found in a saltwater aquarium. Now we consider the measurement of ammonia in the fishtank, using an ammonia-selective compound electrode. The procedure is to mix 100.0 mL of unknown or standard

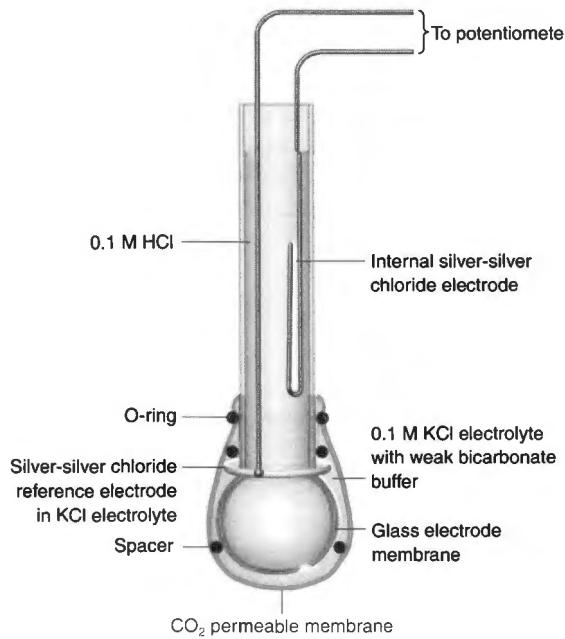


Figure 15-16 A CO_2 gas-sensing electrode. The silver-silver chloride reference electrode just above the glass bulb is the reference electrode like the outer silver-silver chloride electrode in a combination pH electrode in Figure 15-7.



[Rachwal/Dreamstime.com.]

with 1.0 mL of 10 M NaOH and then to measure NH_3 with an electrode. The purpose of NaOH is to raise the pH above 11 so that ammonia is in the form NH_3 , not NH_4^+ . (In a more rigorous procedure, EDTA is added prior to NaOH to bind metal ions and displace NH_3 from the metals.)

(a) A series of standards gave the following readings. Prepare a calibration curve of potential (mV) versus $\log(\text{nitrogen concentration in parts per million})$ and determine the equation of the straight line by the method of least squares. (Calibration is done in terms of nitrogen in the original standards. There is no dilution factor to consider from the NaOH.)

NH_3 nitrogen concentration (ppm)	$\log[\text{N}]$	Electrode potential (mV vs S.C.E.)
0.100	-1.000	72
0.500	-0.301	42
1.000	0.000	25

(b) Two students measured NH_3 in the aquarium and observed values of 106 and 115 mV. What NH_3 nitrogen concentration (in ppm) should be reported by each student?

(c) Artificial seawater for the aquarium is prepared by adding a commercial seawater salt mix to the correct volume of distilled water. There is an unhealthy level of NH_4Cl impurity in the salt mix, so the instructions call for several hours of aerating freshly prepared seawater to remove $\text{NH}_3(g)$ before adding the water to a tank containing live fish. A student measured the concentration of NH_3 in freshly prepared seawater prior to aeration and observed a potential of 56 mV. What is the concentration of NH_3 in the freshly prepared seawater?

Key Equations

Voltage of complete cell	$E = E_+ - E_-$ (repeated from Chapter 14)
	E_+ = voltage of electrode connected to + terminal of meter
	E_- = voltage of electrode connected to - terminal of meter
Titration of X^- with M^+	$Before V_e: [M^+] = K_{sp}/[X^-]$ $At V_e: [M^+] = [X^-] = \sqrt{K_{sp}}$ $After V_e: [M^+] = \frac{\text{mol excess } M^+}{\text{total volume}}$
Response of glass pH electrode	$E = \text{constant} + \beta(0.059 16)\Delta\text{pH}$ $\Delta\text{pH} = (\text{analyte pH}) - (\text{pH of internal solution})$ $\beta (\approx 1.00)$ is measured with standard buffers constant = asymmetry potential (measured by calibration)
Ion-selective electrode response	$E = \text{constant} + \beta\left(\frac{0.059 16}{n}\right) \log \left[[A] + \sum_X K_{A,X}^{\text{Pot}} [X] \right]$ n = number of charges on ion, including sign A = analyte ion X = interfering ion $K_{A,X}^{\text{Pot}}$ = selectivity coefficient

Important Terms

antibody	glass electrode	liquid-based ion-selective electrode	selectivity coefficient
antigen	ion-selective electrode		solid-state ion-selective electrode
combination electrode	junction potential		
compound electrode			

Problems

- 15-1.** A cell was prepared by dipping a Cu wire and a saturated Ag | AgCl electrode into 0.10 M CuSO₄ solution. The Cu wire was attached to the positive terminal of a potentiometer and the reference electrode was attached to the negative terminal.
- Write a half-reaction for the Cu electrode.
 - Write the Nernst equation for the Cu electrode.
 - Calculate the cell voltage.
- 15-2.** Pt and saturated calomel electrodes are dipped into a solution containing 0.002 17 M Br₂(aq) and 0.234 M Br⁻. Pt is attached to the positive terminal of the potentiometer.
- Write the reaction that takes place at Pt and find the half-cell potential E_+ .
 - Find the net cell voltage, E .
- 15-3.** A 50.0-mL solution of 0.100 M NaSCN was titrated with 0.200 M AgNO₃ in the cell in Figure 15-1. Find [Ag⁺] and E at $V_{Ag^+} = 0.1, 10.0, 25.0$, and 30.0 mL and sketch the titration curve.
- 15-4.** A 10.0-mL solution of 0.050 0 M AgNO₃ was titrated with 0.025 0 M NaBr in the cell in Figure 15-1. Find the cell voltage at $V_{Br^-} = 0.1, 10.0, 20.0$, and 30.0 mL and sketch the titration curve.
- 15-5.** A 25.0-mL solution of 0.050 0 M NaCl was titrated with 0.025 0 M AgNO₃ in the cell in Figure 15-1. Find [Ag⁺] and E at $V_{Ag^+} = 1.0, 10.0, 50.0$, and 60.0 mL and sketch the titration curve.
- 15-6.** A more advanced problem. A 50.0-mL solution of 0.100 M NaCl was titrated with 0.100 M Hg₂(NO₃)₂ in a cell analogous to that in Figure 15-1, but with a mercury electrode instead of a silver electrode. The cell is S.C.E. || titration reaction | Hg(l).
- Write the titration reaction and find the equivalence volume.
 - The electrochemical equilibrium at the mercury electrode is $Hg_2^{2+} + 2e^- \rightleftharpoons 2Hg(l)$. Derive an equation for the cell voltage analogous to Equation 15-1.
 - Compute the cell voltage at the following volumes of added Hg₂(NO₃)₂: 0.1, 10.0, 25.0, 30.0 mL. Sketch the titration curve.
- 15-7.** Which side of the liquid junction 0.1 M KNO₃ | 0.1 M NaCl will be negative? Explain your answer.

15-8. In Table 15-3, the liquid junction $0.1\text{ M HCl} \mid 0.1\text{ M KCl}$ has a voltage of $+27\text{ mV}$ and the junction $0.1\text{ M HCl} \mid 3.5\text{ M KCl}$ has a voltage of $+3.1\text{ mV}$. Which side of each junction becomes positive? Why is the voltage so much less with 3.5 M KCl than with 0.1 M KCl ?

15-9. If electrode C in Figure 15-9 is placed in a solution of $\text{pH } 11.0$, what will the pH reading be?

15-10. Suppose that the $\text{Ag} \mid \text{AgCl}$ outer electrode in Figure 15-7 is filled with 0.1 M NaCl instead of saturated KCl . Suppose that the electrode is calibrated in a dilute buffer containing 0.1 M KCl at $\text{pH } 6.54$ at 25°C . The electrode is then dipped in a second buffer *at the same pH* and same temperature, but containing 3.5 M KCl .

(a) Use Table 15-3 to estimate the change in junction potential and how much the indicated pH will change.

(b) Suppose that a change in junction potential causes the apparent pH to change from 6.54 to 6.60 . By what percentage does $[\text{H}^+]$ appear to change?

15-11. Why is measuring $[\text{H}^+]$ with a pH electrode somewhat inaccurate, whereas locating the end point in an acid-base titration with a pH electrode can be very accurate?

15-12. Explain the principle of operation of a liquid-based ion-selective electrode.

15-13. How does a compound electrode differ from a simple ion-selective electrode?

15-14. What does the selectivity coefficient tell us? Is it better to have a large or a small selectivity coefficient?

15-15. A micropipet H^+ ion-selective electrode similar to the NH_4^+ electrode in Box 15-2 was constructed to measure the pH inside large, live cells by impaling a cell with the electrode (and with a similarly small reference electrode).⁷ The ion exchanger at the tip of the H^+ ion-selective electrode was made from 10 wt\% tri(dodecyl)amine $[(\text{C}_{12}\text{H}_{25})_3\text{N}]$ and 0.7 wt\% sodium tetraphenylborate, dissolved in *o*-nitrophenyl octyl ether. The selectivity for H^+ relative to Na^+ , K^+ , Mg^{2+} , and Ca^{2+} was sufficient for intra- and extracellular measurements without significant interference from these metal ions. Explain how this electrode works.

15-16. What is the purpose of the electrically conductive polymer in the ion-selective electrode in Box 15-3? What is the advantage of replacing the filling solution of a liquid-based ion-selective electrode with a conductive polymer?

15-17. By how many volts will the potential of an ideal Mg^{2+} ion-selective electrode change if the electrode is transferred from $1.00 \times 10^{-4}\text{ M MgCl}_2$ to $1.00 \times 10^{-3}\text{ M MgCl}_2$?

15-18. When measured with a F^- ion-selective electrode that has a Nernstian response at 25°C , the potential due to F^- in unfluoridated groundwater in Foxboro, Massachusetts was 40.0 mV more positive than the potential of tap water in Providence, Rhode Island. Providence maintains its fluoridated water at the recommended level of $1.00 \pm 0.05\text{ mg F}^-/\text{L}$.

What is the concentration of F^- in milligrams per liter in groundwater in Foxboro? (Disregard the uncertainty.)

15-19. A cyanide ion-selective electrode obeys the equation $E = \text{constant} - (0.05916) \log[\text{CN}^-]$. The potential was -0.230 V when the electrode was immersed in $1.00 \times 10^{-3}\text{ M NaCN}$.

(a) Evaluate the constant in the equation for the electrode.

(b) Find the concentration of CN^- if $E = -0.300\text{ V}$.

15-20. The selectivity coefficient, $K_{\text{Li}^+, \text{Na}^+}^{\text{Pot}}$, for a lithium electrode is 5×10^{-3} . When this electrode is placed in $3.44 \times 10^{-4}\text{ M Li}^+$ solution, the potential is -0.333 V versus S.C.E. What would the potential be if Na^+ were added to give 0.100 M Na^+ ? If you did not know that Na^+ was interfering, what would be the apparent concentration of Li^+ that gives the same potential as the solution containing Na^+ ?

15-21. A Ca^{2+} ion-selective electrode has a selectivity coefficient $K_{\text{Ca}^{2+}, \text{Mg}^{2+}}^{\text{Pot}} = 0.010$. What will be the electrode potential if 1.0 mM Mg^{2+} is added to 0.100 mM Ca^{2+} ? By what percentage would $[\text{Ca}^{2+}]$ have to change to give the same voltage change?

15-22. An ammonia gas-sensing electrode gave the following calibration points when all solutions contained 1 M NaOH :

$\text{NH}_3\text{ (M)}$	$E\text{ (mV)}$	$\text{NH}_3\text{ (M)}$	$E\text{ (mV)}$
1.00×10^{-5}	268.0	5.00×10^{-4}	368.0
5.00×10^{-5}	310.0	1.00×10^{-3}	386.4
1.00×10^{-4}	326.8	5.00×10^{-3}	427.6

A dry food sample weighing 312.4 mg was digested by the Kjeldahl procedure (Section 10-6) to convert all the nitrogen into NH_4^+ . The digestion solution was diluted to 1.00 L , and 20.0 mL were transferred to a 100-mL volumetric flask. The 20.0-mL aliquot was treated with 10.0 mL of 10.0 M NaOH plus enough NaI to complex the Hg catalyst from the digestion and diluted to 100.0 mL . When measured with the ammonia electrode, this solution gave a reading of 339.3 mV .

(a) From the calibration data, find $[\text{NH}_3]$ in the 100-mL solution.

(b) Calculate the wt% nitrogen in the food sample.

15-23. Selectivities of two NH_4^+ ion-selective electrodes are shown in Box 15-2.

(a) Which alkali metal (Group one) ion interferes most for each electrode?

(b) K^+ interference is reduced by switching from nonactin to crown ether. Estimate $[K_{\text{NH}_4^+, \text{K}^+}^{\text{Pot}}(\text{crown ether})]/[K_{\text{NH}_4^+, \text{K}^+}^{\text{Pot}}(\text{nonactin})]$.

15-24. (a) Write an expression analogous to Equation 15-8 for the response of a La^{3+} ion-selective electrode to La^{3+} ion.

(b) If $\beta \approx 1.00$, by how many millivolts will the potential change when the electrode is removed from $1.00 \times 10^{-4}\text{ M LaClO}_4$ and placed in $1.00 \times 10^{-3}\text{ M LaClO}_4$?

(c) By how many millivolts will the potential of the electrode change if the electrode is removed from 2.36×10^{-4} M LaClO₄ and placed in 4.44×10^{-3} M LaClO₄?

(d) The electrode potential is +100 mV in 1.00×10^{-4} M LaClO₄ and the selectivity coefficient $K_{\text{La}^{3+}, \text{Fe}^{3+}}$ is $\frac{1}{1200}$. What will the potential be when 0.010 M Fe³⁺ is added?

15-25. The following data were obtained when a Ca²⁺ ion-selective electrode was immersed in a series of standard solutions.

Ca ²⁺ (M)	E (mV)
3.38×10^{-5}	-74.8
3.38×10^{-4}	-46.4
3.38×10^{-3}	-18.7
3.38×10^{-2}	+10.0
3.38×10^{-1}	+37.7

(a) Prepare a graph of E versus $\log[\text{Ca}^{2+}]$. Calculate the slope and the y -intercept (and their standard deviations) of the best straight line through the points, by using your least-squares spreadsheet from Section 4-8.

(b) Calculate the concentration of a sample that gave a reading of -22.5 mV.

(c) Your spreadsheet gives the uncertainty in $\log[\text{Ca}^{2+}]$. Using the upper and lower limits for $\log[\text{Ca}^{2+}]$, express the Ca²⁺ concentration as $[\text{Ca}^{2+}] = x \pm y$.

15-26. Fourteen ion-selective electrodes were used to measure Ca²⁺ in the same solution with the following results: $[\text{Ca}^{2+}] = 1.24, 1.13, 1.20, 1.20, 1.30, 1.12, 1.27, 1.19, 1.27, 1.22, 1.23, 1.23, 1.25, 1.24$ mM. Find the 95% confidence interval for the mean. If the actual concentration is known to be 1.19 mM, are the results of the ion-selective electrode within experimental error of the known value at the 95% confidence level?

15-27. Metal ion buffer. Consider the reaction of Pb²⁺ with EDTA to form a metal complex: $\text{Pb}^{2+} + \text{EDTA} \xrightleftharpoons{K_f'} \text{PbY}^{2-}$, where EDTA represents all forms of EDTA not bound to metal (Equations 13-1 and 13-7). The effective formation

constant, K_f' , is related to the formation constant, K_f , by $K_f' = \alpha_{\text{Y}^{4-}} K_f$, where $\alpha_{\text{Y}^{4-}}$ is the fraction of unbound EDTA in the form Y⁴⁻. We can make a lead ion buffer by fixing the concentrations of PbY²⁻ and EDTA. Knowing these two concentrations and the formation constant, we can compute [Pb²⁺]. The lead ion buffer used in the electrode for the colored curve in Figure 15-15 was prepared by mixing 0.74 mL of 0.10 M Pb(NO₃)₂ with 100.0 mL of 0.050 M Na₂EDTA. At the measured pH of 4.34, $\alpha_{\text{Y}^{4-}} = 1.5 \times 10^{-8}$ (Equation 13-5). Show that $[\text{Pb}^{2+}] = 1.0 \times 10^{-12}$ M.

How Would You Do It?

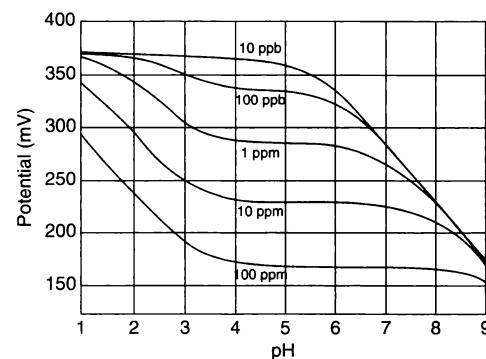
15-28. The graph shows the effect of pH on the response of a liquid-based nitrite (NO₂⁻) ion-selective electrode. Ideally, the response would be flat—Independent of pH.

(a) Nitrite is the conjugate base of nitrous acid. Why do the curves rise at low pH?

(b) Why do the curves fall at high pH?

(c) What is the optimum pH for using this electrode?

(d) Measure points on the graph at the optimum pH and construct a curve of millivolts versus $\log[\text{NO}_2^-]$. What is the lower concentration limit for linear response?

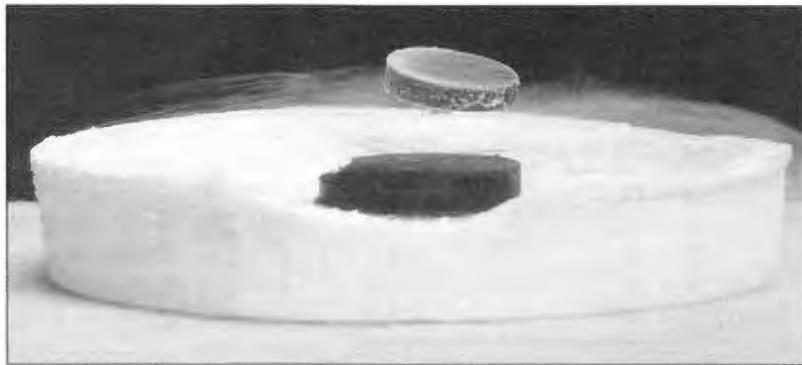


Response of nitrite ion-selective electrode. Shaded region is where response is nearly independent of pH. [From S. J. West and X. Wen, *Am. Environ. Lab.*, September 1997, p. 15.]

Notes and References

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- Build a CO₂ compound electrode: S. Kocmura, E. Cortón, L. Haim, G. Locascio, and L. Galagosky, *J. Chem. Ed.* **1999**, 76, 1253.
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High-Temperature Superconductors



Permanent magnet levitates above superconducting disk cooled in a pool of liquid nitrogen. Redox titrations are crucial in measuring the chemical composition of a superconductor.
[Photo courtesy D. Cornelius and T. Vanderah, Michelson Laboratory.]

Superconductors are materials that lose all electric resistance when cooled below a critical temperature. Prior to 1987, all known superconductors required cooling to temperatures near that of liquid helium (4 K), a process that is costly and impractical for all but a few applications. In 1987, a giant step was taken when “high-temperature” superconductors that retain superconductivity above the boiling point of liquid nitrogen (77 K) were discovered.

The most startling characteristic of a superconductor is magnetic levitation, which is shown here. When a magnetic field is applied to a superconductor, current flows in the outer skin of the material such that the applied magnetic field is exactly canceled by the induced magnetic field, and the net field inside the specimen is 0. Current flow in the skin of the superconductor repels the magnet and causes it to float above the superconductor. Expulsion of a magnetic field from a superconductor is called the *Meissner effect*.

A prototypical high-temperature superconductor is yttrium barium copper oxide, $\text{YBa}_2\text{Cu}_3\text{O}_7$, in which two-thirds of the copper is in the +2 oxidation state and one-third is in the unusual +3 state. Another example is $\text{Bi}_2\text{Sr}_2(\text{Ca}_{0.8}\text{Y}_{0.2})\text{Cu}_2\text{O}_{8.295}$, in which the average oxidation state of copper is +2.105 and the average oxidation state of bismuth is +3.090 (which is formally a mixture of Bi^{3+} and Bi^{5+}). The most reliable means to unravel these complex formulas is through redox titrations described in the last problem of this chapter.

Redox Titrations

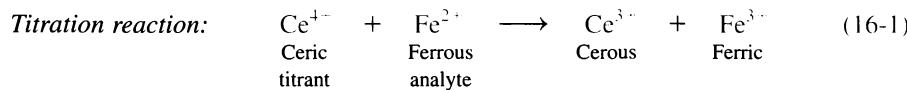
Oxidation-reduction reactions are ubiquitous. Photosynthesis in plants and metabolism of food in your body are redox reactions. In environmental engineering, the *reducing agent* Fe(0) can remediate some pollutants in underground aquifers. The *oxidizing agent* Fe(VI) in FeO_4^{2-} destroys other pollutants (Figure 17-2). For example, thiocyanate (SCN^-) from photofinishing, metal separation, electroplating, and coke production is oxidized by Fe(VI) to SO_4^{2-} , an environmentally benign product.

In analytical chemistry, a **redox titration** is based on an oxidation-reduction reaction between analyte and titrant. Common analytical oxidants include iodine (I_2), permanganate (MnO_4^-), cerium(IV), and dichromate ($\text{Cr}_2\text{O}_7^{2-}$). Titrations with reducing agents such as Fe^{2+} (ferrous ion) and Sn^{2+} (stannous ion) are less common because solutions of most reducing agents need protection from air to prevent reaction with O_2 .

Box 16-1 explains how dichromate is used to measure *chemical oxygen demand* in environmental analysis.

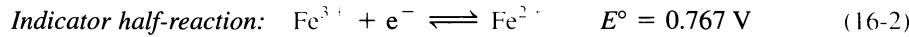
16-1 Theory of Redox Titrations

Consider the titration of iron(II) with standard cerium(IV), the course of which could be monitored potentiometrically as shown in Figure 16-1. (The word “standard” means that the concentration of Ce(IV) is known.) The titration reaction is



for which $K \approx 10^{16}$ in 1 M HClO_4 . Each mole of ceric ion oxidizes 1 mol of ferrous ion rapidly and quantitatively. The titration reaction creates a mixture of Ce^{4+} , Ce^{3+} , Fe^{2+} , and Fe^{3+} in the beaker in Figure 16-1.

To follow the course of the reaction, we use a Pt indicator electrode and a calomel (or other) reference electrode. At the *Pt indicator electrode*, two reactions each come to equilibrium:



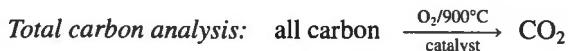
The potentials cited here are formal potentials that apply in 1 M HClO_4 .

The *titration reaction* goes to completion after each addition of titrant. The equilibrium constant is given by Equation 14-23: $K = 10^{nE^\circ/0.05916}$ at 25°C. Strong acid prevents hydrolysis reactions such as $\text{Fe}^{3+} + \text{H}_2\text{O} \rightleftharpoons \text{Fe}(\text{OH})^{2+} + \text{H}^+$.

Equilibria 16-2 and 16-3 are both established at the Pt electrode.

Box 16-1 Environmental Carbon Analysis and Oxygen Demand

Industrial waste streams are partly characterized and regulated on the basis of their carbon content or oxygen demand. *Total carbon* (TC) is defined by the amount of CO_2 evolved when a sample is completely oxidized by combustion:



Color Plate 11 shows an alternative photochemical method to oxidize all carbon to CO_2 without the high temperature required for combustion with O_2 .

Total carbon includes dissolved organic material (called *total organic carbon*, TOC) and dissolved CO_3^{2-} and HCO_3^- (called *inorganic carbon*, IC). By definition, $\text{TC} = \text{TOC} + \text{IC}$. To distinguish TOC from IC, the pH of a fresh sample is lowered below 2 to convert CO_3^{2-} and HCO_3^- into CO_2 , which is purged from (bubbled out of) the solution with N_2 . After IC has been removed, combustion analysis of the remaining material measures TOC. IC is the difference between the two experiments.

TOC is widely used to determine compliance with discharge laws. Municipal wastewater may contain ~ 1 g of TOC per liter. At the other extreme, high-purity water required for microelectronic processes may contain ~ 1 μg of TOC per liter.

Total oxygen demand (TOD) tells us how much O_2 is required for complete combustion of pollutants in a waste stream. A volume of N_2 containing a known quantity of O_2 is mixed with the sample and complete combustion is carried out. The remaining O_2 is measured by a Clark electrode (Figure 17-3). This measurement is sensitive to the oxidation states of species in the waste stream. For example, urea, $(\text{NH}_2)_2\text{C}=\text{O}$, consumes five times as much O_2 as formic acid, HCO_2H , does. Species such as NH_3 and H_2S also contribute to TOD.

Pollutants can be oxidized by refluxing with dichromate, $\text{Cr}_2\text{O}_7^{2-}$. *Chemical oxygen demand* (COD) is defined as the O_2 that is chemically equivalent to the $\text{Cr}_2\text{O}_7^{2-}$ consumed in this process. Each $\text{Cr}_2\text{O}_7^{2-}$ consumes 6e^- (to make 2Cr^{3+}) and each O_2 consumes 4e^- (to make $2\text{H}_2\text{O}$). Therefore 1 mol of $\text{Cr}_2\text{O}_7^{2-}$ is chemically equivalent to 1.5 mol of O_2 for this computation. COD analysis is carried out by refluxing polluted water for 2 h with excess standard $\text{Cr}_2\text{O}_7^{2-}$ in H_2SO_4 solution containing Ag^+ catalyst. Unreacted $\text{Cr}_2\text{O}_7^{2-}$ is then measured by titration with standard Fe^{2+} or by spectrophotometry. Many permits for industrial operations are specified in terms of COD analysis of the waste streams.

Biological oxygen demand (BOD) is defined as the O_2 required for biological degradation of organic materials by microorganisms. The procedure calls for incubating a

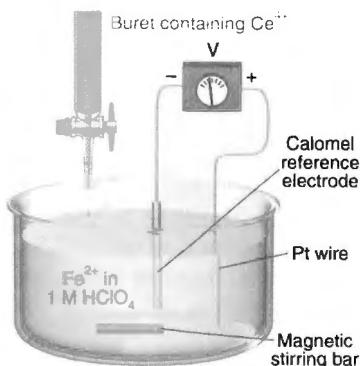


Figure 16-1 Apparatus for potentiometric titration of Fe^{2+} with Ce^{4+} .

E_+ is the potential of the Pt electrode connected to the positive terminal of the potentiometer in Figure 16-1. E_- is the potential of the calomel reference electrode connected to the negative terminal.

We now calculate how the cell voltage changes as Fe^{2+} is titrated with Ce^{4+} . The titration curve has three regions.

Region 1: Before the Equivalence Point

As each aliquot of Ce^{4+} is added, titration reaction 16-1 consumes the Ce^{4+} and creates an equal number of moles of Ce^{3+} and Fe^{3+} . Prior to the equivalence point, excess unreacted Fe^{2+} remains in the solution. Therefore we can calculate the concentrations of Fe^{2+} and Fe^{3+} without difficulty. On the other hand, we cannot find the concentration of Ce^{4+} without solving a fancy little equilibrium problem. Because the amounts of Fe^{2+} and Fe^{3+} are both known, it is *convenient* to calculate the cell voltage with Reaction 16-2 instead of 16-3.

$$E = E_+ - E_- = \left[0.767 - 0.059 \log \left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \right) \right] - 0.241 \quad (16-4)$$

↑
Formal potential for
 Fe^{3+} reduction in
1 M HClO_4

↑
Potential of
saturated calomel
electrode

$$E = 0.526 - 0.059 \log \left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \right) \quad (16-5)$$

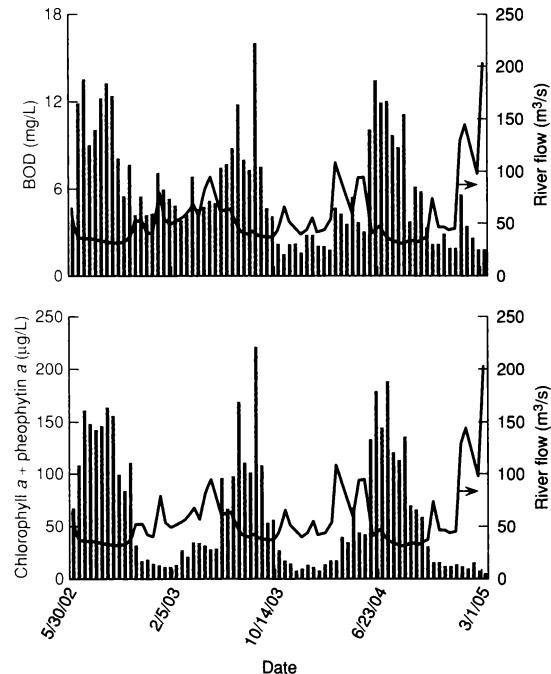
sealed container of wastewater with no extra air space for 5 days at 20°C in the dark while microbes metabolize organic compounds in the waste. Dissolved O₂ is measured before and after incubation. The difference is BOD,¹ which also measures species such as HS⁻ and Fe²⁺ that may be in the water. Inhibitors are added to prevent oxidation of nitrogen species such as NH₃.

The San Joaquin River is an ecologically sensitive aquatic system that drains into San Francisco Bay. Dissolved O₂ in the river often decreases to <5 mg/L in summer and fall, inhibiting upstream migration of salmon and stressing or killing aquatic organisms. High concentrations of algae lead to low dissolved O₂. The bar charts show correlation between high BOD and high levels of algae. Microorganisms consume algae and, in the process, consume O₂ from the river. Each gram of algal carbon is associated with 0.177 g of algal nitrogen. Oxidation of algae at a concentration of 1 mg/L to CO₂ and NO₃⁻ consumes 3.4 mg O₂/L. Algae thrive

BOD and algae in the lower San Joaquin River at Mossdale, California. Chlorophyll *a* and pheophytin *a* (a chlorophyll *a* degradation product) extracted from the river are surrogate measurements for algae. Black curves, whose values are marked on the right side of the graph, show the volume of water flowing in the river. [E. C. Volkmar and R. A. Dahlgren, *Environ. Sci. Technol.* **2006**, 40, 5653.]

One special point is reached before the equivalence point. When the volume of titrant is one-half of the amount required to reach the equivalence point ($V = \frac{1}{2}V_e$), the concentrations of Fe³⁺ and Fe²⁺ are equal. In this case, the log term is 0, and $E_+ = E^\circ$ for the Fe³⁺ | Fe²⁺ couple. *The point at which $V = \frac{1}{2}V_e$ is analogous to the point at which $pH = pK_a$ when $V = \frac{1}{2}V_e$ in an acid-base titration.*

on nitrogen and phosphorus nutrients from fertilizer in agricultural runoff. Possible strategies to increase dissolved O₂ in the river are to decrease nutrient flow into the river and decrease algae seed sources upstream.



For Reaction 16-2, $E_+ = E^\circ$ for the Fe³⁺ | Fe²⁺ couple when $V = \frac{1}{2}V_e$.

Region 2: At the Equivalence Point

Exactly enough Ce⁴⁺ has been added to react with all the Fe²⁺. Virtually all cerium is in the form Ce³⁺, and virtually all iron is in the form Fe³⁺. Tiny amounts of Ce⁴⁺ and Fe²⁺ are present at equilibrium. From the stoichiometry of Reaction 16-1, we can say that

$$[\text{Ce}^{3+}] = [\text{Fe}^{3+}] \quad (16-6)$$

$$[\text{Ce}^{4+}] = [\text{Fe}^{2+}] \quad (16-7)$$

To understand why Equations 16-6 and 16-7 are true, imagine that *all* the cerium and the iron have been converted into Ce³⁺ and Fe³⁺. Because we are at the equivalence point, $[\text{Ce}^{3+}] = [\text{Fe}^{3+}]$. Now let Reaction 16-8 come to equilibrium:



If a little bit of Fe³⁺ goes back to Fe²⁺, an equal number of moles of Ce⁴⁺ must be made. So $[\text{Ce}^{4+}] = [\text{Fe}^{2+}]$.

At any time, Reactions 16-2 and 16-3 are *both* in equilibrium at the Pt electrode. At the equivalence point, it is *convenient* to use both reactions to find the cell voltage. The Nernst equations are

$$E_+ = 0.767 - 0.059 \text{ } 16 \log\left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]}\right) \quad (16-9)$$

$$E_+ = 1.70 - 0.059 \text{ } 16 \log\left(\frac{[\text{Ce}^{3+}]}{[\text{Ce}^{4+}]}\right) \quad (16-10)$$

At the equivalence point, we use both Reactions 16-2 and 16-3 to calculate the cell voltage. This is just an algebraic convenience.

Here is where we stand: Equations 16-9 and 16-10 are statements of algebraic truth. But neither one alone allows us to find E_+ , because we do not know exactly what tiny concentrations of Fe^{2+} and Ce^{4+} are present. It is possible to solve the four simultaneous equations 16-6, 16-7, 16-9, and 16-10, by first *adding* Equations 16-9 and 16-10:

$$2E_+ = 0.767 + 1.70 - 0.059 \text{ } 16 \log\left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]}\right) - 0.059 \text{ } 16 \log\left(\frac{[\text{Ce}^{3+}]}{[\text{Ce}^{4+}]}\right)$$

$$2E_+ = 2.467 - 0.059 \text{ } 16 \log\left(\frac{[\text{Fe}^{2+}][\text{Ce}^{3+}]}{[\text{Fe}^{3+}][\text{Ce}^{4+}]}\right)$$

But, because $[\text{Ce}^{3+}] = [\text{Fe}^{3+}]$ and $[\text{Ce}^{4+}] = [\text{Fe}^{2+}]$ at the equivalence point, the quotient of concentrations in the log term is unity. Therefore the logarithm is 0 and

$$2E_+ = 2.467 \text{ V} \Rightarrow E_+ = 1.23 \text{ V}$$

In this particular example, E_+ is the average of the standard potentials for the two half-reactions at the Pt electrode.

The cell voltage is

$$E = E_+ - E(\text{calomel}) = 1.23 - 0.241 = 0.99 \text{ V} \quad (16-11)$$

In this particular titration, the equivalence-point voltage is independent of the concentrations and volumes of the reactants.

Region 3: After the Equivalence Point

After V_e , we use Reaction 16-3 because we know $[\text{Ce}^{3+}]$ and $[\text{Ce}^{4+}]$. It is not convenient to use Reaction 16-2, because we do not know $[\text{Fe}^{2+}]$, which has been “used up.”

Now virtually all iron atoms are Fe^{3+} . The moles of Ce^{3+} equal the moles of Fe^{3+} , and there is a known excess of unreacted Ce^{4+} . Because we know both $[\text{Ce}^{3+}]$ and $[\text{Ce}^{4+}]$, it is *convenient* to use Reaction 16-3 to describe the chemistry at the Pt electrode:

$$E = E_+ - E(\text{calomel}) = \left[1.70 - 0.059 \text{ } 16 \log\left(\frac{[\text{Ce}^{3+}]}{[\text{Ce}^{4+}]}\right) \right] - 0.241 \quad (16-12)$$

At the special point when $V = 2V_e$, $[\text{Ce}^{3+}] = [\text{Ce}^{4+}]$ and $E_+ = E^\circ(\text{Ce}^{4+} \mid \text{Ce}^{3+}) = 1.70 \text{ V}$.

Before the equivalence point, the voltage is fairly steady near the value $E = E_+ - E(\text{calomel}) \approx E^\circ(\text{Fe}^{3+} \mid \text{Fe}^{2+}) - 0.241 \text{ V} = 0.53 \text{ V}$. After the equivalence point, the voltage levels off near $E \approx E^\circ(\text{Ce}^{4+} \mid \text{Ce}^{3+}) - 0.241 \text{ V} = 1.46 \text{ V}$. At the equivalence point, there is a rapid rise in voltage.

Example Potentiometric Redox Titration

Suppose that we titrate 100.0 mL of 0.050 0 M Fe^{2+} with 0.100 M Ce^{4+} by using the cell in Figure 16-1. The equivalence point occurs when $V_{\text{Ce}^{4+}} = 50.0 \text{ mL}$, because the Ce^{4+} is twice as concentrated as the Fe^{2+} . Calculate the cell voltage at 36.0, 50.0, and 63.0 mL.

SOLUTION At 36.0 mL: This is 36.0/50.0 of the way to the equivalence point. Therefore 36.0/50.0 of the iron is in the form Fe^{3+} and 14.0/50.0 is in the form Fe^{2+} . Putting $[\text{Fe}^{2+}]/[\text{Fe}^{3+}] = 14.0/36.0$ into Equation 16-5 gives $E = 0.550 \text{ V}$.

At 50.0 mL: Equation 16-11 tells us that the cell voltage at the equivalence point is 0.99 V, regardless of the concentrations of reagents for this particular titration.

At 63.0 mL: The first 50.0 mL of cerium have been converted into Ce^{3+} . Because 13.0 mL of excess Ce^{4+} have been added, $[\text{Ce}^{3+}]/[\text{Ce}^{4+}] = 50.0/13.0$ in Equation 16-12, and $E = 1.424 \text{ V}$.

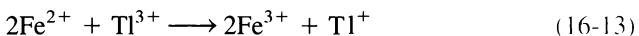
 **Test Yourself** Compute E at 37.0 and 64.0 mL. Do your answers make sense in comparison to the values at 36.0 and 63.0 mL? (Answer: 0.553 V, 1.426 V)

Shapes of Redox Titration Curves

Our calculations allow us to plot the solid titration curve for Reaction 16-1 in Figure 16-2, which shows the potential as a function of the volume of added titrant. The equivalence point is marked by a steep rise in the voltage. The calculated value of E_+ at $\frac{1}{2}V_e$ is the formal potential of the $\text{Fe}^{3+} \mid \text{Fe}^{2+}$ couple, because the quotient $[\text{Fe}^{2+}]/[\text{Fe}^{3+}]$ is unity at this point. The calculated voltage at any point in this titration depends only on the *concentration ratio* of reactants; their *absolute concentrations* make no difference in this example. We expect, therefore, that the curve in Figure 16-2 would not change if both reactants were diluted by a factor of 10.

The voltage at zero titrant volume cannot be calculated because we do not know how much Fe^{3+} is present. If $[\text{Fe}^{3+}] = 0$, the voltage calculated with Equation 16-9 would be $-\infty$. In fact, there must be some Fe^{3+} in each reagent, either as an impurity or from oxidation of Fe^{2+} by atmospheric oxygen. In any case, the voltage could not be lower than that needed to reduce the solvent ($\text{H}_2\text{O} + \text{e}^- \rightarrow \frac{1}{2}\text{H}_2 + \text{OH}^-$).

For Reaction 16-1, the titration curve in Figure 16-2 is symmetric near the equivalence point because the reaction stoichiometry is 1:1. For oxidation of Fe(II) by Tl(III)



the dashed curve in Figure 16-2 is not symmetric about the equivalence point because the stoichiometry of reactants is 2:1, not 1:1. Still, the curve is so steep near the equivalence point that negligible error is introduced if the center of the steepest portion is taken as the end point. Demonstration 16-1 provides an example of an asymmetric titration curve whose shape also depends on the pH of the reaction medium.

The change in voltage near the equivalence point for the dashed curve in Figure 16-2 is smaller than the voltage change for the solid curve because Tl^{3+} is a weaker oxidizing agent than Ce^{4+} . Clearest results are achieved with the strongest oxidizing and reducing agents. The same rule applies to acid-base titrations where strong-acid or strong-base titrants give the sharpest break at the equivalence point.

Ask Yourself

16-A. A 20.0-mL solution of 0.005 00 M Sn^{2+} in 1 M HCl was titrated with 0.020 0 M Ce^{4+} to give Sn^{4+} and Ce^{3+} . What is the potential (versus S.C.E.) at the following volumes of Ce^{4+} : 0.100, 1.00, 5.00, 9.50, 10.00, 10.10, and 12.00 mL? Sketch the titration curve.

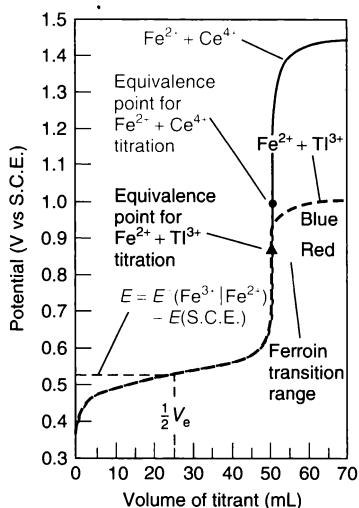


Figure 16-2 Solid line: Theoretical curve for titration of 100.0 mL of 0.050 0 M Fe^{2+} with 0.100 M Ce^{4+} in 1 M HClO_4 . You cannot calculate the potential for zero titrant, but you can start at a small volume such as 0.1 mL. Dashed line: Theoretical curve for titration of 100.0 mL of 0.050 0 M Fe^{2+} with 0.050 0 M Tl^{3+} in 1 M HClO_4 .

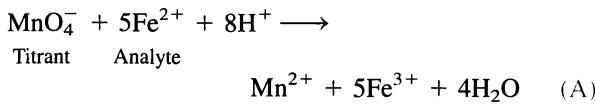
The shape of the curve in Figure 16-2 is essentially independent of the concentrations of analyte and titrant. The solid curve is symmetric near V_e because the stoichiometry is 1:1.

You would not choose a weak acid to titrate a weak base because the break at V_e would not be very large.



Demonstration 16-1 Potentiometric Titration of Fe^{2+} with MnO_4^-

The titration of Fe^{2+} with KMnO_4 nicely illustrates principles of potentiometric titrations.

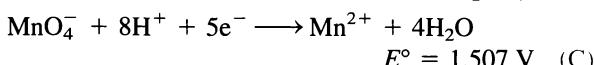
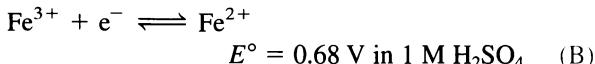


Dissolve 0.60 g of $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (FM 392.14; 1.5 mmol) in 400 mL of 1 M H_2SO_4 . Titrate the well-stirred solution with 0.02 M KMnO_4 ($V_e \approx 15$ mL), using Pt and saturated calomel electrodes with a pH meter as a potentiometer. The reference socket of the pH meter is the negative input terminal. Before starting the titration, calibrate the meter by connecting the two input sockets directly to each other with a wire and setting the millivolt scale of the meter at 0.

The demonstration is more meaningful if you calculate points on the theoretical titration curve before performing the experiment. Then compare the theoretical and experimental results. Also note the coincidence of the potentiometric and visual end points.

Question Potassium permanganate is purple, and all the other species in this titration are colorless (or very faintly colored). What color change is expected at the equivalence point?

To calculate points on the theoretical titration curve, we use the following half-reactions:



Prior to the equivalence point, calculations are similar to those in Section 16-1 for the titration of Fe^{2+} by Ce^{4+} , but $E^\circ = 0.68 \text{ V}$. After the equivalence point, you can find the potential by using Reaction C. For example, suppose that you titrate 0.400 L of 3.75 mM Fe^{2+} with 0.0200 M KMnO_4 . From the stoichiometry of Reaction A, the equivalence point is $V_e = 15.0 \text{ mL}$. When you have added 17.0 mL of KMnO_4 , the concentrations of species in Reaction C are $[\text{Mn}^{2+}] = 0.719 \text{ mM}$, $[\text{MnO}_4^-] = 0.0959 \text{ mM}$, and $[\text{H}^+] = 0.959 \text{ M}$ (neglecting the small quantity of H^+ consumed in the titration). The cell voltage is

$$\begin{aligned} E &= E_+ - E(\text{calomel}) \\ &= \left[1.507 - \frac{0.05916}{5} \log \left(\frac{[\text{Mn}^{2+}]}{[\text{MnO}_4^-][\text{H}^+]^8} \right) \right] - 0.241 \\ &= \left[1.507 - \frac{0.05916}{5} \log \left(\frac{7.19 \times 10^{-4}}{(9.59 \times 10^{-5})(0.959)^8} \right) \right] \\ &\quad - 0.241 = 1.254 \text{ V} \end{aligned}$$

To calculate the voltage at the equivalence point, we add the Nernst equations for Reactions B and C, as we did for the cerium and iron reactions in Section 16-1. Before doing so, however, multiply the permanganate equation by 5 so that we can add the log terms:

$$\begin{aligned} E_+ &= 0.68 - 0.05916 \log \left(\frac{[\text{Fe}^{2+}]}{[\text{Fe}^{3+}]} \right) \\ 5E_+ &= 5 \left[1.507 - \frac{0.05916}{5} \log \left(\frac{[\text{Mn}^{2+}]}{[\text{MnO}_4^-][\text{H}^+]^8} \right) \right] \end{aligned}$$

Now we can add the two equations to get

$$6E_+ = 8.215 - 0.05916 \log \left(\frac{[\text{Mn}^{2+}][\text{Fe}^{2+}]}{[\text{MnO}_4^-][\text{Fe}^{3+}][\text{H}^+]^8} \right) \quad (\text{D})$$

But the stoichiometry of titration reaction A tells us that at the equivalence point $[\text{Fe}^{3+}] = 5[\text{Mn}^{2+}]$ and $[\text{Fe}^{2+}] = 5[\text{MnO}_4^-]$. Substituting these values into Equation D gives

$$\begin{aligned} 6E_+ &= 8.215 - 0.05916 \log \left(\frac{[\text{Mn}^{2+}](5[\text{MnO}_4^-])}{[\text{MnO}_4^-](5[\text{Mn}^{2+}])[\text{H}^+]^8} \right) \\ &= 8.215 - 0.05916 \log \left(\frac{1}{[\text{H}^+]^8} \right) \quad (\text{E}) \end{aligned}$$

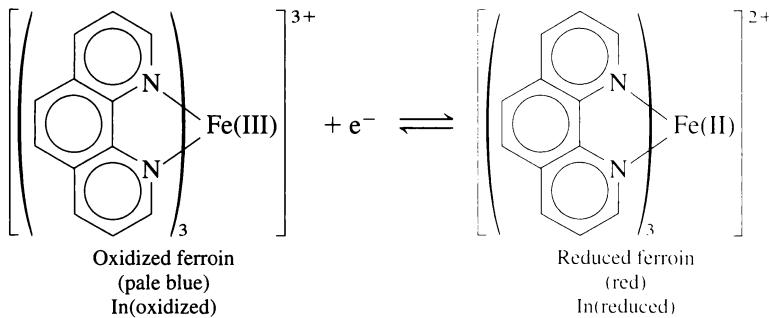
Inserting the concentration of $[\text{H}^+]$, which is $(400/415) \times (1.00 \text{ M}) = 0.964 \text{ M}$, we find

$$\begin{aligned} 6E_+ &= 8.215 - 0.05916 \log \left(\frac{1}{(0.964)^8} \right) \Rightarrow \\ E_+ &= 1.368 \text{ V} \end{aligned}$$

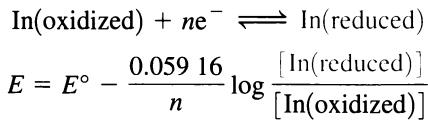
The predicted cell voltage at V_e is $E = E_+ - E(\text{calomel}) = 1.368 - 0.241 = 1.127 \text{ V}$.

16-2 Redox Indicators

An indicator (*In*) can be used to detect the end point of a redox titration, just as an indicator can be used in an acid-base titration. A **redox indicator** changes color when it goes from its oxidized to its reduced state. One common indicator is ferroin, whose color change is from pale blue (almost colorless) to red.



To predict the potential range over which the indicator color will change, we first write a Nernst equation for the indicator.



As with acid-base indicators, the color of *In(reduced)* will be observed when

$$\frac{[\text{In(reduced)}]}{[\text{In(oxidized)}]} \gtrsim \frac{10}{1}$$

and the color of *In(oxidized)* will be observed when

$$\frac{[\text{In(reduced)}]}{[\text{In(oxidized)}]} \lesssim \frac{1}{10}$$

Putting these quotients into the Nernst equation for the indicator tells us that the color change will occur over the range

Redox indicator color change range:

$$E = \left(E^\circ \pm \frac{0.059\ 16}{n} \right) \text{volts} \quad (16-14)$$

For ferroin, with $E^\circ = 1.147$ V (Table 16-1), we expect the color change to occur in the approximate range 1.088 V to 1.206 V with respect to the standard hydrogen electrode. If a saturated calomel electrode is used as the reference instead, the transition range is

$$\begin{aligned} \left(\begin{array}{l} \text{indicator transition} \\ \text{range versus calomel} \\ \text{electrode (S.C.E.)} \end{array} \right) &= \left(\begin{array}{l} \text{transition range} \\ \text{versus standard hydrogen} \\ \text{electrode (S.H.E.)} \end{array} \right) - E(\text{calomel}) \quad (16-15) \\ &= (1.088 \text{ to } 1.206) - (0.241) \\ &= 0.847 \text{ to } 0.965 \text{ V (versus S.C.E.)} \end{aligned}$$

Ferroin would therefore be a useful indicator for the solid curve in Figure 16-2.

The larger the difference in standard potential between titrant and analyte, the sharper the break in the titration curve at the equivalence point. A redox titration is

A redox indicator changes color over a range of $\pm(59/n)$ mV, centered at E° for the indicator. n is the number of electrons in the indicator half-reaction.

Figure 14-13 helps you understand Equation 16-15.

The indicator transition range should overlap the steep part of the titration curve.

Table 16-1 Redox indicators

Indicator	Color		E°
	Reduced	Oxidized	
Phenoxyfranine	colorless	red	0.28
Indigo tetrasulfonate	colorless	blue	0.36
Methylene blue	colorless	blue	0.53
Diphenylamine	colorless	violet	0.75
4'-Ethoxy-2,4-diaminoazobenzene	red	yellow	0.76
Diphenylamine sulfonic acid	colorless	red-violet	0.85
Diphenylbenzidine sulfonic acid	colorless	violet	0.87
Tris(2,2'-bipyridine)iron	red	pale blue	1.120
Tris(1,10-phenanthroline)iron (ferroin)	red	pale blue	1.147
Tris(5-nitro-1,10-phenanthroline)iron	red-violet	pale blue	1.25
Tris(2,2'-bipyridine)ruthenium	yellow	pale blue	1.29

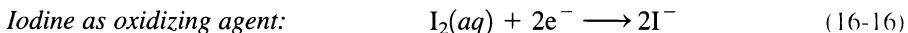
usually feasible if the difference between analyte and titrant is ≥ 0.2 V. However, the end point of such a titration is not very sharp and is best detected potentiometrically. If the difference in formal potentials is ≥ 0.4 V, then a redox indicator usually gives a satisfactory end point.

Ask Yourself

16-B. What would be the best redox indicator in Table 16-1 for the titration of $\text{Fe}(\text{CN})_6^{4-}$ with Tl^{3+} in 1 M HCl? (Hint: The potential at the equivalence point must be between the potentials for each redox couple.) What color change would you look for?

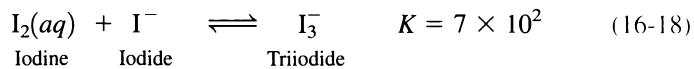
16-3 Titrations Involving Iodine

Redox titrations (Tables 16-2 and 16-3) are available for many analytes with iodine (I_2 , a mild oxidizing agent) or iodide (I^- , a mild reducing agent).



For example, vitamin C in foods and the compositions of superconductors (at the opening of the chapter) can be measured with iodine. When a reducing analyte is titrated with iodine (I_2), the method is called *iodometry*. *Iodometry* is the titration of iodine produced when an oxidizing analyte is added to excess I^- . Iodine is usually titrated with standard thiosulfate solution.

I_2 is only slightly soluble in water (1.3 mM at 20°C), but its solubility is enhanced by complexation with iodide:



A typical 0.05 M solution of I_3^- for titrations is prepared by dissolving 0.12 mol of KI plus 0.05 mol of I_2 in 1 L of water. When we speak of using “iodine,” we

Table 16-2 Iodometric titrations: Titrations with standard iodine (actually I_3^-)

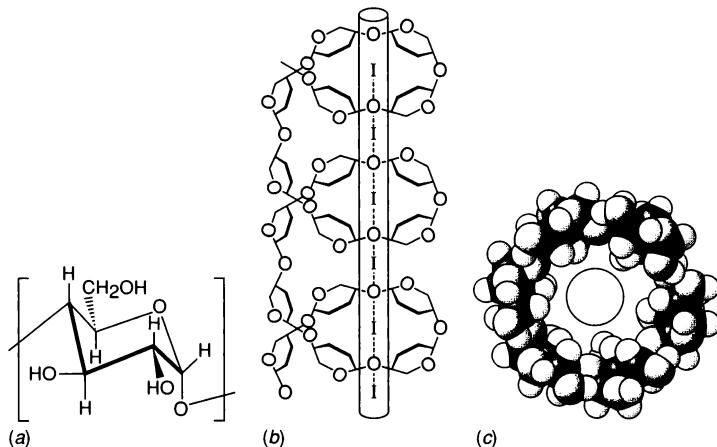
Species analyzed	Oxidation reaction	Notes
SO_2	$SO_2 + H_2O \rightleftharpoons H_2SO_3$ $H_2SO_3 + H_2O \rightleftharpoons SO_4^{2-} + 4H^+ + 2e^-$	Add SO_2 (or H_2SO_3 or HSO_3^- or SO_3^{2-}) to excess standard I_3^- in dilute acid and back titrate unreacted I_3^- with standard thiosulfate.
H_2S	$H_2S \rightleftharpoons S(s) + 2H^+ + 2e^-$	Add H_2S to excess I_3^- in 1 M HCl and back titrate with thiosulfate.
Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+}	$M^{2+} + H_2S \longrightarrow MS(s) + 2H^+$ $MS(s) \rightleftharpoons M^{2+} + S + 2e^-$	Precipitate and wash metal sulfide. Dissolve in 3 M HCl with excess standard I_3^- and back titrate with thiosulfate.
Cysteine, glutathione, mercaptoethanol	$2RSH \rightleftharpoons RSSR + 2H^+ + 2e^-$	Titrate the sulphydryl compound at pH 4–5 with I_3^- .
$H_2C=O$	$H_2CO + 3OH^- \rightleftharpoons HCO_2^- + 2H_2O + 2e^-$	Add excess I_3^- plus NaOH to the unknown. After 5 min, add HCl and back titrate with thiosulfate.
Glucose (and other reducing sugars)	$\begin{array}{c} O \\ \parallel \\ RCH \end{array} + 3OH^- \rightleftharpoons RCO_2^- + 2H_2O + 2e^-$	Add excess I_3^- plus NaOH to the sample. After 5 min, add HCl and back titrate with thiosulfate.

Table 16-3 Iodometric titrations: Titrations of iodine (actually I_3^-) produced by analyte

Species analyzed	Reaction	Notes
$HOCl$	$HOCl + H^+ + 3I^- \rightleftharpoons Cl^- + I_3^- + H_2O$	Reaction in 0.5 M H_2SO_4 .
Br_2	$Br_2 + 3I^- \rightleftharpoons 2Br^- + I_3^-$	Reaction in dilute acid.
IO_3^-	$2IO_3^- + 16I^- + 12H^+ \rightleftharpoons 6I_3^- + 6H_2O$	Reaction in 0.5 M HCl.
IO_4^-	$2IO_4^- + 22I^- + 16H^+ \rightleftharpoons 8I_3^- + 8H_2O$	Reaction in 0.5 M HCl.
O_2	$O_2 + 4Mn(OH)_2 + 2H_2O \rightleftharpoons 4Mn(OH)_3$ $2Mn(OH)_3 + 3I^- \rightleftharpoons 2Mn^{2+} + I_3^- + 6OH^-$	The sample is treated with Mn^{2+} , NaOH, and KI. After 1 min, it is acidified with H_2SO_4 , and the I_3^- is titrated.
H_2O_2	$H_2O_2 + 3I^- + 2H^+ \rightleftharpoons I_3^- + 2H_2O$	Reaction in 1 M H_2SO_4 with NH_4MoO_3 catalyst.
$O_3^{\prime\prime}$	$O_3 + 3I^- + H_2O \rightleftharpoons O_2 + I_3^- + 2OH^-$	O_3 is passed through neutral 2 wt% KI solution. Add H_2SO_4 and titrate.
NO_2^-	$2HNO_2 + 2H^+ + 3I^- \rightleftharpoons 2NO + I_3^- + 2H_2O$	The nitric oxide is removed (by bubbling CO_2 generated in situ) prior to titration of I_3^- .
$S_2O_8^{2-}$	$S_2O_8^{2-} + 3I^- \rightleftharpoons 2SO_4^{2-} + I_3^-$	Reaction in neutral solution. Then acidify and titrate.
Cu^{2+}	$2Cu^{2+} + 5I^- \rightleftharpoons 2CuI(s) + I_3^-$	NH_4HF_2 is used as a buffer.
MnO_4^-	$2MnO_4^- + 16H^+ + 15I^- \rightleftharpoons 2Mn^{2+} + 5I_3^- + 8H_2O$	Reaction in 0.1 M HCl.
MnO_2	$MnO_2(s) + 4H^+ + 3I^- \rightleftharpoons Mn^{2+} + I_3^- + 2H_2O$	Reaction in 0.5 M H_3PO_4 or HCl.

a. The pH must be ≥ 7 when O_3 is added to I^- . In acidic solution, each O_3 produces 1.25 I_3^- , not 1.00 I_3^- . [N. V. Klassen, D. Marchington, and H. C. E. McGowan, *Anal. Chem.* **1994**, 66, 2921.]

Figure 16-3 (a) Structure of the repeating unit of the sugar amylose found in starch. (b) In the starch-iodine complex, the sugar chain forms a helix around nearly linear I_6 units. [V. T. Calabrese and A. Khan, *J. Polymer Sci.* **1999**, *A37*, 2711.] (c) View down the starch helix. [Drawing from R. D. Hancock, Power Engineering, Salt Lake City.]



usually mean I_2 plus excess I^- . A mole of I_2 is equivalent to a mole of I_3^- through Reaction 16-18.

Starch Indicator

Starch is the indicator of choice for iodine because it forms an intense blue complex with iodine. The active fraction of starch is amylose, a polymer of the sugar α -D-glucose (Figure 16-3). The polymer coils into a helix, inside of which chains of I_6 (made from $3I_2$) form an intense blue color. In a solution with no other colored species, it is possible to see the color of $\sim 5 \times 10^{-6}$ M I_3^- . With starch, the limit of detection is extended by a factor of 10.

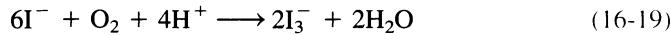
Starch is biodegradable, so either it should be freshly dissolved or the solution should contain a preservative, such as HgI_2 or thymol. A hydrolysis product of starch is glucose, which is a reducing agent. Partly hydrolyzed starch could be a source of error in a redox titration.

In iodimetry (titration *with* I_3^-), starch can be added at the beginning of the titration. The first drop of excess I_3^- after the equivalence point causes the solution to turn dark blue. In iodometry (titration *of* I_3^-), I_3^- is present throughout the reaction up to the equivalence point. *Starch should not be added until immediately before the equivalence point*, as detected visually, by fading of the I_3^- (Color Plate 12). Otherwise, some iodine tends to remain bound to starch particles after the equivalence point has been reached.

Preparation and Standardization of I_3^- Solutions

Triiodide (I_3^-) is prepared by dissolving solid I_2 in excess KI. I_2 is seldom used as a primary standard because some sublimes (evaporates) during weighing. Instead, an approximate amount is rapidly weighed, and the solution of I_3^- is standardized with a pure sample of the intended analyte or with As_4O_6 or $Na_2S_2O_3$.

Acidic solutions of I_3^- are unstable because the excess I^- is slowly oxidized by air:



At neutral pH, oxidation is insignificant in the absence of heat, light, and metal ions. Above pH 11, iodine disproportionates to hypoiodous acid (HOI), iodate (IO_3^-), and iodide.

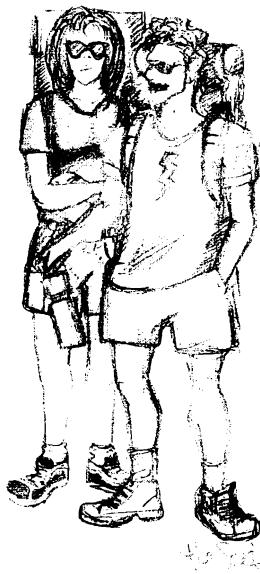
Disproportionation means that an element in one oxidation state changes to the same element in both higher and lower oxidation states.

Box 16-2 Disinfecting Drinking Water with Iodine

Some hikers use iodine to disinfect water from streams and lakes to make it safe to drink. Iodine is more effective than filter pumps, which remove bacteria but not viruses, because viruses are small enough to pass through the filter. Iodine kills everything in the water. A disadvantage of iodine is that it is a diuretic—increasing the frequency of urination.

When I hike, I carry a 60-mL glass bottle of water containing a few large crystals of solid iodine and a Teflon-lined cap. The crystals keep the solution saturated with I_2 . I keep the bottle inside two layers of plastic bags to prevent I_2 vapor from attacking everything in my backpack.

I use the bottle cap to measure out liquid to add to a 1-L bottle of water from a stream or lake. The required volume of saturated aqueous I_2 is shown in the table. For



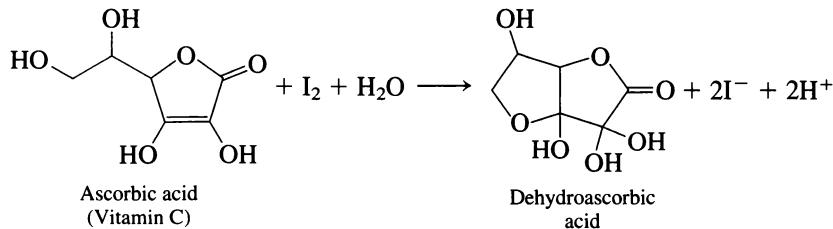
example, I use 4 caps of iodine solution when the air temperature is near 20°C to deliver approximately 13 mL

Recipe for disinfecting drinking water

Temperature of saturated $I_2(aq)$	Volume to add to 1 L
3°C (37°F)	20 mL
20°C (68°F)	13 mL
25°C (77°F)	12.5 mL
40°C (104°F)	10 mL

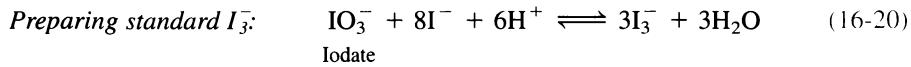
of disinfectant to my 1-L water bottle. It is important to use just the supernatant liquid, not the crystals of solid iodine, because too much iodine is harmful to humans. After allowing 30 min for the iodine to kill any critters, the water is safe to drink. Each time I use I_2 solution, I refill the small bottle with water so that saturated aqueous I_2 is available at the next water stop.

Vitamin C, a reducing agent present in many foods, reacts rapidly with I_2 :



Beverages such as the orange drink Tang are loaded with vitamin C. *Don't put Tang or other beverages in the disinfected stream water until the 30-min waiting period is over.* If you add the beverage too soon, it consumes the I_2 before the water is disinfected.

Standard I_3^- is made by adding a weighed quantity of pure potassium iodate to a small excess of KI. Addition of excess strong acid (to give $pH \approx 1$) produces I_3^- :



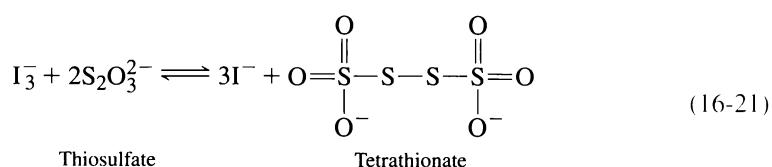
KIO_3 is a primary standard for the generation of I_3^- .

Freshly acidified iodate plus iodide can be used to standardize thiosulfate. The I_3^- reagent must be used immediately, because it is oxidized by air. The only disadvantage of KIO_3 is its low formula mass relative to the number of electrons it accepts. The small quantity of KIO_3 leads to a larger-than-desirable relative weighing error in preparing solutions.

Use of Sodium Thiosulfate

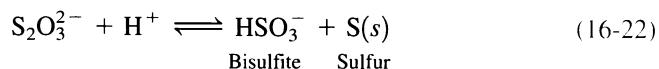
Sodium thiosulfate is the almost universal titrant for iodine. At pH < 9, iodine oxidizes thiosulfate cleanly to tetrathionate:

The unusual ability of *Salmonella enterica* bacteria to use tetrathionate in the human gut as an oxidant—just as we use O₂—permits this pathogenic organism to thrive and make people very sick.



One mole of I_3^- in Reaction 16-21 is equivalent to one mole of I_2 . I_2 and I_3^- are interchangeable through the equilibrium $I_2 + I^- \rightleftharpoons I_3^-$. The common form of thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, is not pure enough to be a primary standard. Instead, thiosulfate is standardized by reaction with a fresh solution of I_3^- prepared from KIO_3 plus KI .

A stable solution of $\text{Na}_2\text{S}_2\text{O}_3$ is prepared by dissolving the reagent in high-quality, freshly boiled distilled water. Dissolved CO_2 promotes disproportionation of $\text{S}_2\text{O}_3^{2-}$:



and metal ions catalyze atmospheric oxidation of thiosulfate. Thiosulfate solutions are stored in the dark with 0.1 g of Na_2CO_3 per liter to maintain optimum pH. Three drops of chloroform should be added to a thiosulfate solution to prevent bacterial growth. Although an acidic solution of thiosulfate is unstable, the reagent can be used to titrate iodine in acid because Reaction 16-21 is faster than Reaction 16-22.

Ask Yourself

- 16-C.** (a) Potassium iodate solution was prepared by dissolving 1.022 g of KIO_3 (FM 214.00) in a 500-mL volumetric flask. Then 50.00 mL of the solution were pipetted into a flask and treated with excess KI (2 g) and acid (10 mL of 0.5 M H_2SO_4) to drive Reaction 16-20 to completion. How many moles of I_3^- are created by the reaction?

(b) The triiodide from (a) required 37.66 mL of sodium thiosulfate solution for Reaction 16-21. What is the concentration of the sodium thiosulfate solution?

(c) A 1.223-g sample of solid containing ascorbic acid and inert ingredients was dissolved in dilute H_2SO_4 and treated with 2 g of KI and 50.00 mL of potassium iodate solution from (a). After Reaction 16-20 and the reaction with ascorbic acid in Box 16-2 went to completion, the excess, unreacted triiodide required 14.22 mL of sodium thiosulfate solution from (b) for complete titration. Find the moles of ascorbic acid and weight percent of ascorbic acid (FM 176.13) in the unknown.

Key Equations

Redox titration potential calculations

Cell voltage = $E_+ - E$ (reference electrode)

where E_+ is the indicator electrode potential

Before equivalence point: Analyte is in excess; use analyte Nernst equation to find indicator electrode potential.

At equivalence point: Add analyte and titrant Nernst equations (with equal number of electrons) and use stoichiometry to cancel many terms; if necessary, use known concentrations to evaluate log term.

After equivalence point: Titrant is in excess; use titrant Nernst equation to find indicator electrode potential.

Redox indicator color change range

$$E = \left(E^\circ \pm \frac{0.05916}{n} \right) \text{volts}$$

n = number of electrons in indicator half-reaction

Important Terms

redox indicator

redox titration

Problems

- 16-1. Find E° and K for the titration reaction 16-1 in 1 M HClO_4 at 25°C.
- 16-2. Consider the titration of Fe^{2+} with Ce^{4+} in Figure 16-2.
- Write a balanced titration reaction.
 - Write two half-reactions for the indicator electrode.
 - Write two Nernst equations for the cell voltage.
 - Calculate E at the following volumes of Ce^{4+} : 10.0, 25.0, 49.0, 50.0, 51.0, 60.0, and 100.0 mL. Compare your results with Figure 16-2.
- 16-3. Consider the titration of 100.0 mL of 0.010 0 M Ce^{4+} in 1 M HClO_4 by 0.040 0 M Cu^+ to give Ce^{3+} and Cu^{2+} , using Pt and saturated $\text{Ag} \mid \text{AgCl}$ electrodes.
- Write a balanced titration reaction.
 - Write two half-reactions for the indicator electrode.
 - Write two Nernst equations for the cell voltage.
 - Calculate E at the following volumes of Cu^+ : 1.00, 12.5, 24.5, 25.0, 25.5, 30.0, and 50.0 mL. Sketch the titration curve.
 - Select a suitable indicator for this titration from Table 16-1.
- 16-4. Consider the titration of 25.0 mL of 0.010 0 M Sn^{2+} by 0.050 0 M Ti^{3+} in 1 M HCl, using Pt and saturated calomel electrodes.
- Write a balanced titration reaction.
 - Write two half-reactions for the indicator electrode.
 - Write two Nernst equations for the cell voltage.
 - Calculate E at the following volumes of Ti^{3+} : 1.00, 2.50, 4.90, 5.00, 5.10, and 10.0 mL. Sketch the titration curve.
 - Select a suitable indicator for this titration from Table 16-1.
- 16-5. Compute the titration curve for Demonstration 16-1, in which 400.0 mL of 3.75 mM Fe^{2+} are titrated with 20.0 mM MnO_4^- at a fixed pH of 0.00 in 1 M H_2SO_4 . Calculate the cell voltage at titrant volumes of 1.0, 7.5, 14.0, 15.0, 16.0, and 30.0 mL and sketch the titration curve.
- 16-6. Consider the titration of 25.0 mL of 0.050 0 M Sn^{2+} with 0.100 M Fe^{3+} in 1 M HCl to give Fe^{2+} and Sn^{4+} , using Pt and saturated calomel electrodes.
- Write a balanced titration reaction.
 - Write two half-reactions for the indicator electrode.
 - Write two Nernst equations for the cell voltage.
 - Calculate E at the following volumes of Fe^{3+} : 1.0, 12.5, 24.0, 25.0, 26.0, and 30.0 mL. Sketch the titration curve.
- 16-7. Ascorbic acid (0.010 0 M) (structure in Box 16-2) was added to 10.0 mL of 0.020 0 M Fe^{3+} in a solution buffered to pH 0.30, and the potential was monitored with Pt and saturated $\text{Ag} \mid \text{AgCl}$ electrodes.
- dehydroascorbic acid + $2\text{H}^+ + 2\text{e}^- \rightleftharpoons$
ascorbic acid + H_2O $E^\circ = 0.390 \text{ V}$
- Write a balanced equation for the titration reaction.
 - Using $E^\circ = 0.767 \text{ V}$ for the $\text{Fe}^{3+} \mid \text{Fe}^{2+}$ couple, calculate the cell voltage when 5.0, 10.0, and 15.0 mL of ascorbic acid have been added. (*Hint:* Whenever $[\text{H}^+]$ appears in a Nernst equation, use the numerical value $10^{-\text{pH}} = 10^{-0.30}$.)
 - Select indicators from Table 16-1 that would be suitable for finding the two end points in Figure 16-2. What color changes would be observed?
 - Would tris(2,2'-bipyridine)iron be a useful indicator for the titration of Sn^{2+} in 1 M HCl with $\text{Mn}(\text{EDTA})^-$? (*Hint:* The potential at the equivalence point must be between the potentials for each redox couple.)
 - Why is iodine almost always used in a solution containing excess I^- ?
 - Above pH 11, iodine *disproportionates* to hypoiodous acid (HOI), iodate (IO_3^-), and iodide. Define disproportionation, find the oxidation states of I in each compound, and write balanced equations for the reactions.

16-12. Ozone (O_3) is a colorless gas with a pungent odor. It can be generated by passing a high-voltage electric spark through air. O_3 can be analyzed by its stoichiometric reaction with I^- in neutral solution:



(The reaction must be carried out in neutral solution. In acidic solution, more I_3^- is made than the preceding reaction indicates.) A 1.00-L bulb of air containing O_3 produced by an electric spark was treated with 25 mL of 2 M KI, shaken well, and left closed for 30 min so that all O_3 would react. The aqueous solution was then drained from the bulb, was acidified with 2 mL of 1 M H_2SO_4 , and required 29.33 mL of 0.050 44 M $S_2O_3^{2-}$ for titration of the I_3^- .

- (a) What color would you expect the KI solution to be before and after reaction with O_3 ?
- (b) Calculate the mass of O_3 in the 1.00-L bulb.
- (c) Does it matter whether starch indicator is added at the beginning or near the end point in this titration? Why?

16-13. The Kjeldahl analysis in Section 10-6 is used to measure the nitrogen content of organic compounds, which are digested in boiling sulfuric acid to decompose to ammonia, which, in turn, is distilled into standard acid. The remaining acid is then back titrated with base. Kjeldahl himself had difficulty discerning by lamplight in 1883 the methyl red indicator end point in the back titration. He could have refrained from working at night, but instead he chose to complete the analysis differently. After distilling the ammonia into standard sulfuric acid, he added a mixture of KIO_3 and KI to the acid. The liberated iodine was then titrated with thiosulfate, with starch for easy end-point detection—even by lamplight. Explain how the thiosulfate titration is related to the nitrogen content of the unknown. Derive a relation between moles of NH_3 liberated in the digestion and moles of thiosulfate required for titration of iodine.

16-14. Sulfite (SO_3^{2-}) is added to many foods as a preservative. Some people have an allergic reaction to sulfite, so it is important to control the level of sulfite. Sulfite in wine was measured by the following procedure: To 50.0 mL of wine were added 5.00 mL of solution containing (0.804 3 g $KIO_3 + 6.0$ g KI)/100 mL. Acidification with 1.0 mL of 6.0 M H_2SO_4 quantitatively converted IO_3^- into I_3^- by Reaction 16-20. The I_3^- reacted with sulfite to generate sulfate, leaving excess I_3^- in solution. The excess I_3^- required 12.86 mL of 0.048 18 M $Na_2S_2O_3$ to reach a starch end point.

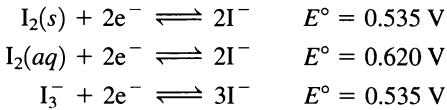
(a) Write the reaction that takes place when H_2SO_4 is added to $KIO_3 + KI$ and explain why 6.0 g of KI were added to the stock solution. Is it necessary to measure out 6.0 g very accurately? Is it necessary to measure 1.0 mL of H_2SO_4 very accurately?

(b) Write a balanced reaction between I_3^- and sulfite.

(c) Find the concentration of sulfite in the wine. Express your answer in moles per liter and in milligrams SO_3^{2-} per liter.

(d) *t test.* Another wine was found to contain 277.7 mg of SO_3^{2-}/L with a standard deviation of ± 2.2 mg/L for three determinations by the iodometric method. A spectrophotometric method gave 273.2 ± 2.1 mg/L in three determinations. Are these results significantly different at the 95% confidence level?

16-15. From the following reduction potentials,



(a) Calculate the equilibrium constant for the reaction $I_2(ag) + I^- \rightleftharpoons I_3^-$.

(b) Calculate the equilibrium constant for the reaction $I_2(s) + I^- \rightleftharpoons I_3^-$.

(c) Calculate the solubility (g/L) of I_2 in water.

How Would You Do It?

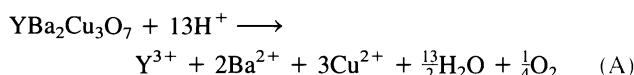
16-16. Ozone (O_3) in smog is formed by the action of solar ultraviolet light on organic vapors plus nitric oxide (NO) in the air. An O_3 level of 100 to 200 ppb (nL per liter of air) for 1 h creates a “1st stage smog alert” and is considered unhealthy. A level above 200 ppb, which defines a “2nd stage smog alert,” is very unhealthy.

(a) The ideal gas law tells us that $PV = nRT$, where P is pressure (bar), V is volume (L), n is moles, R is the gas constant (0.083 14 L · bar/(mol · K)), and T is temperature (K). If the pressure of air in a flask is 1 bar, the partial pressure of a 1-ppb component is 10^{-9} bar. Find the number of moles of O_3 in a liter of air if the concentration of O_3 is 200 ppb and the temperature is 300 K.

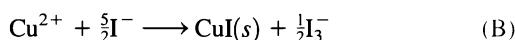
(b) Is it feasible to use the iodometric procedure of Problem 16-12 to measure O_3 at a level of 200 ppb in smog? State your reason.

16-17. *Iodometric analysis of a superconductor.* An analysis was carried out to find the effective copper oxidation state, and therefore the number of oxygen atoms, in the superconductor $YBa_2Cu_3O_{7-z}$, where z ranges from 0 to 0.5. Common oxidation states of yttrium and barium are Y^{3+} and Ba^{2+} , and common states of copper are Cu^{2+} and Cu^+ . If copper were Cu^{2+} , the formula of the superconductor would be $(Y^{3+})(Ba^{2+})_2(Cu^{2+})_3(O^{2-})_{6.5}$, with a cation charge of +13 and an anion charge of -13. The composition $YBa_2Cu_3O_7$ formally requires Cu^{3+} , which is rather rare. $YBa_2Cu_3O_7$ can be thought of as $(Y^{3+})(Ba^{2+})_2(Cu^{2+})_2(Cu^{3+})(O^{2-})_7$, with a cation charge of +14 and an anion charge of -14.

An iodometric analysis of $\text{YBa}_2\text{Cu}_3\text{O}_x$ entails two experiments. In *Experiment 1*, $\text{YBa}_2\text{Cu}_3\text{O}_x$ is dissolved in dilute acid, in which Cu^{3+} is converted to Cu^{2+} . For simplicity, we write the equations for the formula $\text{YBa}_2\text{Cu}_3\text{O}_7$, but you could balance these equations for $x \neq 7$:



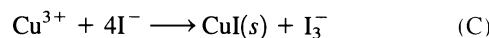
The total copper content is measured by treatment with iodide



followed by titration of the liberated I_3^- with standard thiosulfate (Reaction 16-21). Each mole of Cu in $\text{YBa}_2\text{Cu}_3\text{O}_7$ is equivalent to 1 mol of $\text{S}_2\text{O}_3^{2-}$ in Experiment 1.

In *Experiment 2*, $\text{YBa}_2\text{Cu}_3\text{O}_x$ is dissolved in dilute acid containing I^- . Each mole of Cu^{2+} produces 0.5 mol of

I_3^- by Reaction B and each mole of Cu^{3+} produces 1 mol of I_3^- :



The moles of thiosulfate required in Experiment 1 equal the total moles of Cu in the superconductor. The difference in thiosulfate required between Experiments 2 and 1 gives the Cu^{3+} content.

(a) In Experiment 1, 1.00 g of superconductor required 4.55 mmol of $\text{S}_2\text{O}_3^{2-}$. In Experiment 2, 1.00 g of superconductor required 5.68 mmol of $\text{S}_2\text{O}_3^{2-}$. What is the value of z in the formula $\text{YBa}_2\text{Cu}_3\text{O}_{7-z}$ (FM 666.246 – 15.999 4 z)?

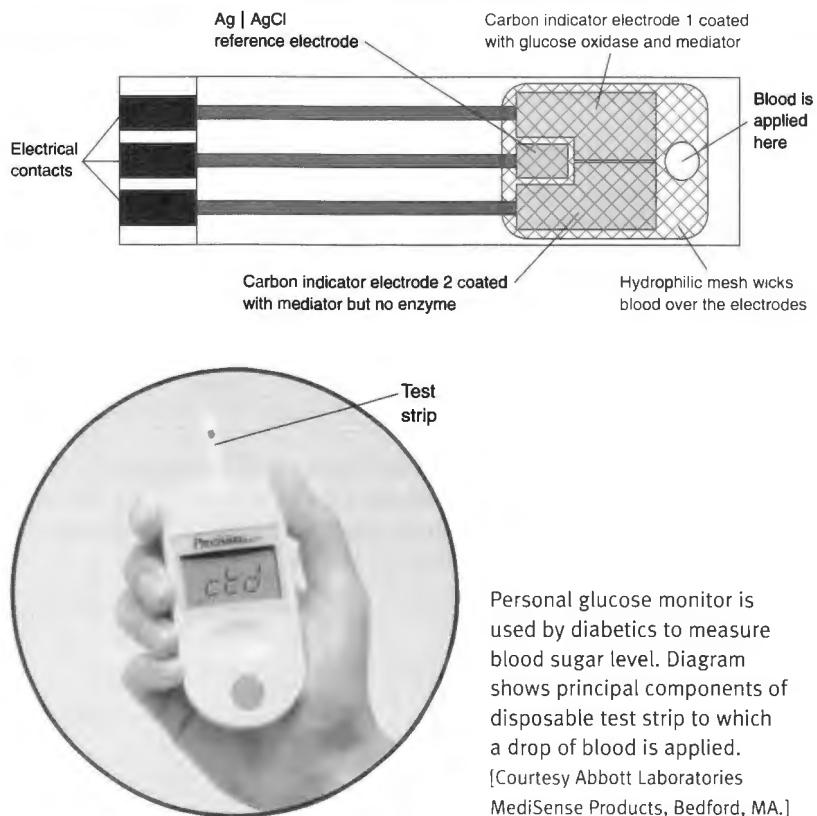
(b) *Propagation of uncertainty.* In several replications of Experiment 1, the thiosulfate required was 4.55 (± 0.10) mmol of $\text{S}_2\text{O}_3^{2-}$ per gram of $\text{YBa}_2\text{Cu}_3\text{O}_{7-z}$. In Experiment 2, the thiosulfate required was 5.68 (± 0.05) mmol of $\text{S}_2\text{O}_3^{2-}$ per gram. Find the uncertainty of x in the formula $\text{YBa}_2\text{Cu}_3\text{O}_x$.

Notes and References

- Biochemical oxygen demand and chemical oxygen demand procedures are described in *Standard Methods for the Examination of Water and Wastewater*, 21st ed.

(Washington, DC: American Public Health Association, 2005); www.standardmethods.org/.

A Biosensor for Personal Glucose Monitoring



Personal glucose monitor is used by diabetics to measure blood sugar level. Diagram shows principal components of disposable test strip to which a drop of blood is applied.
[Courtesy Abbott Laboratories MediSense Products, Bedford, MA.]

Abiosensor is an analytical device that uses a biological component such as an enzyme, an antibody, or even whole cells for specific sensing of one substance. Many people with diabetes must monitor their blood sugar (glucose) levels several times a day to control the disease through diet and insulin injections. The photograph shows a home glucose monitor featuring a disposable test strip to which as little as 4 μL of blood is applied for each measurement. This biosensor uses the enzyme glucose oxidase to catalyze the oxidation of glucose. The electrodes measure an oxidation product. Section 17-2 explains how the sensor works. The market for glucose sensors was \$2.5 billion per year in the United States in 2009 and has been projected to rise to \$14 billion worldwide in 2014.

Instrumental Methods in Electrochemistry

We now introduce a variety of electrochemical methods used in chemical analysis. These techniques are used in applications such as home glucose monitors, quality control in food processing, and chromatography detectors.

17-1 Electrogravimetric and Coulometric Analysis

Electrolysis is a chemical reaction in which we apply a voltage to drive a redox reaction that would not otherwise occur. An **electroactive species** is one that can be oxidized or reduced at an electrode.

Electrogravimetric Analysis

One of the oldest electrolytic methods in quantitative analysis is **electrogravimetric analysis**, in which analyte is plated out on an electrode and weighed. For example, an excellent procedure for the measurement of copper is to pass current through a solution of a copper salt to deposit all of the copper on the cathode:



The increase in mass of the cathode tells us how much copper was present in the solution.

Figure 17-1 shows how this experiment might be done. Analyte is typically deposited on a carefully cleaned, chemically inert Pt gauze cathode with a large surface area.

How do you find out when electrolysis is complete? One way is to observe the disappearance of color in a solution from which a colored species such as Cu^{2+} is removed. Another way is to expose most, but not all, of the surface of the cathode to the solution during electrolysis. To test whether the reaction is complete, raise the beaker or add water so that fresh surface of the cathode is exposed to the solution. After an additional period of electrolysis (15 min, say), see whether the newly exposed electrode surface has a deposit. If it does, repeat the procedure. If not, the electrolysis is finished. A third method is to remove a small sample of solution and perform a qualitative test for analyte.

Tests for completion of the deposition:

1. Disappearance of color
2. Deposition on freshly exposed electrode surface
3. Qualitative test for analyte in solution

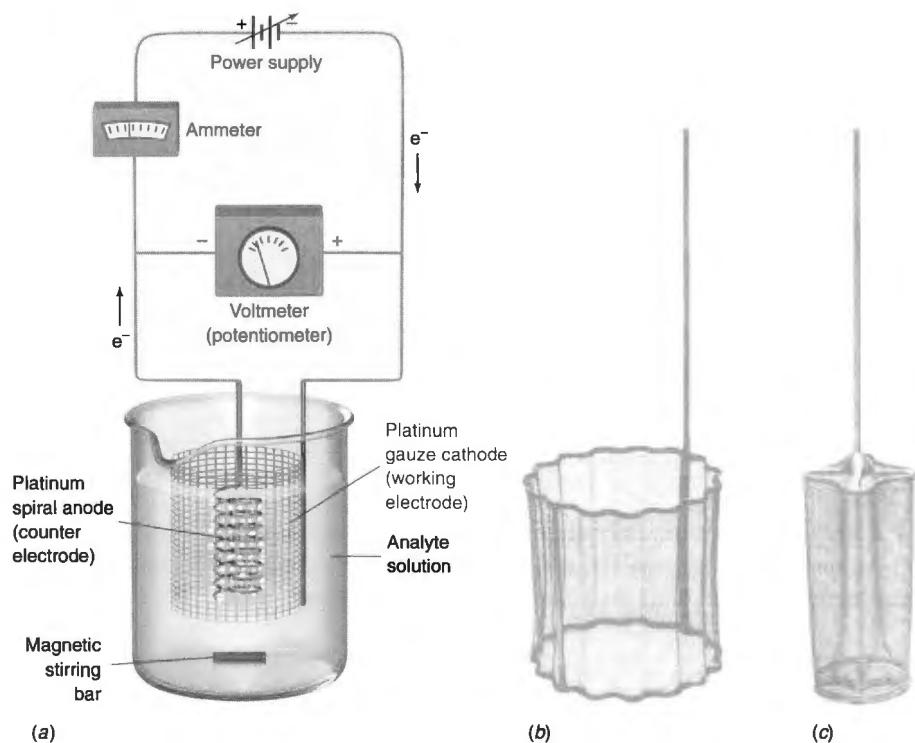


Figure 17-1 (a) Electrogravimetric analysis. Analyte is deposited on the large Pt gauze electrode. If analyte is to be oxidized, rather than reduced, the polarity of the power supply is reversed so that deposition is always on the large electrode. (b) Outer Pt gauze electrode. (c) Optional inner Pt gauze electrode designed to be spun by a motor in place of magnetic stirring.

Electrogravimetric analysis would be simple if there were only a single analyte in an otherwise inert solution. In practice, there may be other electroactive species that interfere. Water decomposes to H₂ at the cathode and to O₂ at the anode at sufficiently high voltage. Gas bubbles at an electrode interfere with deposition of solid. Because of these complications, control of electrode potential is important for successful analysis.

Coulometric Analysis

In **coulometry**, electrons participating in a chemical reaction are counted to learn how much analyte reacted. A constant current of I amperes (= I C/s) flowing for t seconds provides an electric charge of $q = It$:

$$q = \text{coulombs of } e^- = I \left(\frac{C}{s} \right) \times t(s) \quad (17-2)$$

For example, hydrogen sulfide (H₂S) can be measured by its reaction with I₂ generated at an anode:



Review of Section 14-1:

Electric charge is measured in **coulombs** (C).

Electric current (charge per unit time) is measured in **amperes** (A):

$$1 A = 1 C/s$$

Faraday constant relates coulombs to moles:

$$F \approx 96\,485 \text{ C/mol}$$

$$q = n \cdot N \cdot F$$

Coulombs Charges Mol C/mol
 per per mol

n (= charge per molecule) is **dimensionless**

We measure the electric current and the time required to generate enough I₂ in Reaction 17-3a to reach the equivalence point of Reaction 17-3b. From the current and time, we calculate how many electrons participated in Reaction 17-3a and therefore how many moles of H₂S took part in Reaction 17-3b. A way to find the end point in this example would be to have some starch in the solution. As long as I₂ is consumed

rapidly by H_2S , the solution remains colorless. After the equivalence point, the solution turns blue because excess I_2 accumulates.

Example Coulometry

Find the moles of H_2S in an unknown if the end point in Reaction 17-3b came after a current of 0.0582 A flowed for 184 s .

SOLUTION The quantity of charge in Reaction 17-3a is $q = It = (0.0582 \text{ C/s})(184 \text{ s}) = 10.7_1 \text{ C}$. We then convert coulombs into moles of electrons with Equation 14-1. For electrons, n in Equation 14-1 is 1 charge per electron, which is a *dimensionless* quantity.

$$N = \text{mol e}^- = \frac{q}{nF} = \frac{10.7_1 \text{ C}}{(1 \text{ charge/electron})(96485 \text{ C/mol})} = 1.11_0 \times 10^{-4} \text{ mol e}^-$$

In Reaction 17-3a, 2 electrons correspond to 1 I_2 . In Reaction 17-3b, 1 I_2 reacts with 1 H_2S . Therefore 2 electrons correspond to reaction of 1 H_2S . The moles of H_2S in the unknown must have been $(\frac{1 \text{ mol H}_2\text{S}}{2 \text{ mol e}^-})(1.11_0 \times 10^{-4} \text{ mol e}^-) = 5.55 \times 10^{-5} \text{ mol H}_2\text{S}$ in the unknown.

 **Test Yourself** How long would it take to titrate $1.00 \text{ mmol H}_2\text{S}$ with a current of 100.0 mA ? (Answer: $1.94 \times 10^3 \text{ s}$)

Equation 14.1:

$$\frac{q}{\text{Coulombs}} = \frac{n}{\text{Charges per electron}} \cdot \frac{N}{\text{Mol}} \cdot \frac{F}{\text{C/mol}}$$

n is dimensionless

Ask Yourself

17-A. Electrolysis in Figure 17-2 generates the powerful oxidant Fe(VI) as FeO_4^{2-} , which can oxidize hazardous species in wastewater. For example, sulfide (S^{2-}) is converted to thiosulfate ($\text{S}_2\text{O}_3^{2-}$), cyanide (CN^-) is converted to cyanate (CNO^-), and arsenite (AsO_3^{2-}) is converted to arsenate (AsO_4^{3-}).

- (a) Write a balanced half-reaction for the Fe anode in basic solution.
- (b) Write a balanced reaction for $\text{FeO}_4^{2-} + \text{S}^{2-} \rightarrow \text{Fe(OH)}_3(s) + \text{S}_2\text{O}_3^{2-}$.
- (c) How many moles of S^{2-} can be removed from the wastewater if a current of 16.0 A is applied for 1.00 h ?
- (d) What volume of wastewater containing 10.0 mM S^{2-} can be purified in 1.00 h ?

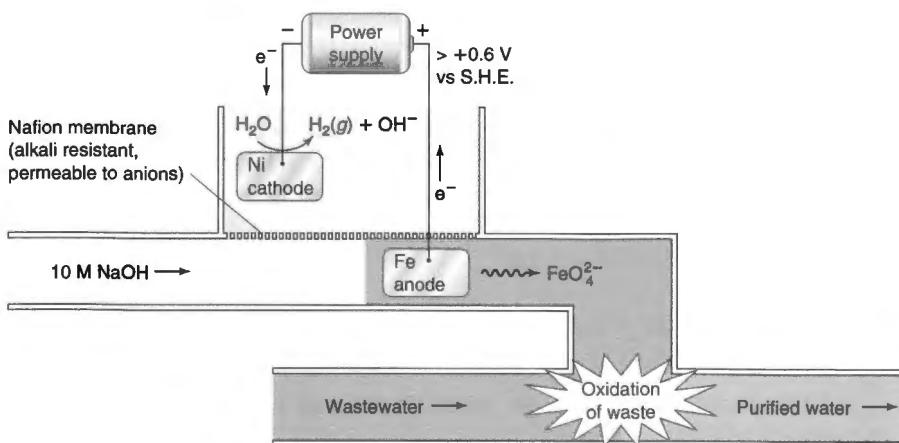


Figure 17-2 Oxidative purification of wastewater with electrochemically generated Fe(VI) . [From S. Licht and X. Yu, *Environ. Sci. Technol.* **2005**, 39, 8071.]

17-2 Amperometry

In **amperometry**, we measure an electric current that is proportional to the concentration of a species in solution.

In **coulometry**, we measure the total number of electrons (= current \times time) that flow during a chemical reaction.

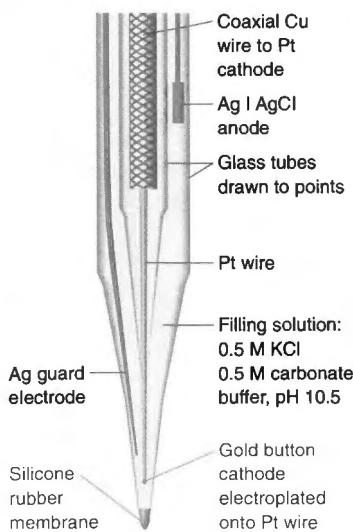


Figure 17-3 Clark oxygen microelectrode used to measure dissolved O₂ in marine sediment in Box 6-1. The tip of the cathode is plated with Au, which is less prone than Pt to fouling by adsorption of species from the test solution.
[Adapted from N. P. Revsbech, *Limnol. Oceanogr.* **1989**, *34*, 474.]

In **amperometry**, we measure the electric current between a pair of electrodes that are driving an electrolysis reaction. One of the reactants is the intended analyte, and the measured current is proportional to the concentration of analyte.

An important amperometric method is the measurement of dissolved O₂ with the **Clark electrode**,¹ such as that in Figure 17-3 used to measure O₂ in marine sediment in Box 6-1. The glass body is drawn to a fine point with a 5-μm opening at the base. Inside the opening is a 10- to 40-μm-long plug of silicone rubber, which is permeable to O₂. Oxygen diffuses into the electrode through the rubber and is reduced at the Au tip on the Pt wire, which is held at -0.75 V with respect to the Ag | AgCl reference electrode:



The current is proportional to the dissolved oxygen concentration in the unknown solution. A Clark electrode is calibrated by placing it in solutions of known O₂ concentration, and a graph of current versus [O₂] is constructed.

The electrode in Figure 17-3 also contains a silver *guard electrode* extending most of the way to the bottom. The guard electrode is kept at a negative potential that is sufficient to reduce any O₂ diffusing in from the top of the electrode but does not interfere with measurement of O₂ diffusing in through the silicone membrane at the bottom.

A Clark electrode can fit into the tip of a surgical catheter to measure O₂ in the umbilical artery of a newborn child and detect respiratory distress. The sensor responds within 20–50 s to administration of O₂ for breathing or to mechanical ventilation of the lungs.

Example A Digression on Henry's Law

Henry's law states that, in dilute solution, the concentration of a dissolved gas is proportional to the pressure of that gas in contact with the solution. For oxygen in water at 25°C, Henry's law takes the form

$$[O_2(aq)] = (0.001\ 26 \text{ M/bar}) \times P_{O_2} (\text{bar})$$

where P_{O₂} is expressed in bar and [O₂(aq)] is in mol/L. Clark electrodes are often calibrated in terms of P_{O₂} rather than [O₂(aq)], because P_{O₂} is easier to measure. For example, an electrode might be calibrated in solutions bubbled with pure nitrogen (P_{O₂} = 0), dry air (P_{O₂} ≈ 0.21 bar), and pure oxygen (P_{O₂} ≈ 1.0 bar). If a Clark electrode gives a reading of "0.100 bar," what is the molarity of O₂(aq)?

SOLUTION Henry's law tells us that

$$[O_2(aq)] = 0.001\ 26 \times P_{O_2} = (0.001\ 26 \text{ M/bar}) \times (0.100 \text{ bar}) = 0.126 \text{ mM}$$

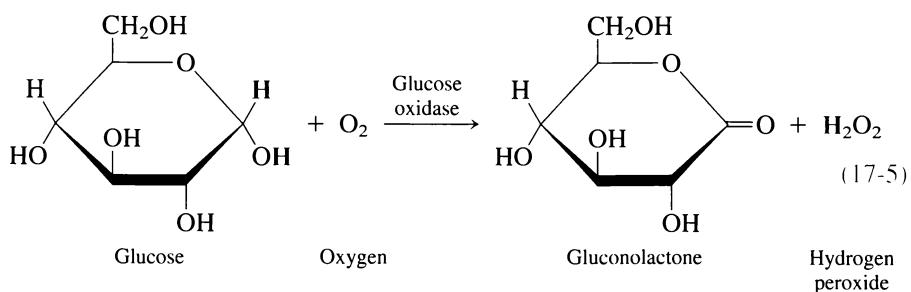
 **Test Yourself** What is the molarity of O₂ in water saturated with air? (Answer: 0.26 mM)

Glucose Monitors

The blood glucose monitor at the opening of this chapter is probably the most widely used **biosensor**—a device that uses a biological component such as an *enzyme* or *antibody* for highly selective response to one analyte. Glucose monitors account for over 95% of all amperometric instruments sold each year. The disposable test strip shown at the opening of the chapter has two carbon indicator electrodes and a Ag | AgCl reference electrode. As little as 4 μL of blood applied in the circular opening at the right of the figure is wicked over all three electrodes by a thin *hydrophilic* (“water loving”) mesh. A 20-s measurement begins when liquid reaches the reference electrode.

Indicator electrode 1 is coated with the enzyme glucose oxidase and a *mediator*, described below. The enzyme is a protein that catalyzes the reaction of glucose with oxygen:

Reaction in coating above indicator electrode 1:



In the absence of enzyme, the rate of Reaction 17-5 is negligible.

Early glucose monitors measured H_2O_2 from Reaction 17-5 by oxidation at a single indicator electrode, which was held at +0.6 V versus Ag | AgCl:

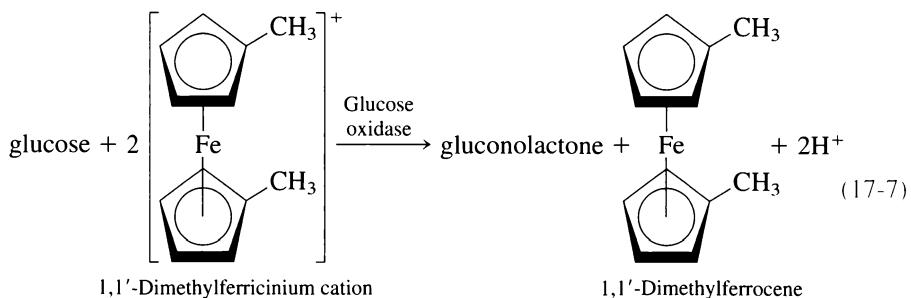


The current is proportional to the concentration of H_2O_2 , which, in turn, is proportional to the glucose concentration in blood (Figure 17-4).

A problem with early glucose monitors was that their response depended on the concentration of O_2 in the enzyme layer, because O_2 participates in Reaction 17-5. If the O_2 concentration was low, the monitor responded as though the glucose concentration were low.

A good way to reduce O_2 dependence is to incorporate into the enzyme layer a species that substitutes for O_2 in Reaction 17-5. A substance that transports electrons between the analyte (glucose, in this case) and the electrode is called a **mediator**. Ferricinium salts serve this purpose nicely:

Reaction in coating above indicator electrode 1:



Enzyme: A protein that catalyzes a biochemical reaction. The enzyme increases the rate of reaction by many orders of magnitude.

Antibody: A protein that binds to a specific target molecule called an *antigen*. Foreign cells that infect your body are marked by antibodies and destroyed by *lysis* (bursting them open with fluid) or gobbled up by macrophage cells.

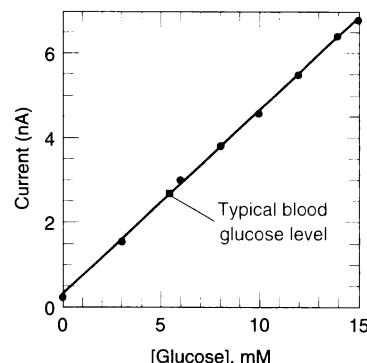


Figure 17-4 Calibration curve for amperometric glucose electrode when the dissolved O_2 concentration corresponds to $P_{\text{O}_2} = 0.027$ bar, which is 20% lower than the typical concentration in subcutaneous tissue. [Data from S.-K. Jung and G. W. Wilson, *Anal. Chem.* **1996**, 68, 591.]

A **mediator** transports electrons between analyte and the working electrode. The mediator undergoes no net reaction itself.

Ferrocene contains flat five-membered aromatic carbon rings, similar to benzene. Each ring formally carries one negative charge, so the oxidation state of iron is +2. The iron atom sits between the two flat rings. Because of its shape, this type of molecule is called a *sandwich complex*.

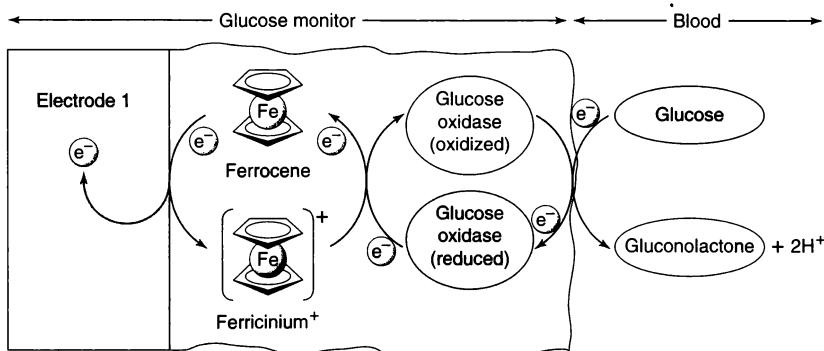


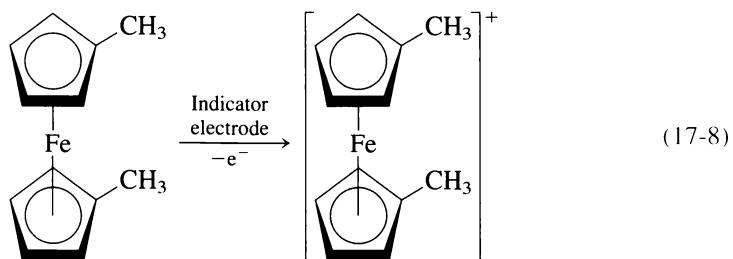
Figure 17-5 Electrons flow from glucose (in blood) to glucose oxidase to the ferricinium ion (coated on the electrode), and finally to electrode 1 of the glucose monitor. Colored species are in their reduced state.

Ferricinium mediator lowers the required working electrode potential from 0.6 V to 0.2 V versus Ag | AgCl, thereby improving the stability of the glucose sensor and eliminating some interference by other species in the blood.

You can build your own glucose biosensor for student experiments.²

The mediator consumed in Reaction 17-7 is then regenerated at the indicator electrode:

Reaction at indicator electrode 1:



The sequence by which glucose is oxidized and electrons flow to the indicator electrode is shown in Figure 17-5. The current at the electrode is proportional to the concentration of ferrocene, which, in turn, is proportional to the concentration of glucose in the blood.

Another problem with glucose monitors is that other species found in blood can be oxidized at the same potential required to oxidize the mediator in Reaction 17-8. Interfering species include ascorbic acid (vitamin C), uric acid, and acetaminophen (Tylenol). To correct for this interference, the test strip at the opening of this chapter has a second indicator electrode coated with mediator *but not with glucose oxidase*. Interfering species that are reduced at electrode 1 are also reduced at electrode 2. The current due to glucose is the current at electrode 1 minus the current at electrode 2 (both measured with respect to the reference electrode). Now you see why the test strip has three electrodes.

A major challenge is to manufacture glucose monitors in such a reproducible manner that they do not require calibration. A user expects to add a drop of blood to the test strip and get a reliable reading without constructing a calibration curve from known concentrations of glucose in blood. Each lot of test strips must be highly reproducible and is calibrated at the factory.

Cells with Three Electrodes

Cells discussed so far are based on two electrodes: an indicator electrode and a reference electrode. Current is measured between the two electrodes. The apparent exception—the glucose monitor at the opening of the chapter—has two indicator electrodes.

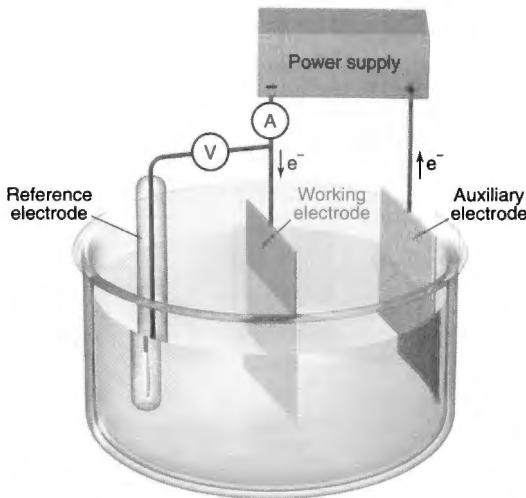


Figure 17-6 Controlled-potential electrolysis with a three-electrode cell. Voltage is measured between the working and reference electrodes. Current is measured between the working and auxiliary electrodes. Negligible current flows through the reference electrode. \textcircled{V} is a voltmeter (potentiometer) and \textcircled{A} is an ammeter.

For many techniques, a cell with three electrodes is required for fine control of the electrochemistry. The cell in Figure 17-6 features a conventional **reference electrode** (such as calomel or silver-silver chloride), a **working electrode** at which the reaction of interest takes place, and an **auxiliary electrode** (also called a *counter electrode*) that is the current-carrying partner of the working electrode. The working electrode is equivalent to the indicator electrode of two-electrode cells. The auxiliary electrode is something new that we have not encountered before. *Current flows between the working and auxiliary electrodes. Voltage is measured between the working and reference electrodes.*

The voltage between the working and reference electrodes is controlled by a device called a **potentiostat**. Virtually no current flows through the reference electrode; it simply establishes a fixed reference potential with which to measure the working electrode potential. Current flows between the working and auxiliary electrodes. The potential of the auxiliary electrode varies with time in an uncontrolled manner in response to changing concentrations and current in an electrolysis cell. It is beyond the scope of this text to explain why the electrode potential varies. Suffice it to say, however, that in a two-electrode cell the potential of the working electrode can drift as the reaction proceeds. As it drifts, reactions other than the intended analytical reaction can take place. In a three-electrode cell, the potentiostat maintains the working electrode at the desired potential, while the auxiliary electrode potential drifts out of our control.

Figure 17-6 shows reduction of analyte at the working electrode, which is therefore the cathode in this figure. In other cases, the working electrode could be the anode. The working electrode is always the indicator electrode at which analyte reacts.

Amperometric Detector for Chromatography

Figure 0-4 provided an example of chromatography used to separate caffeine from theobromine in a chemical analysis. Absorption of light and electrochemical reactions

Reference electrode: Provides fixed reference potential with negligible current flow

Working electrode: Analyte reacts here, voltage is measured between working and reference electrodes

Auxiliary electrode: Other half of the electrochemistry occurs here, current flows between working and auxiliary electrodes

Potentiostat: Controls potential difference between working and reference electrodes

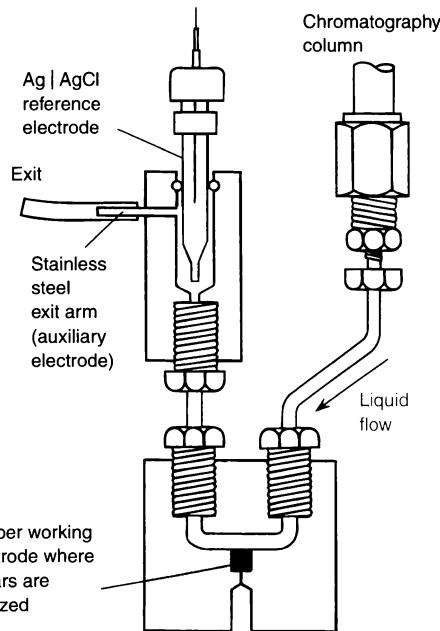


Figure 17-7 Electrochemical detector measures sugars emerging from a chromatography column by using amperometry. Sugars are oxidized at the copper electrode, and water is reduced at the stainless steel exit arm. [Adapted from Bioanalytical Systems, West Lafayette, IN.]

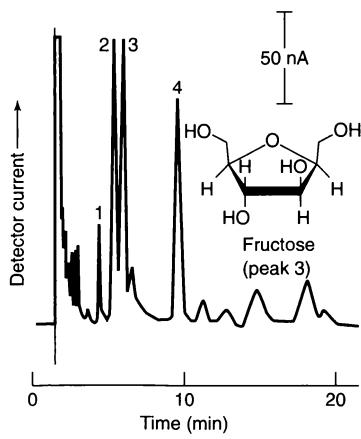


Figure 17-8 Anion-exchange chromatogram of Bud Dry beer diluted by a factor of 100 with water and filtered through a $0.45\text{-}\mu\text{m}$ membrane to remove particles. Column stationary phase is CarboPac PA1 and the mobile phase is 0.1 M NaOH. Labeled peaks are the sugars (1) arabinose, (2) glucose, (3) fructose, and (4) lactose. [From P. Luo, M. Z. Luo, and R. P. Baldwin, *J. Chem. Ed.* **1993**, 70, 679.]

are common means to detect analytes as they emerge from the column. Sugars in beverages can be measured by separating them by anion-exchange chromatography (described in Chapter 23) and detecting them with an electrode as they emerge. The $-\text{OH}$ groups of sugars such as glucose partly dissociate to $-\text{O}^-$ anions in 0.1 M NaOH. Anions are separated from one another as they pass through a column packed with particles having fixed positive charges.

The amperometric detector in Figure 17-7 features a Cu working electrode over which the liquid from the column flows. The Ag | AgCl reference electrode and a stainless steel auxiliary electrode are farther downstream at the upper left of the diagram. The working electrode is poised by a potentiostat at a potential of +0.55 V versus Ag | AgCl. As sugars emerge from the column, they are oxidized at the Cu surface. Reduction of water ($\text{H}_2\text{O} + \text{e}^- \longrightarrow \frac{1}{2}\text{H}_2 + \text{OH}^-$) takes place at the auxiliary electrode. Electric current flowing between the working and auxiliary electrodes is proportional to the concentration of each sugar exiting the column. Figure 17-8 shows the chromatogram, which displays detector current versus time as different sugars emerge from the chromatography column. Table 17-1 shows the sugar contents in various beverages measured by this method.

Ask Yourself

- How does the glucose monitor work?
- Why is a mediator advantageous in the glucose monitor?

Table 17-1 Partial list of sugars in beverages^a

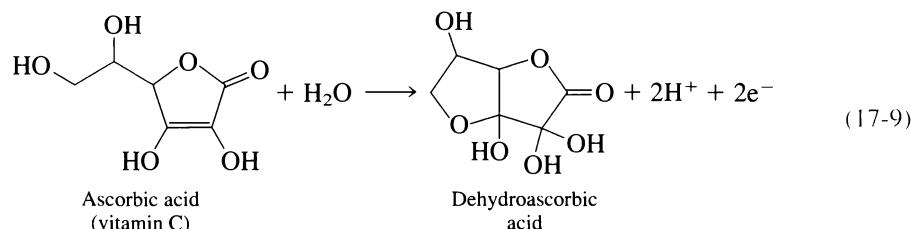
Brand	Sugar concentration (g/L)			
	Glucose	Fructose	Lactose	Maltose
Budweiser	0.54	0.26	0.84	2.05
Bud Dry	0.14	0.29	0.46	—
Coca Cola	45.1	68.4	—	1.04
Pepsi	44.0	42.9	—	1.06
Diet Pepsi	0.03	0.01	—	—

a. From P. Luo, M. Z. Luo, and R. P. Baldwin, *J Chem. Ed.* **1993**, 70, 679.

17-3 Voltammetry

In **voltammetry**, current is measured while voltage between two electrodes is varied. (In amperometry, we held voltage fixed during the measurement of current.) Consider the apparatus in Figure 17-9 used to measure vitamin C (ascorbic acid) in fruit drinks. Oxidation of analyte takes place at the exposed tip of the graphite working electrode:

Working electrode:



and reduction of H^+ occurs at the auxiliary electrode:



We measure current between the working and auxiliary electrodes as the potential of the working electrode is varied with respect to the reference electrode.

To record the **voltammogram** (the graph of current versus potential) of orange juice in Figure 17-10, the working electrode was first held at a potential of -1.5 V (versus $\text{Ag} | \text{AgCl}$) for 2 min while the solution was stirred. This *conditioning* reduces and removes organic material from the tip of the electrode. The potential was then changed to -0.4 V , and stirring continued for 30 s while bubbles of gas were dislodged from the electrode by gentle tapping. Stirring was then discontinued for 30 s so that the solution would be calm for the measurement. Finally, the voltage was scanned from -0.4 V to $+1.2\text{ V}$ at a rate of $+33\text{ mV/s}$ to record the lowest trace in Figure 17-10.

What happens as the voltage is scanned? At -0.4 V , there is no significant reaction and little current flows. At a potential near $+0.2\text{ V}$ in Figure 17-10, ascorbic acid begins to be oxidized at the tip of the working electrode and current rises. Beyond $+0.8\text{ V}$, ascorbic acid in the vicinity of the electrode tip is depleted by the electrochemical reaction. The current falls slightly because analyte cannot diffuse fast enough to the electrode to maintain the peak reaction rate.

Graphite is chosen because it is not expensive. The small exposed tip decreases distortion of the signal caused by electric resistance of the solution and capacitance of the electrode. A graphite “pencil lead” also works in this experiment.

Conditioning is repeated before each measurement (including each standard addition) to obtain a clean, fairly reproducible electrode surface.

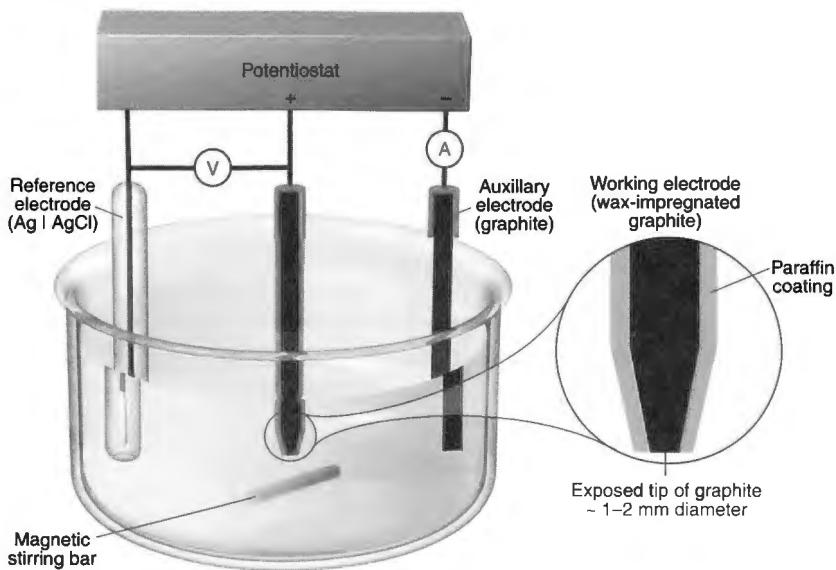


Figure 17-9 Three-electrode cell for voltammetric measurement of vitamin C in fruit drinks. The voltage between the working and reference electrodes is measured by the voltmeter (V) , and the current between the working and auxiliary electrodes is measured by the ammeter (A) . The potentiostat varies the voltage in a chosen manner.

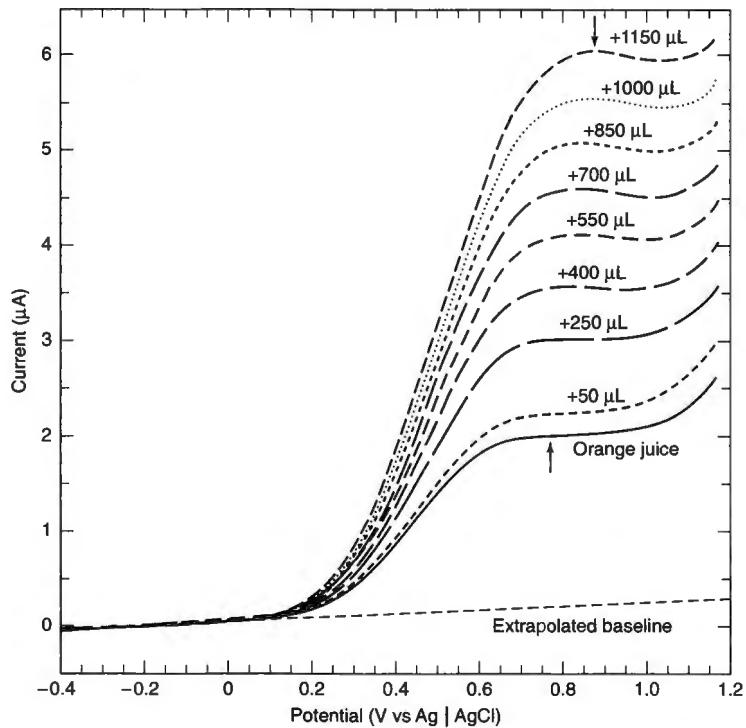


Figure 17-10 Voltammogram of 50.0 mL of orange juice and standard additions of 0.279 M ascorbic acid in 0.029 M HNO_3 . Voltage was scanned at +33 mV/s with apparatus in Figure 17-9. Peak position marked by arrows in the lowest and highest curves changes slightly as standard is added because the solution becomes more acidic.

The peak current is proportional to the concentration of ascorbic acid in the orange juice. We measure peak current at the arrow in Figure 17-10 relative to the baseline extrapolated from the region between -0.4 and 0 V, where little reaction occurs. Any species in juice that is oxidized near $+0.8$ V will interfere with the analysis. We do not yet know the proportionality constant between current and ascorbic acid concentration. To complete the measurement, we make several *standard additions* of known quantities of ascorbic acid, shown by the dashed curves in Figure 17-10.



Ask Yourself

17-C. If you have not worked Ask Yourself 5-C, now is the time to do it to get practice in the method of standard addition.

The method of standard addition was described in Section 5-3. Problem 5-19 gives an equation for the uncertainty in a standard addition graph.

17-4 Polarography

Polarography is voltammetry conducted with a *dropping-mercury electrode*. The cell in Figure 17-11 has a dropping-mercury working electrode, a Pt auxiliary electrode, and a calomel reference electrode. An electronically controlled dispenser suspends one drop of mercury from the tip of a glass capillary tube immersed in analyte solution. A measurement is made in ~ 1 s, the drop is released, and a fresh drop is suspended for the next measurement. There is always fresh, reproducible metal surface for each measurement.

Mercury is particularly useful for reduction processes. At other working electrodes, such as Pt, Au, or carbon, H^+ is reduced to H_2 at modest negative potentials. High current from this reaction obscures the signal from reduction of analyte. Reduction of H^+ is difficult at a Hg surface and requires much more negative potentials. Conversely, Hg has little useful range for oxidations, because Hg itself is oxidized to Hg^{2+} at modest positive potentials. Therefore a dropping-mercury electrode is usually used to reduce analytes. Platinum, gold, or carbon are used to oxidize analytes such as vitamin C in Reaction 17-9.

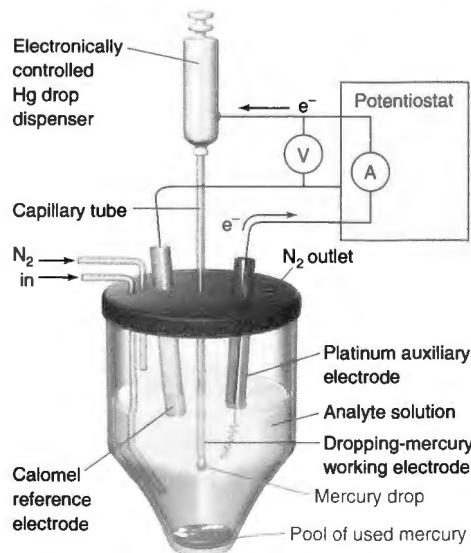


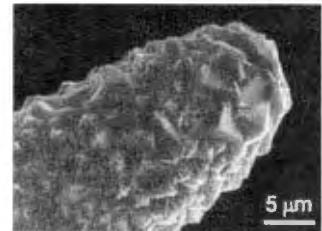
Figure 17-11 A cell for polarography.

Polarography was invented in 1922 by Jaroslav Heyrovský, who received the Nobel Prize in 1959.

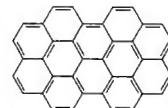
Potential limit (versus S.C.E.) for electrodes in 1 M H_2SO_4 :

Pt	-0.2 to $+0.9$ V
Au	-0.3 to $+1.4$ V
Glassy carbon	-0.8 to $+1.1$ V
B-doped diamond	-1.5 to $+1.7$ V
Hg	-1.3 to $+0.1$ V

In 1 M Cl^- , Hg is oxidized near 0 V by the reaction



Boron-doped diamond has one of the widest available potential ranges and is chemically inert. [From J. Cvačka et al., *Anal. Chem.* 2003, 75, 2678. Courtesy G. M. Swain, Michigan State University.]



Graphene is a single plane of hexagonal carbon.

Single atomic layers of graphite, called *graphene*, make electrodes with a working range similar to that of boron-doped diamond.

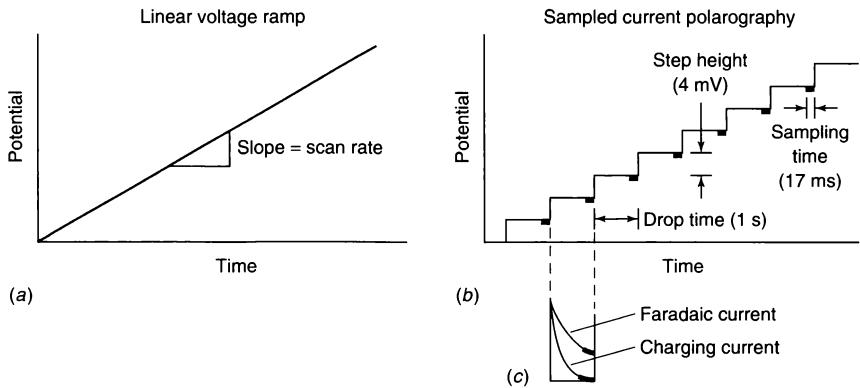
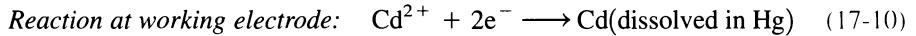


Figure 17-12 Voltage profiles for voltammetry: (a) linear voltage ramp used in vitamin C experiment; (b) staircase profile for *sampled current polarography*. Inset (c) shows how Faradaic and charging currents decay after each potential step.

The Polarogram

To record the voltammogram of vitamin C in Figure 17-10, the potential applied to the working electrode was varied at a constant rate from -0.4 V to $+1.2\text{ V}$. We call this voltage profile a *linear voltage ramp* (Figure 17-12a).

One of many ways to conduct a polarography experiment is with a *staircase voltage ramp* (Figure 17-12b). When each drop of Hg is dispensed, the potential is made more negative by 4 mV. After almost 1 s, current is measured during the last 17 ms of the life of each Hg drop. The **polarogram** in Figure 17-13a is a graph of current versus voltage when Cd^{2+} is the analyte. The chemistry at the working electrode is



The product Cd(0) is dissolved in the liquid Hg drop. A solution of anything in Hg is called an **amalgam**. We call Figure 17-13a a *sampled current polarogram* because current is measured only at the end of each drop life.

The curve in Figure 17-13a is called a **polarographic wave**. The potential at which half the maximum current is reached is called the **half-wave potential** ($E_{1/2}$) in Figure 17-13a. The constant current in the plateau region is called the **diffusion current** because it is limited by the rate of diffusion of analyte to the electrode. *For quantitative analysis, diffusion current is proportional to the concentration of analyte.* Diffusion current is measured from the baseline recorded without analyte in

$E_{1/2}$ is characteristic of a particular analyte in a particular medium. Analytes can be distinguished from one another by their half-wave potentials.

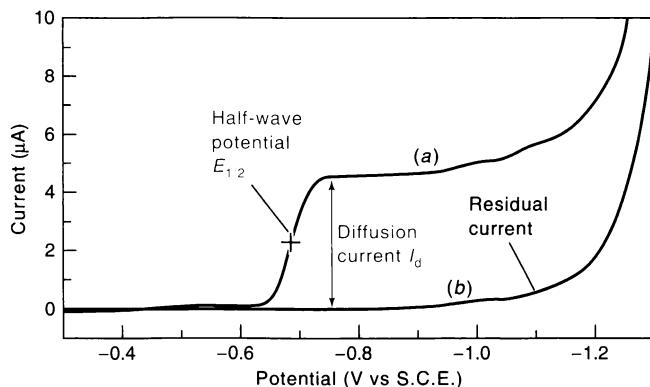


Figure 17-13 Sampled current polarogram of (a) 5 mM Cd^{2+} in 1 M HCl and (b) 1 M HCl alone.

Figure 17-13b. The small **residual current** in the absence of analyte is due mainly to reduction of impurities in the solution and on the surface of the electrodes. At sufficiently negative potential (-1.2 V in Figure 17-13), current increases rapidly as reduction of H^+ to H_2 in the aqueous solution commences.

Quantitative analysis requires that peak current (the diffusion current) be governed by the rate at which analyte diffuses to the electrode. Analyte can also reach the electrode by convection and electrostatic attraction. We minimize convection by using an unstirred solution. Electrostatic attraction is decreased by a high concentration of inert ions (called *supporting electrolyte*), such as 1 M HCl in Figure 17-13.

Oxygen must be absent because O_2 gives two polarographic waves when it is reduced first to H_2O_2 and then to H_2O . In Figure 17-11, N_2 is bubbled through analyte solution for 10 min to remove O_2 . Then bubbling is suspended, but the liquid is maintained under a blanket of flowing N_2 to keep O_2 out. The liquid must be calm during a measurement to minimize convection of analyte to the electrode.

Faradaic and Charging Currents

The current that we seek to measure in voltammetry is **faradaic current** due to reduction (or oxidation) of analyte at the working electrode. In Figure 17-13a, faradaic current is from reduction of Cd^{2+} at the Hg electrode. Another current, called **charging current** (or *capacitor current*) interferes with every measurement. To step the working electrode to a more negative potential, electrons are forced into the electrode from the potentiostat. In response, cations in solution flow toward the electrode, and anions flow away from the electrode. This flow of ions and electrons, called the *charging current*, is not from redox reactions. We try to minimize charging current because it obscures the faradaic current. The charging current usually controls the detection limit in polarography or voltammetry.

Figure 17-12c shows the behavior of faradaic and charging currents after each potential step in Figure 17-12b. Faradaic current decays because analyte cannot diffuse to the electrode fast enough to sustain the high reaction rate. Charging current decays even faster because ions near the electrode redistribute themselves rapidly. Waiting 1 s after each potential step ensures that faradaic current is still significant and charging current is small.

Square Wave Voltammetry

The most efficient voltage profile for polarography or voltammetry, called **square wave voltammetry**, uses the waveform in Figure 17-14, which consists of a square wave superimposed on a staircase.³ During each cathodic pulse in Figure 17-14, there is a rush of analyte to be reduced at the electrode surface. During the anodic pulse, analyte that was just reduced is reoxidized. The square wave polarogram in Figure 17-15 is the *difference* in current between intervals 1 and 2 in Figure 17-14. Electrons flow from the electrode to analyte at point 1 and in the reverse direction at point 2. Because the two currents have opposite signs, their difference is larger than either current alone. Because the difference is plotted, the shape of the square wave polarogram in Figure 17-15 is essentially the derivative of the sampled current polarogram.

The signal in square wave voltammetry is increased relative to a sampled current voltammogram, and the wave becomes peak shaped. The detection limit is reduced from $\sim 10^{-5}\text{ M}$ for sampled current polarography to $\sim 10^{-7}\text{ M}$ in square wave polarography. It is easier to resolve neighboring peaks than neighboring waves, so square wave polarography can resolve species whose half-wave potentials differ by $\sim 0.05\text{ V}$, whereas the potentials must differ by $\sim 0.2\text{ V}$ to be resolved in sampled

Polarograms in the older literature have large oscillations superimposed on the curve in Figure 17-13a. For the first 50 years of polarography, current was measured continuously as Hg flowed from an open capillary tube. Each drop grew until it fell off and was replaced by a new drop. The current oscillated from a low value when the drop was small to a high value when the drop was big.

Faradaic current: Due to redox reaction at the electrode

Charging current: Due to migration of ions toward or away from an electrode because of electrostatic attraction or repulsion; redox reactions have no role in charging current

By waiting after each potential step before measuring current, we observe significant faradaic current from the redox reaction with little interference from the charging current.

The optimum height of the square wave, E_p in Figure 17-14, is $50/n\text{ mV}$, where n is the number of electrons in the half-reaction. For Reaction 17-10, $n = 2$, so $E_p = 25\text{ mV}$.

Advantages of square wave voltammetry:

- Increased signal
- Derivative (peak) shape provides better resolution of neighboring signals
- Faster measurement

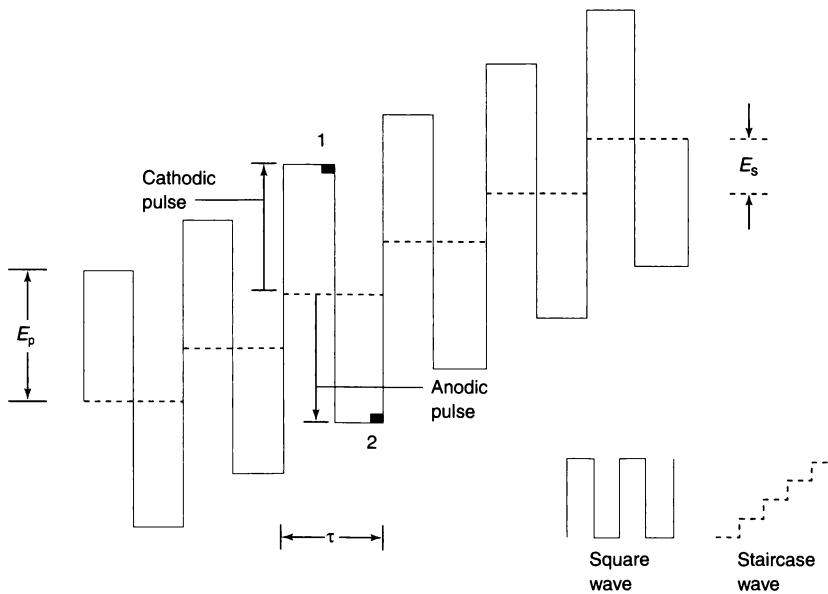


Figure 17-14 Waveform for square wave voltammetry. Typical parameters are pulse height (E_p) = 25 mV, step height (E_s) = 10 mV, and pulse period (τ) = 5 ms. Current is measured in regions 1 and 2.

current polarography. Square wave voltammetry is faster than other voltammetric techniques. The square wave polarogram in Figure 17-15 was recorded in one-fifth of the time required for the sampled current polarogram. In principle, the shorter the pulse period, τ , in Figure 17-14, the greater the current that will be observed. In practice, a pulse period of 5 ms is a practical lower limit for common equipment.

Stripping Analysis

Stripping analysis:

1. Concentrate analyte into a drop of Hg by reduction.
2. Reoxidize analyte by making the potential more positive.
3. Measure polarographic signal during oxidation.

In **stripping analysis**, analyte from a dilute solution is first concentrated into a single drop of Hg (or a thin film of Hg or onto a solid electrode) by electroreduction. Analyte is then *stripped* from the electrode by making the potential more positive, thereby oxidizing it back into solution. Current measured during oxidation is proportional to the quantity of analyte that was initially deposited. Figure 17-16 shows an anodic stripping voltammogram of traces of Cd, Pb, and Cu from honey. Anodic stripping is used to measure Pb in blood and is a valuable tool in screening children for exposure to lead.

Stripping is the most sensitive voltammetric technique because analyte is concentrated from a dilute solution. The longer the period of concentration, the more sensitive is the analysis. Only a fraction of analyte from solution is deposited, so deposition must be done for a reproducible time (such as 5 min) with reproducible stirring. Detection limits are $\sim 10^{-10}$ M.

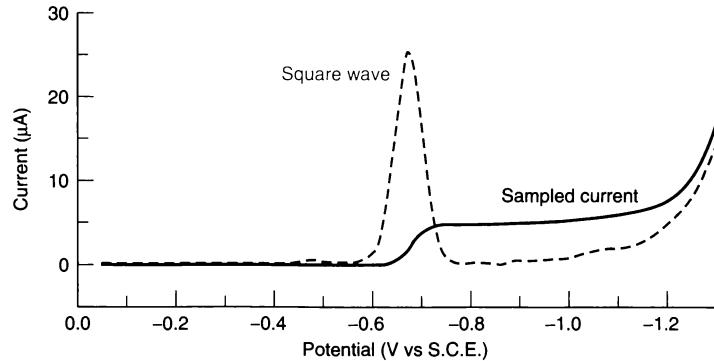


Figure 17-15 Comparison of polarograms of 5 mM Cd²⁺ in 1 M HCl. Operating parameters are defined in Figures 17-12b and 17-14. Sampled current: drop time = 1 s, step height = 4 mV, sampling time = 17 ms. Square wave: drop time = 1 s, step height (E_s) = 4 mV, pulse period (τ) = 67 ms, pulse height (E_p) = 25 mV, sampling time = 17 ms.

Figure 17-16 (a) Anodic stripping voltammogram of honey dissolved in water and acidified to pH 1.2 with HCl. Cd, Pb, and Cu were reduced from solution into a thin film of Hg for 5 min at -1.4 V (versus S.C.E.) prior to recording the voltammogram. (b) Voltammogram obtained without 5-min reduction step. The concentrations of Cd and Pb in the honey were 7 and 27 ng/g (ppb), respectively. The precision of the analysis was 2–4%. [From Y. Li, F. Wahdat, and R. Neeb, *Fresenius J. Anal. Chem.* **1995**, *351*, 678.]



Ask Yourself

- What is the difference between faradaic and charging current?
- Why is it desirable to wait 1 s after a potential pulse before recording the current in voltammetry?
- What are the advantages of square wave polarography over sampled current polarography?
- Explain what is done in anodic stripping voltammetry. Why is stripping the most sensitive polarographic technique?

Important Terms

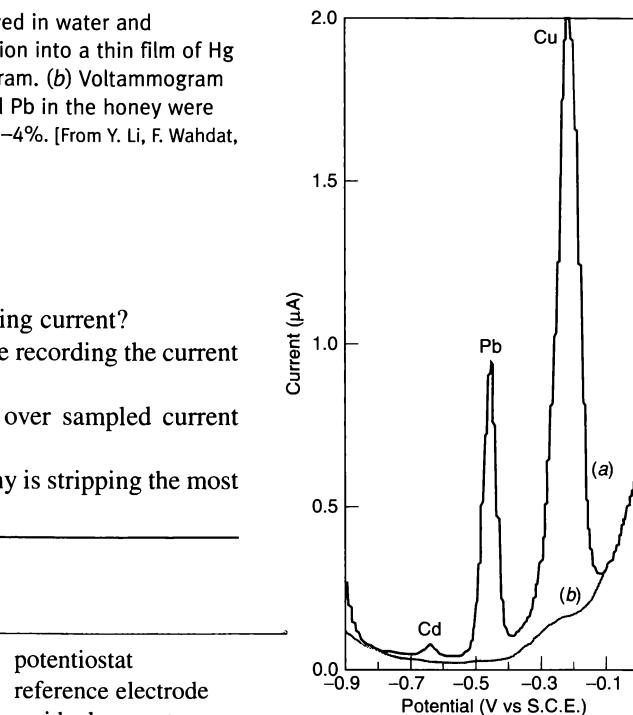
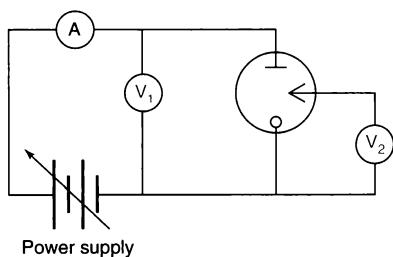
amalgam	electrogravimetric analysis	potentiostat
amperometry	electrolysis	reference electrode
auxiliary electrode	faradaic current	residual current
biosensor	half-wave potential	square wave voltammetry
charging current	mediator	stripping analysis
Clark electrode	polarogram	voltammetry
coulometry	polarographic wave	voltammogram
diffusion current	polarography	working electrode
electroactive species		

Problems

- 17-1. (a) State the general idea behind electrogravimetric analysis.
 (b) How can you know when an electrogravimetric deposition is complete?

17-2. How do the measurements of current and time in Reaction 17-3a allow us to measure the quantity of H_2S in Reaction 17-3b?

17-3. In the following diagram, —○— is the symbol for the working electrode, —|— is the auxiliary electrode, and —→— is the reference electrode. Which voltage, V_1 or V_2 , is held constant in an electrolysis with three electrodes?



17-4. Explain the function of each electrode in the polarography cell in Figure 17-11.

17-5. What is the difference between faradaic and charging current and why do we wait 1 s after each voltage step in Figure 17-12b before measuring current?

17-6. A 50.0-mL aliquot of unknown Cu(II) solution was exhaustively electrolyzed to deposit all copper on the cathode. The mass of the cathode was 15.327 g prior to electrolysis and 16.414 g after electrolysis. Find the molarity of Cu(II) in the unknown.

17-7. A solution containing 0.402 49 g of $\text{CoCl}_2 \cdot x\text{H}_2\text{O}$ (a solid with an unknown number of waters of hydration) was exhaustively electrolyzed to deposit 0.099 37 g of metallic cobalt on a platinum cathode by the reaction $\text{Co}^{2+} + 2\text{e}^- \rightarrow \text{Co}(s)$. Calculate the number of moles of water per mole of cobalt in the reagent. A good approach is to find moles of Co, moles of CoCl_2 , mass of CoCl_2 , and, by difference, mass of H_2O in the sample.

17-8. Ions that react with Ag^+ can be determined electrogravimetrically by deposition on a silver anode: $\text{Ag}(s) + \text{X}^- \rightarrow \text{AgX}(s) + \text{e}^-$. What will be the final mass of a

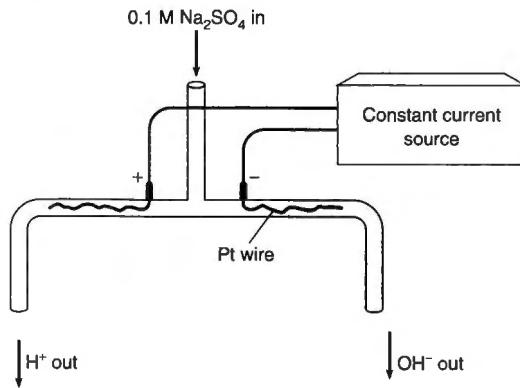
silver anode used to electrolyze 75.00 mL of 0.023 80 M KSCN if the initial mass of the anode is 12.463 8 g?

17-9. A 0.326 8-g unknown containing lead lactate, $\text{Pb}(\text{CH}_3\text{CHOHCO}_2)_2$ (FM 385.3), plus inert material was electrolyzed to produce 0.111 1 g of PbO_2 (FM 239.2). Was the PbO_2 deposited at the anode or at the cathode? Find the weight percent of lead lactate in the unknown.

17-10. $\text{H}_2\text{S}(aq)$ is analyzed by titration with coulometrically generated I_2 in Reactions 17-3a and 17-3b. To 50.00 mL of unknown H_2S sample were added 4 g of KI. Electrolysis required 812 s at 52.6 mA. Find the concentration of H_2S ($\mu\text{g/mL}$) in the sample.

17-11. OH^- generated at the right side of the apparatus in the diagram was used to titrate an unknown acid.

- (a) What chemical reactions produce OH^- and H^+ ?
 (b) If a current of 89.2 mA for 666 s was required to reach the end point in the titration of 5.00 mL of an unknown acid, HA, what was the molarity of HA?

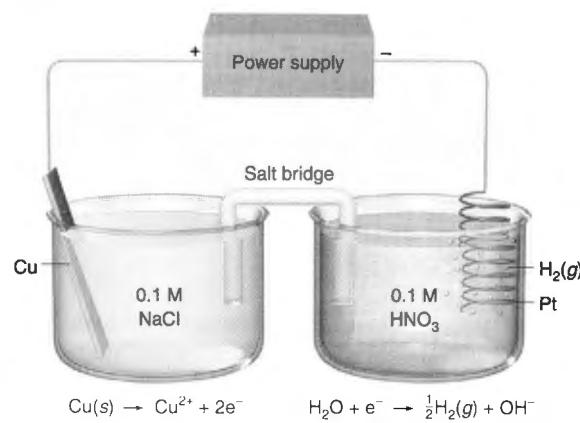


17-12. The sensitivity of a coulometer is governed by the delivery of its minimum current for its minimum time. Suppose that 5 mA can be delivered for 0.1 s.

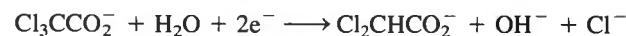
- (a) How many moles of electrons are delivered at 5 mA for 0.1 s?
 (b) How many milliliters of a 0.01 M solution of a two-electron reducing agent are required to deliver the same number of electrons?

17-13. The electrolysis cell shown here was run at a constant current of 0.021 96 A. On one side, 49.22 mL of H_2 were produced (at 303 K and 0.996 bar); on the other side, Cu metal was oxidized to Cu^{2+} .

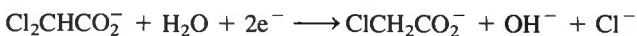
- (a) How many moles of H_2 were produced? (See Problem 16-16 for the ideal gas law.)
 (b) If 47.36 mL of EDTA were required to titrate the Cu^{2+} produced by the electrolysis, what was the molarity of the EDTA?
 (c) For how many hours was the electrolysis run?



17-14. A mixture of trichloroacetate and dichloroacetate can be analyzed by selective reduction in a solution containing 2 M KCl, 2.5 M NH_3 , and 1 M NH_4Cl . At a mercury cathode potential of -0.90 V (versus S.C.E.), only trichloroacetate is reduced:

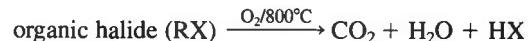


At a potential of -1.65 V, dichloroacetate reacts:

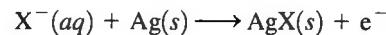


A hygroscopic mixture of trichloroacetic acid (FM 163.39) and dichloroacetic acid (FM 128.94) containing an unknown quantity of water weighed 0.721 g. On controlled potential electrolysis, 224 C passed at -0.90 V, and 758 C were required to complete the electrolysis at -1.65 V. Calculate the weight percent of each acid in the mixture.

17-15. Chlorine has been used for decades to disinfect drinking water. An undesirable side effect of this treatment is the reaction of chlorine with organic impurities to create organochlorine compounds, some of which could be toxic. Monitoring total organic halide (designated TOX) is now required for many water providers. A standard procedure for TOX is to pass water through activated charcoal that adsorbs organic compounds. Then the charcoal is combusted to liberate hydrogen halides:



HX is absorbed into aqueous solution and measured by coulometric titration with a silver anode:



When 1.00 L of drinking water was analyzed, a current of 4.23 mA was required for 387 s. A blank prepared by oxidizing charcoal required 6 s at 4.23 mA. Express TOX of the drinking water as micromoles of halogen per liter. If all halogen is chlorine, express the TOX as micrograms of Cl per liter.

17-16. Propagation of uncertainty. In an extremely accurate measurement of the Faraday constant, a pure silver anode was oxidized to Ag^+ with a constant current of 0.203 639 0 ($\pm 0.000\ 000\ 4$) A for 18 000.075 (± 0.010) s to give a mass loss of 4.097 900 ($\pm 0.000\ 003$) g from the anode. Given that the atomic mass of Ag is 107.868 2 ($\pm 0.000\ 2$), find the value of the Faraday constant and its uncertainty.

17-17. (a) How does the Clark electrode in Figure 17-3 measure dissolved O_2 ? Is electrode response an electric current or a voltage?

(b) What does it mean when we say that the concentration of dissolved O_2 is “0.20 bar”? What is the actual molarity of O_2 ?

17-18. Figure 17-10 shows successive standard additions of 0.279 M ascorbic acid (vitamin C) to 50.0 mL of orange juice. Data are given in columns B and D of Figure 5-5.

(a) Use a spreadsheet to prepare a graph like Figure 5-6 and find the concentration of ascorbic acid in the orange juice.

(b) Use the equation in Problem 5-19 to find the uncertainty in the x -intercept. Assuming that this is the principal uncertainty in the method, estimate the uncertainty in the concentration of ascorbic acid in the orange juice.

17-19. Suppose that a peak current of 3.9 μA was observed in the oxidation of 50 mL of 2.4 mM ascorbic acid in the experiment in Figures 17-9 and 17-10. Suppose that this much current flowed for 10 min in the course of several measurements. From the current and time, calculate what fraction of the ascorbic acid is oxidized at the electrode. Is it fair to say that the ascorbic acid concentration is nearly constant during the measurements?

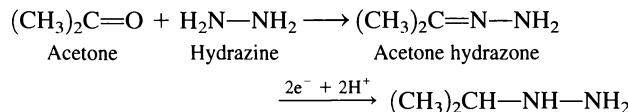
17-20. What are the advantages of a dropping-mercury electrode in polarography? Why is polarography used mainly to study reductions rather than oxidations?

17-21. *Calibration curve and error estimate.* The following polarographic diffusion currents were measured at -0.6 V for CuSO_4 in 2 M $\text{NH}_4\text{Cl}/2\text{M NH}_3$. Use the method of least squares to estimate the molarity (and its uncertainty) of an unknown solution giving $I_d = 15.6\ \mu\text{A}$.

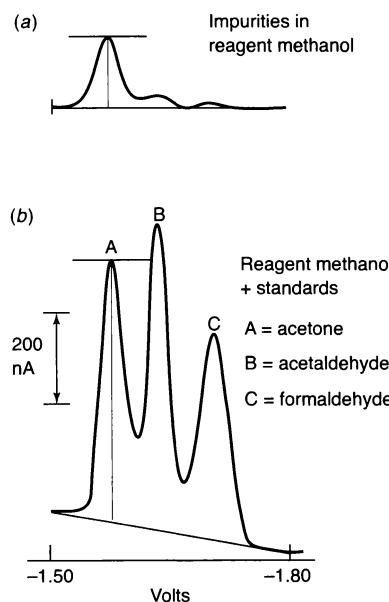
$[\text{Cu}^{2+}] (\text{mM})$	$I_d (\mu\text{A})$	$[\text{Cu}^{2+}] (\text{mM})$	$I_d (\mu\text{A})$
0.039 3	0.256	0.990	6.37
0.078 0	0.520	1.97	13.00
0.158 5	1.058	3.83	25.0
0.489	3.06	8.43	55.8

17-22. The drug Librium gives a polarographic wave with $E_{1/2} = -0.265\text{ V}$ (versus S.C.E.) in 0.05 M H_2SO_4 . A 50.0-mL sample containing Librium gave a wave height of 0.37 μA . When 2.00 mL of 3.00 mM Librium in 0.05 M H_2SO_4 were added to the sample, the wave height increased to 0.80 μA . Find the molarity of Librium in the unknown.

17-23. A polarogram of reagent-grade methanol is shown in trace *a*. Trace *b* shows reagent methanol with added 0.001 00 wt% acetone, 0.001 00 wt% acetaldehyde, and 0.001 00 wt% formaldehyde. Scales are the same in both panels. Solutions were prepared by diluting 25 mL of methanol up to 100 mL with water containing buffer and hydrazine sulfate, which reacts with carbonyl compounds to form electroactive hydrazones. An example is shown below:



From the two polarograms, estimate the wt% of acetone in reagent-grade methanol.



Polarograms of (a) reagent grade methanol and (b) methanol containing added standards. [D. B. Palladino, *Am. Lab.*, August 1992, p. 56].

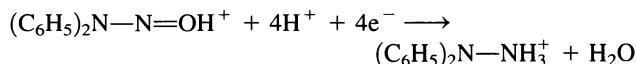
17-24. Problem 5-20 shows standard additions of Cu^{2+} to acidified tap water measured by anodic stripping voltammetry at a solid Ir electrode.

(a) What reaction occurs during the concentration stage of the analysis?

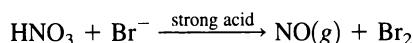
(b) What reaction takes place during the stripping stage of the analysis?

17-25. *Standard addition.* Chromium is an essential trace element present to the extent of $\sim 3\text{--}10\text{ ppb}$ in blood. It can be measured by cathodic stripping voltammetry after digesting blood with a powerful oxidant to destroy organic

5. The acidic diphenylnitrosamine is analyzed by amperometry at -0.66 V (versus Ag | AgCl) to measure the NO liberated from the food sample:



6. To measure nitrate, an additional 6 mL of 18 M H_2SO_4 are added to the sample tube in step 3. The acid promotes reduction of HNO_3 to NO, which is then purged with N_2 and trapped and analyzed as in steps 4 and 5.



A 10.0-g bacon sample gave a current of 8.9 μA in step 5, which increased to 23.2 μA in step 6. (Step 6 measures the sum of signals from nitrite and nitrate, not just the signal from nitrate.) In a second experiment, the 5.00-mL sample in step 3 was spiked with a standard addition of 5.00 μg of NO_2^- ion. The analysis was repeated to give currents of 14.6 μA in step 5 and 28.9 μA in step 6.

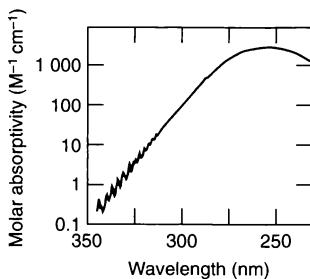
(a) Find the nitrite content of the bacon, expressed as micrograms per gram of bacon.

(b) From the ratio of signals due to nitrate and nitrite in the first experiment, find the micrograms of nitrate per gram of bacon.

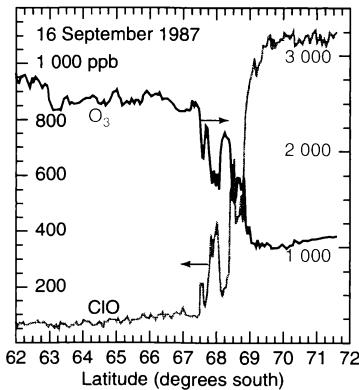
Notes and References

1. L. C. Clark, R. Wolf, D. Granger, and A. Taylor, "Continuous Recording of Blood Oxygen Tension by Polarography," *J. Appl. Physiol.* **1953**, 6, 189. To construct an oxygen electrode, see J. E. Brunet, J. I. Gardiazabal, and R. Schrebler, *J. Chem. Ed.* **1983**, 60, 677.
2. Make an enzymatic amperometric glucose electrode: M. C. Blanco-López, M. J. Lobo-Castañón, and A. J. Miranda-Ordieres, *J. Chem. Ed.* **2007**, 84, 677.
3. For an excellent account of square wave voltammetry, see J. G. Osteryoung and R. A. Osteryoung, *Anal. Chem.* **1985**, 57, 101A.
4. L. Yong, K. C. Armstrong, R. N. Dansby-Sparks, N. A. Carrington, J. Q. Chambers, and Z.-L. Xue, *Anal. Chem.* **2006**, 78, 7582.
5. D. Lowinsohn and M. Bertotti, *J. Chem. Ed.* **2002**, 79, 103. Some other species in wine, in addition to sulfite, react with I_3^- . A blank titration to correct for such reactions is described in this article.

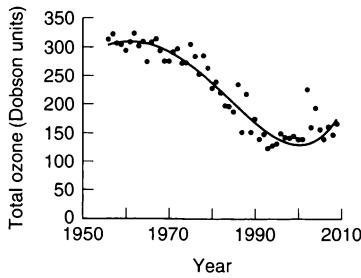
The Ozone Hole



Spectrum of ozone, showing maximum absorption of ultraviolet radiation at a wavelength near 260 nm. At this wavelength, a layer of ozone is more opaque than a layer of gold of the same mass. [From R. P. Wayne, *Chemistry of Atmospheres* (Oxford: Clarendon Press, 1991).]

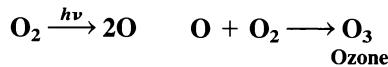


Spectroscopically measured concentrations of O₃ and ClO (measured in ppb = nL/L) in the stratosphere near the South Pole in 1987. Destruction of O₃ and increased ClO above latitude 68° are consequences of Reaction (2). [From J. G. Anderson, W. H. Brune, and M. H. Proffitt, *J. Geophys. Res.* 1989, 94D, 11465.]



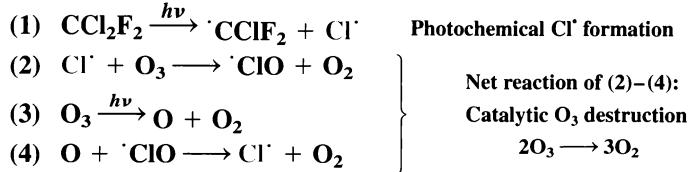
October average atmospheric ozone at Halley in Antarctica. Dobson units are a measure of total ozone. [From J. D. Shanklin, British Antarctic Survey, <http://www.antarctica.ac.uk/met/jds/ozone/>.]

Ozone, formed at altitudes of 20 to 40 km by the action of solar ultraviolet radiation ($h\nu$) on O₂, absorbs the ultraviolet radiation that causes sunburns and skin cancer:



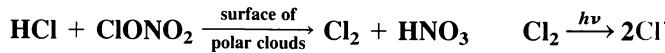
In 1985, the British Antarctic Survey reported that ozone over Antarctica had decreased by 50% in early spring (October), relative to levels observed in the preceding 20 years. This “ozone hole” appeared only in early spring and deepened for four decades.

Ozone destruction begins with chlorofluorocarbons such as Freon-12 (CCl₂F₂) from refrigerants. These long-lived compounds diffuse to the stratosphere, where they catalyze ozone decomposition:



Cl[·] produced in step 4 goes back to destroy another ozone molecule in step 2. A single Cl[·] atom in this chain reaction can destroy $>10^5$ molecules of O₃. The chain is terminated when Cl[·] or ·ClO reacts with hydrocarbons or NO₂ to form HCl or ClONO₂.

Stratospheric clouds catalyze the reaction of HCl with ClONO₂ to form Cl₂, which is split by sunlight into Cl[·] atoms to initiate O₃ destruction:



The clouds require winter cold to form. When the sun rises at the South Pole in September and October and clouds are still present, conditions are right for O₃ destruction.

To protect life from ultraviolet radiation, international treaties now ban or phase out chlorofluorocarbons. However, so much has already been released that ozone depletion is not expected to return to historic values until late in the twenty-first century.

Let There Be Light

Asorption and emission of *electromagnetic radiation* (a fancy term for light) are molecular characteristics used in quantitative and qualitative analysis. This chapter discusses basic aspects of **spectrophotometry**—the use of electromagnetic radiation to measure chemical concentrations—and Chapter 19 provides further detail on instrumentation and applications.

18-1 Properties of Light

Light can be described both as waves and as particles. Light waves consist of perpendicular, oscillating electric and magnetic fields (Figure 18-1). **Wavelength**, λ , is the crest-to-crest distance between waves. **Frequency**, ν , is the number of oscillations that the wave makes each second. The unit of frequency is *reciprocal seconds*, s^{-1} . One oscillation per second is also called 1 **hertz** (Hz). A frequency of 10^9 s^{-1} is therefore said to be 10^9 Hz , or one *gigahertz* (GHz). The product of frequency times wavelength is c , the speed of light ($2.998 \times 10^8 \text{ m/s}$ in vacuum):

$$\text{Relation between frequency and wavelength:} \quad \nu\lambda = c \quad (18-1)$$

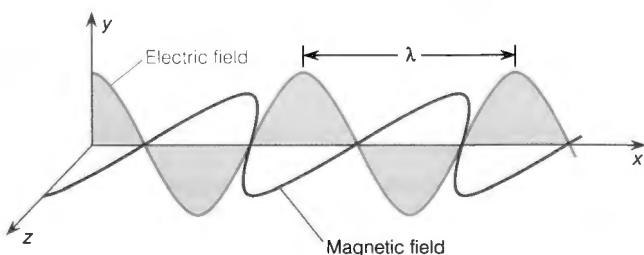
Example Relating Wavelength and Frequency

What is the wavelength of radiation in your microwave oven, whose frequency is 2.45 GHz?

SOLUTION First recognize that 2.45 GHz means $2.45 \times 10^9 \text{ Hz} = 2.45 \times 10^9 \text{ s}^{-1}$. From Equation 18-1, we write

$$\lambda = \frac{c}{\nu} = \frac{2.998 \times 10^8 \text{ m/s}}{2.45 \times 10^9 \text{ s}^{-1}} = 0.122 \text{ m}$$

 **Test Yourself** What is the frequency of green light with $\lambda = 500 \text{ nm}$? (Answer: $6.00 \times 10^{14} \text{ s}^{-1} = 600 \text{ THz}$)



Following the discovery of the Antarctic ozone “hole” in 1985, atmospheric chemist Susan Solomon led the first expedition in 1986 specifically intended to make chemical measurements of the Antarctic atmosphere by using high-altitude balloons and ground-based spectroscopy. The expedition discovered that ozone depletion occurred after polar sunrise and that the concentration of chemically active chlorine in the stratosphere was 100 times greater than that predicted from gas-phase chemistry. Solomon’s group identified chlorine as the culprit in ozone destruction and polar stratospheric clouds as the catalytic surface for the release of so much chlorine.

Figure 18-1 *Plane-polarized* electromagnetic radiation of wavelength λ , propagating along the x -axis. The electric field oscillates in the xy -plane and the magnetic field oscillates in the xz -plane. Ordinary, unpolarized light has electric and magnetic field components in all planes.

Light can also be thought of as particles called **photons**. The energy, E (measured in joules, J), of a photon is proportional to its frequency:

$$\text{Relation between energy and frequency:} \quad E = h\nu \quad (18-2)$$

Physical constants are listed inside the book cover.

where h is *Planck's constant* ($= 6.626 \times 10^{-34} \text{ J} \cdot \text{s}$).

Combining Equations 18-1 and 18-2, we can write

$$E = h \frac{c}{\lambda} = hc \frac{1}{\lambda} = hc\tilde{\nu} \quad (18-3)$$

Energy increases with

- increasing frequency (ν)
- decreasing wavelength (λ)
- increasing wavenumber ($\tilde{\nu}$)

where $\tilde{\nu} (= 1/\lambda)$ is called the **wavenumber**. Energy is inversely proportional to wavelength and directly proportional to wavenumber. Red light, with a wavelength longer than that of blue light, is less energetic than blue light. The SI unit for wavenumber is m^{-1} . However, the most common unit of wavenumber is cm^{-1} , read “reciprocal centimeters” or “wavenumbers.” Wavenumber units are most common in infrared spectroscopy.

Regions of the **electromagnetic spectrum** are shown in Figure 18-2. Visible light—the kind that our eyes detect—represents only a small fraction of the electromagnetic spectrum.

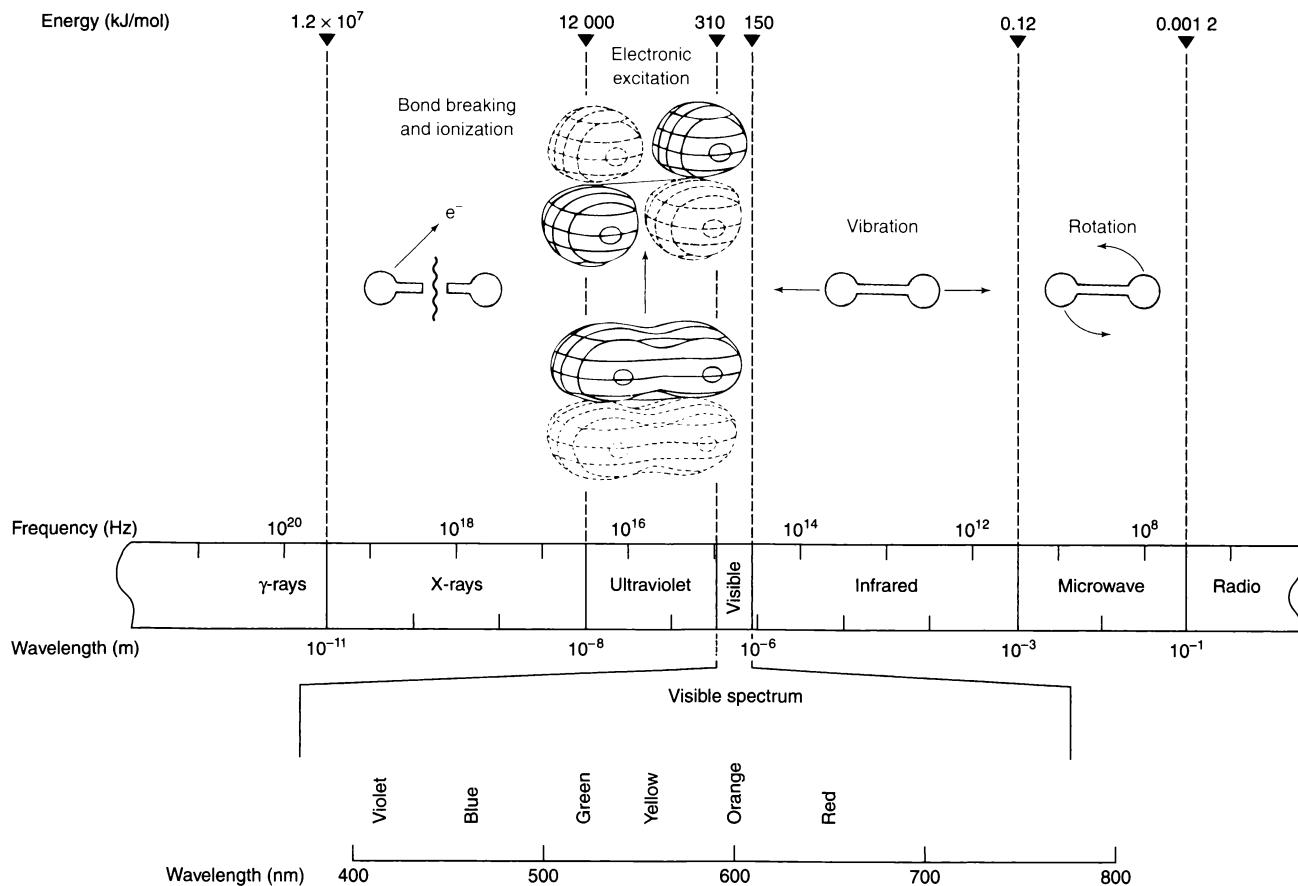


Figure 18-2 Electromagnetic spectrum, showing representative molecular processes that occur when radiation in each region is absorbed. The visible spectrum spans the wavelength range 380 to 780 nanometers ($1 \text{ nm} = 10^{-9} \text{ m}$).

The lowest energy state of a molecule is called the **ground state**. When a molecule absorbs a photon, its energy increases and we say that the molecule is promoted to an **excited state** (Figure 18-3). If the molecule emits a photon, its energy decreases. Figure 18-2 indicates that microwave radiation stimulates molecules to rotate faster. A microwave oven heats food by increasing the rotational energy of water in the food. Infrared radiation excites vibrations of molecules. Visible and ultraviolet radiation promote electrons to higher energy states. (Molecules that absorb visible light are colored.)

X-rays and short-wavelength ultraviolet radiation are harmful because they break chemical bonds and ionize molecules, which is why you should minimize your exposure to medical X-rays. An amazing source of X-rays is the peeling of a roll of Scotch tape® in a vacuum of 10^{-5} to 10^{-6} bar.¹ Figure 18-4 shows visible emission, which is accompanied by bursts of 10^5 X-ray photons in nanoseconds. The radiation has sufficient intensity to make an X-ray image of bones in a finger on dental film in 1 second. Peeling the tape causes electric charge to separate between the adhesive and its polyethylene backing. Periodically, the electric field is high enough to enable an electric discharge between the adhesive and the backing. Highly accelerated electrons liberate a burst of X-rays when they strike the positively charged adhesive and suddenly decelerate. X-rays are not emitted when the tape is peeled at atmospheric pressure because electrons collide with gas molecules before they accelerate to high enough energy to emit X-rays.

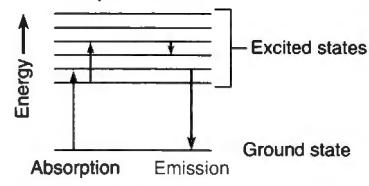
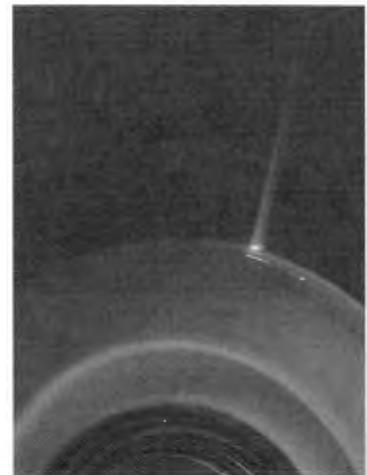


Figure 18-3 Absorption of light increases the energy of a molecule. Emission of light decreases its energy.



Example Photon Energies

By how many joules is the energy of a molecule increased when it absorbs (a) visible light with a wavelength of 500 nm or (b) infrared radiation with a wavenumber of $1\ 251\ \text{cm}^{-1}$?

SOLUTION (a) The visible wavelength is $500\ \text{nm} = 500 \times 10^{-9}\ \text{m}$.

$$E = h\nu = h\frac{c}{\lambda}$$

$$= (6.626 \times 10^{-34}\ \text{J} \cdot \text{s}) \left(\frac{2.998 \times 10^8\ \text{m/s}}{500 \times 10^{-9}\ \text{m}} \right) = 3.97 \times 10^{-19}\ \text{J}$$

This is the energy of one photon absorbed by one molecule. If a mole of molecules absorbed a mole of photons, the energy increase is

$$E = \left(3.97 \times 10^{-19} \frac{\text{J}}{\text{molecule}} \right) \left(6.022 \times 10^{23} \frac{\text{molecules}}{\text{mol}} \right) = 2.39 \times 10^5 \frac{\text{J}}{\text{mol}}$$

$$= \left(2.39 \times 10^5 \frac{\text{J}}{\text{mol}} \right) \left(\frac{1\ \text{kJ}}{1\ 000\ \text{J}} \right) = 239 \frac{\text{kJ}}{\text{mol}}$$

(b) When given the wavenumber, we use Equation 18-3. First convert the wavenumber unit cm^{-1} to m^{-1} with the conversion factor $100\ \text{cm/m}$. The energy of one photon is

$$E = hc\tilde{\nu} = (6.626 \times 10^{-34}\ \text{J} \cdot \text{s}) \left(2.998 \times 10^8 \frac{\text{m}}{\text{s}} \right) \left(1\ 251\ \text{cm}^{-1} \right) \underbrace{\left(100 \frac{\text{cm}}{\text{m}} \right)}_{\text{Conversion of } \text{cm}^{-1} \text{ to } \text{m}^{-1}}$$

$$= 2.485 \times 10^{-20}\ \text{J}$$

Figure 18-4 Visible (blue) emission from peeling a roll of Scotch tape® in $1.3\ \mu\text{bar}$ of air. Visible light is accompanied by bursts of X-rays. [Courtesy C. Camara and S. Puttermann, University of California, Los Angeles.]

Multiplying by Avogadro's number, we find that this photon energy corresponds to 14.97 kJ/mol, which falls in the infrared region and excites molecular vibrations.

 **Test Yourself** We say that the first excited vibrational state of H₂ lies "4 160 cm⁻¹ above the ground state." Find the energy (kJ/mol) of H₂ in this state. (Answer: 49.76 kJ/mol)

Ask Yourself

18-A. What is the frequency (Hz), wavenumber (cm⁻¹), and energy (kJ/mol) of light with a wavelength of (a) 100 nm; (b) 500 nm; (c) 10 μm; and (d) 1 cm? In which spectral region does each kind of radiation lie and what molecular process occurs when the radiation is absorbed?

18-2 Absorption of Light

A **spectrophotometer** measures transmission of light. If a substance absorbs light, the *radiant power* of a light beam decreases as it passes through the substance. Radian power, P , is the energy per second per unit area of the beam. Light with a narrow range of wavelengths is said to be **monochromatic** ("one color"). In Figure 18-5, light passes through a *monochromator*, a device that selects a narrow band of wavelengths. This light with radiant power P_0 strikes a sample of length b . The radiant power of the beam emerging from the other side of the sample is P . Some light may be absorbed by the sample, so $P \leq P_0$.

Transmittance, Absorbance, and Beer's Law

Transmittance, T , is the fraction of incident light that passes through a sample.

$$\text{Transmittance:} \quad T = \frac{P}{P_0} \quad (18-4)$$

Transmittance lies in the range 0 to 1. If no light is absorbed by the sample, the transmittance is 1. If all light is absorbed, the transmittance is 0. *Percent transmittance* ($100T$) ranges from 0% to 100%. A transmittance of 30% means that 70% of the light does not pass through the sample.

The most useful quantity for chemical analysis is **absorbance**, A , defined as

$$\text{Absorbance:} \quad A = \log\left(\frac{P_0}{P}\right) = -\log\left(\frac{P}{P_0}\right) = -\log T \quad (18-5)$$

Of course, you remember that

$$\log\left(\frac{1}{x}\right) = -\log x$$

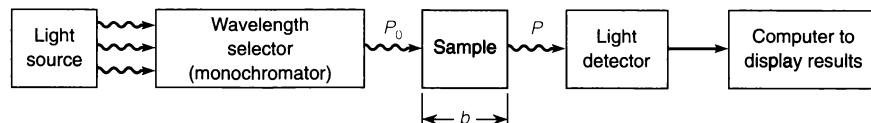


Figure 18-5 Schematic representation of a single-beam spectrophotometric experiment.

When no light is absorbed, $P = P_0$ and $A = 0$. If 90% of the light is absorbed, 10% is transmitted and $P = P_0/10$. This ratio gives $A = 1$. If 1% of the light is transmitted, $A = 2$.

P/P_0	% T	A
1	100	0
0.1	10	1
0.01	1	2

Example Absorbance and Transmittance

What absorbance corresponds to 99% transmittance? To 0.10% transmittance?

SOLUTION Use the definition of absorbance in Equation 18-5:

$$99\% T: \quad A = -\log T = -\log 0.99 = 0.0044$$

$$0.10\% T: \quad A = -\log T = -\log 0.0010 = 3.0$$

The higher the absorbance, the less light is transmitted through a sample.



Test Yourself What absorbance corresponds to 1% transmittance? To 50% transmittance? (Answer: 2.0, 0.30)

Absorbance is proportional to the concentration of light-absorbing molecules in the sample. Figure 18-6 shows that the absorbance of KMnO_4 is proportional to concentration over four orders of magnitude (from 0.6 μM to 3 mM).

Absorbance is also proportional to the pathlength of substance through which light travels. The dependence on concentration and pathlength is expressed in **Beer's law**:

Beer's law:
$$A = \epsilon bc \quad (18-6)$$

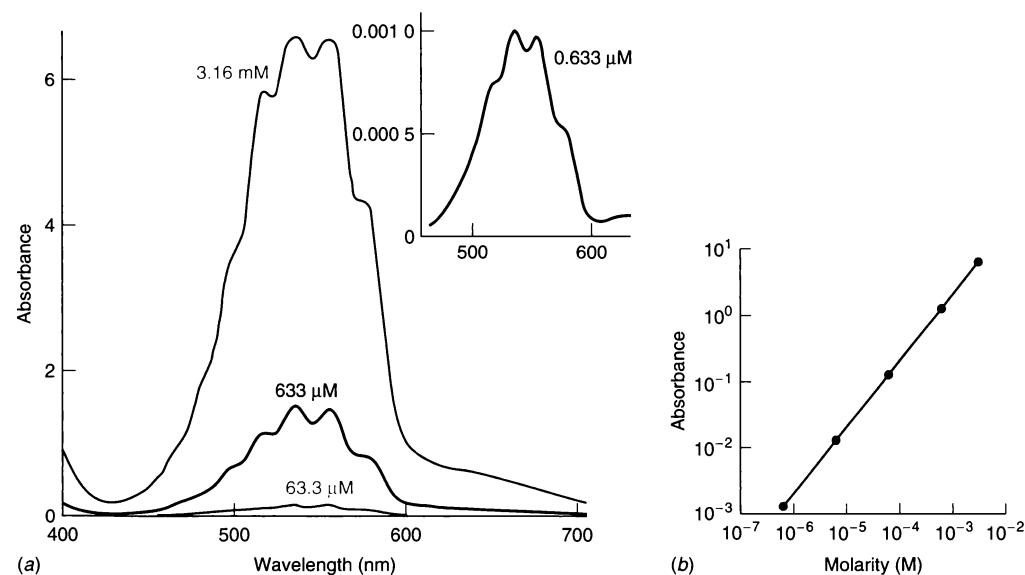


Figure 18-6 (a) Absorption spectrum of KMnO_4 at four different concentrations. (b) Peak absorbance at 555 nm is proportional to concentration from 0.6 μM to 3 mM. The Cary 5000 ultraviolet-visible-near infrared spectrophotometer used for this work has a wider operating range than many instruments. It is difficult to measure absorbance accurately above 2 or below 0.01. [From A. R. Hind, *Am. Lab.*, December 2002, p. 32. Courtesy Varian, Inc., Palo Alto, CA.]

Box 18-1 gives a physical picture of Beer's law that could be the basis for a classroom exercise.

Absorbance (A) is dimensionless. Concentration (c) has units of moles per liter (M), and pathlength (b , Figure 18-5) is commonly expressed in centimeters. The quantity ϵ (epsilon) is called the **molar absorptivity**. It has the units $M^{-1} \text{ cm}^{-1}$ because the product ϵbc must be dimensionless. Molar absorptivity tells how much light is absorbed at a particular wavelength by a particular substance.

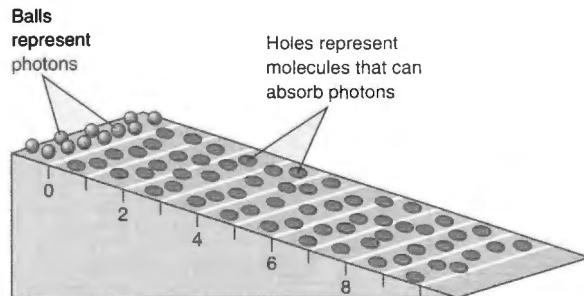
Color Plate 13 shows that color intensity increases as the concentration of the absorbing molecule increases. Absorbance is a measure of the color. The more intense the color, the greater the absorbance.

Example Using Beer's Law

Peak absorbance of 3.16×10^{-3} M KMnO_4 at 555 nm in a 1.000-cm-pathlength cell in Figure 18-6 is 6.54. (a) Find the molar absorptivity and percent transmittance of this solution. (b) What would be the absorbance if the pathlength were 0.100 cm? (c) What would be the absorbance in a 1.000-cm cell if the concentration were decreased by a factor of 4?

Box 18-1 Discovering Beer's Law²

Each photon passing through a solution has a certain probability of striking a light-absorbing molecule and being absorbed. Let's model this process by thinking of an inclined plane with holes representing absorbing molecules. The number of molecules is equal to the number of holes and the pathlength is equal to the length of the plane. Suppose that 1 000 small balls, representing 1 000 photons, are rolled down the incline. Whenever a ball drops through a hole, we consider it to have been "absorbed" by a molecule.



Inclined plane model for photon absorption.

Let the plane be divided into 10 equal intervals and let the probability that a ball will fall through a hole in the first interval be 1/10. Of the 1 000 balls entering the first interval, one-tenth—100 balls—are absorbed (dropping through the holes) and 900 pass into the second interval. Of the 900 balls entering the second

interval, one-tenth—90 balls—are absorbed and 810 proceed to the third interval. Of these 810 balls, 81 are absorbed and 729 proceed to the fourth interval. The table below summarizes the action.

Transmittance is defined as

$$T = \frac{\text{number of surviving balls}}{\text{initial number of balls} (= 1\,000)}$$

Graph *a* shows that a plot of transmittance versus interval number (which is analogous to plotting transmittance versus pathlength in a spectrophotometric experiment) is not linear. However, the plot of $-\log(\text{transmittance})$

Interval	Photons absorbed	Photons transmitted	Transmittance (P/P_0)
0		1 000	1.000
1	100	900	0.900
2	90	810	0.810
3	81	729	0.729
4	73	656	0.656
5	66	590	0.590
6	59	531	0.531
7	53	478	0.478
8	48	430	0.430
9	43	387	0.387
10	39	348	0.348

SOLUTION The molar absorptivity in Beer's law is the constant of proportionality between absorbance and the product pathlength \times concentration:

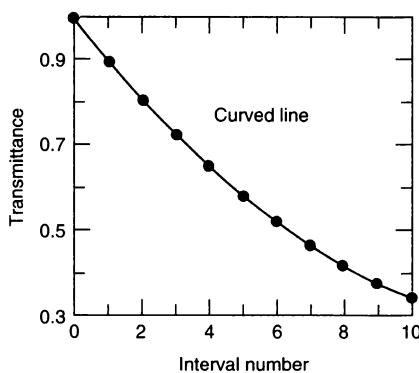
$$(a) A = \varepsilon bc$$

$$6.54 = \varepsilon(1.000 \text{ cm})(3.16 \times 10^{-3} \text{ M}) \Rightarrow \varepsilon = 2.07 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$$

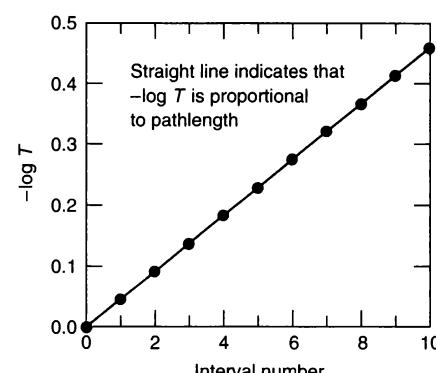
Equation 18-5 tells us that $A = -\log T$ or $\log T = -A$. But you know that if $x = y$, then $10^x = 10^y$. So we solve for T by raising 10 to the power on both sides of the equation:

$$\begin{aligned}\log T &= -A \\ 10^{\log T} &= 10^{-A} \\ 10^{\log T} &\text{ is the same as } T \\ T &= 10^{-A} = 10^{-6.54} = 2.88 \times 10^{-7}\end{aligned}$$

To evaluate $10^{-6.54}$ on your calculator, use y^x or *antilog*. If you use y^x , $y = 10$ and $x = -6.54$. If you use *antilog*, find the antilog of -6.54 . Be sure that you can show that $10^{-6.54} = 2.88 \times 10^{-7}$.



(a) Transmittance versus interval is analogous to T versus pathlength.



(b) $-\log T$ versus interval is analogous to $-\log T$ versus pathlength.

versus interval number in graph *b* is linear and passes through the origin. Graph *b* shows that $-\log T$ is proportional to pathlength.

To investigate how transmittance depends on the concentration of absorbing molecules, you could do the same mental experiment with a different number of holes in the inclined plane. For example, try setting up a table to show what happens if the probability of absorption in each interval is $1/20$ instead of $1/10$. This change corresponds to decreasing the concentration of absorbing molecules to half of its initial value. You will

discover that a graph of $-\log T$ versus interval number has a slope equal to one-half that in graph *b*. That is, $-\log T$ is proportional to concentration as well as to pathlength. So, we have just shown that $-\log T$ is proportional to both concentration and pathlength. Defining absorbance as $-\log T$ gives us the essential terms in Beer's law:

$$A \equiv -\log T \propto \underset{\uparrow}{\text{concentration}} \times \text{pathlength}$$

This symbol means "is proportional to"

Percent transmittance is $100T = 2.88 \times 10^{-5}\%$. When the absorbance is 6.54, transmittance is very tiny.

- (b) If we decrease pathlength by a factor of 10, we decrease absorbance by a factor of 10 to $6.54/10 = 0.654$.
- (c) If we decrease concentration by a factor of 4, we decrease absorbance by a factor of 4 to $6.54/4$ to 1.64.

 **Test Yourself** Find the absorbance of a 13.0- μM solution of a compound whose molar absorptivity is $4.64 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ in a 1.00-cm cell and in a 2.00-cm cell. (**Answer:** 0.603, 1.206)

 **Example** Finding Concentration from Absorbance

Gaseous ozone has a molar absorptivity of $2700 \text{ M}^{-1} \text{ cm}^{-1}$ at the absorption peak near 260 nm in the spectrum at the beginning of this chapter. Find the concentration of ozone (mol/L) in air if a sample has an absorbance of 0.23 in a 10.0-cm cell. Air has negligible absorbance at 260 nm.

SOLUTION Rearrange Beer's law to solve for concentration:

$$c = \frac{A}{\epsilon b} = \frac{0.23}{(2700 \text{ M}^{-1} \text{ cm}^{-1})(10.0 \text{ cm})} = 8.5 \times 10^{-6} \text{ M}$$

 **Test Yourself** What is the concentration of O_3 if $A = 0.18$ at 260 nm in a 2.00-meter-pathlength cell? (**Answer:** 0.33 μM)

Limitations of Beer's Law

Deviations from Beer's law can occur when

- light is not monochromatic and measurement is not made at absorption peak
- analyte or other solute concentration is too high
- analyte participates in a concentration-dependent chemical equilibrium
- too much stray light reaches the detector
- sample temperature changes

Beer's law states that absorbance is proportional to the concentration of the absorbing species. Beer's law works for monochromatic radiation passing through a dilute solution ($\leq 0.01 \text{ M}$) in which the absorbing species is not participating in a concentration-dependent equilibrium.

At an absorption peak, such as 555 nm in Figure 18-6, the molar absorptivity does not vary much for small excursions away from the peak. Light from the monochromator has a small range of wavelengths and is not perfectly monochromatic. However, Beer's law is obeyed because the molar absorptivity does not vary much over the narrow width of the incoming light. If we measured absorbance on the steep side of the band at 590 nm, there is a large change in molar absorptivity over just a few nanometers of wavelength, leading to a nonlinear dependence of absorbance on concentration.

Beer's law fails when the concentration of analyte or other species in the solution becomes too high. When solute molecules get close to one another, their molar absorptivity changes somewhat. At very high concentration, the solute *becomes* the solvent. The molar absorptivity of a molecule is different in different solvents. Non-absorbing solutes in a solution can also interact with the absorbing species and alter the absorptivity.

If the absorbing molecule participates in a concentration-dependent chemical equilibrium, the absorptivity changes with concentration. For example, in concentrated solution, a weak acid, HA, could be mostly undissociated. As the solution is diluted, dissociation increases. If the absorptivity of A^- is not the same as that of HA, the solution will appear not to obey Beer's law as it is diluted.

Imperfect instruments can lead to deviations from Beer's law. In every instrument, some *stray light* (wavelengths outside the bandwidth expected from the monochromator) reaches the detector. When the sample absorbance is very high, much of the light reaching the detector could be stray light. Therefore stray light causes a deviation from Beer's law. If the temperature of the sample is not kept constant, molar absorptivity will change.

The way to guard against deviations from Beer's law is to construct a calibration curve with your spectrophotometer under fixed operating conditions, showing the measured absorbance over the expected range of analyte concentration. If you operate within this demonstrated range, Beer's law should be valid.

A linear calibration curve assures us that we are working in a range in which Beer's law is obeyed.

Absorption Spectra and Color

An **absorption spectrum** is a graph showing how A (or ϵ) varies with wavelength (or frequency or wavenumber). Figure 18-6 shows the visible absorption spectrum of $KMnO_4$, and the opening of this chapter shows the ultraviolet absorption spectrum of ozone. Figure 18-7 shows the absorption spectrum of a typical sunscreen lotion, which absorbs harmful solar radiation below about 350 nm. Demonstration 18-1 illustrates the meaning of an absorption spectrum.

The plural of "spectrum" is "spectra."

Example How Effective Is Sunscreen?

What fraction of ultraviolet radiation is transmitted through the sunscreen in Figure 18-7 at the peak absorbance near 300 nm?

SOLUTION The absorbance at 300 nm is approximately 0.35. Therefore the transmittance is $T = 10^{-A} = 10^{-0.35} = 0.45 = 45\%$. Just over half the ultraviolet radiation (55%) is absorbed by this thickness of sunscreen and would not reach your skin.

 **Test Yourself** If you goop on more sunscreen to double its thickness, absorbance will be doubled. What will be the transmittance near 300 nm and what percentage of ultraviolet radiation is blocked? (**Answer:** $T = 0.20$; 80% is blocked)

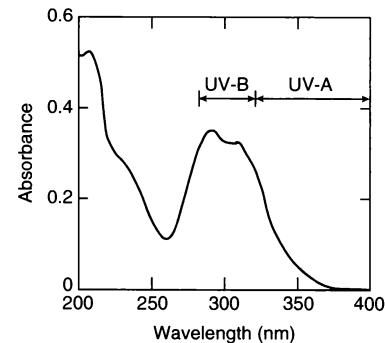


Figure 18-7 Absorption spectrum of typical sunscreen lotion shows absorbance versus wavelength in the ultraviolet region. Sunscreen was thinly coated onto a transparent window to make this measurement. [From D. W. Daniel, *J. Chem. Ed.* **1994**, 71, 83.] Sunscreen makers refer to the region 400–320 nm as UV-A and 320–280 nm as UV-B.

Problem 18-13 defines the "SPF" number of sunscreens.

White light contains all colors of the rainbow. A substance that absorbs visible light appears colored when white light is transmitted through it or reflected from it. The substance absorbs certain wavelengths of white light, and our eyes detect wavelengths that are not absorbed. Table 18-1 is an *approximate* guide to colors. The observed color is the *complement* of the absorbed color. As an example, bromophenol blue in Color Plate 14 has a visible absorbance maximum at 591 nm, and its observed color is blue.

The color of a substance is the complement of the color that it absorbs.

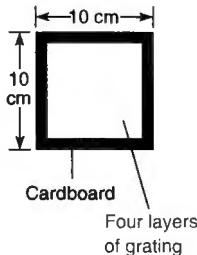


Demonstration 18-1 Absorption Spectra^{3,4}

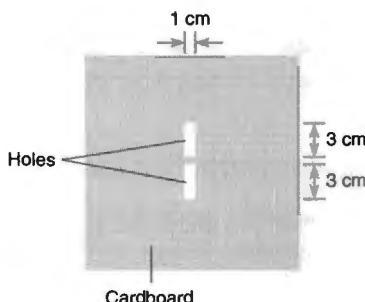
The spectrum of visible light can be projected on a screen in a darkened room in the following manner: Four layers of plastic diffraction grating* are mounted on a cardboard frame that has a square hole large enough to cover the lens of an overhead projector. This assembly is taped over the projector lens facing the screen. An opaque cardboard surface with two 1×3 cm slits is placed on the working surface of the projector.



(a)



(b)



(c)

(a) Overhead projector. (b) Diffraction grating mounted on cardboard. (c) Mask for working surface.

Color Plate 14a shows the spectrum of white light and the absorption spectra of three different colored solutions. We see that potassium dichromate, which appears orange or yellow, absorbs blue wavelengths. Bromophenol blue absorbs yellow and orange wavelengths and appears blue to our eyes. The absorption of

When the lamp is turned on, the white image of each slit is projected on the center of the screen. A visible spectrum appears on either side of each image. When a beaker of colored solution is placed over one slit, you can see color projected on the screen where the white image previously appeared. The spectrum beside the colored image loses its intensity in regions where the colored solution absorbs light.

phenolphthalein is located near the center of the visible spectrum. For comparison, the spectra of these three solutions recorded with a spectrophotometer are shown in Color Plate 14b.

*Edmund Scientific Co., edmundoptics.com, catalog no. NT40-267.

Table 18-1 Colors of visible light

Wavelength of maximum absorption (nm)	Color absorbed	Color observed
380–420	Violet	Green-yellow
420–440	Violet-blue	Yellow
440–470	Blue	Orange
470–500	Blue-green	Red
500–520	Green	Purple-red
520–550	Yellow-green	Violet
550–580	Yellow	Violet-blue
580–620	Orange	Blue
620–680	Red	Blue-green
680–780	Red	Green

Ask Yourself

- 18-B. (a) What is the absorbance of a 2.33×10^{-4} M solution of a compound with a molar absorptivity of $1.05 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ in a 1.00-cm cell?
(b) What is the transmittance of the solution in (a)?
(c) Find A and $\%T$ when the pathlength is doubled to 2.00 cm.
(d) Find A and $\%T$ when the pathlength is 1.00 cm but the concentration is doubled.
(e) What would be the absorbance in (a) for a different compound with twice as great a molar absorptivity ($\epsilon = 2.10 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$)? The concentration and pathlength are unchanged from (a).
(f) Color Plate 15 shows suspensions of silver nanoparticles whose color depends on the size and shape of the particles. From Table 18-1, predict the color of each solution from the wavelength of maximum absorption. Do observed colors agree with predicted colors?

18-3 Practical Matters

Minimum requirements for a spectrophotometer were shown in Figure 18-5. The instrument measures the fraction of incident light (the transmittance) that passes through a sample to the detector. Sample is usually contained in a cell called a **cuvet** (Figure 18-8), which has flat, fused-silica faces. Fused silica (a glass made of SiO_2) transmits visible and ultraviolet radiation. Plastics and ordinary glass absorb ultraviolet radiation, so plastic or glass cuvets can be used only for measurements at visible wavelengths. Infrared cells are typically made of sodium chloride or potassium bromide crystals. Gases are more dilute than liquids and require cells with longer pathlengths, typically ranging from 10 cm to many meters. A pathlength of many meters is obtained by reflecting light so that it traverses the sample many times before reaching the detector.

The instrument represented in Figure 18-5 is called a *single-beam spectrophotometer* because it has only one beam of light. We do not measure the incident radiant power, P_0 , directly. Rather, the radiant power passing through a reference cuvet containing pure solvent is *defined* as P_0 in Equation 18-4. This cuvet is then removed and replaced by an identical one containing sample. Radiant power striking the detector is then taken as P in Equation 18-4, thus allowing T or A to be determined. The reference cuvet containing pure solvent compensates for reflection, scattering, and absorption of light by the cuvet and solvent. The radiant power reaching the detector would not be the same if the reference cuvet were removed from the beam. The reference cuvet containing pure solvent acts as a blank in the measurement of transmittance. A double-beam spectrophotometer housing both a sample cuvet and a reference cuvet is described in Section 19-1.

Good Operating Techniques

Cuvets must be kept scrupulously clean and should be handled with a tissue to avoid putting fingerprints on the faces. Fingerprints or contamination from previous samples can scatter or absorb light. Wash the cuvet and rinse it with distilled water

For chemical analysis, transmittance is converted into absorbance:

$$A = -\log T$$

Radiant power passing through cuvet filled with solvent $\equiv P_0$

Radiant power passing through cuvet filled with sample $\equiv P$

$$\text{Transmittance} = P/P_0$$

Do not touch the clear faces of a cuvet with your fingers. Keep the cuvet scrupulously clean.

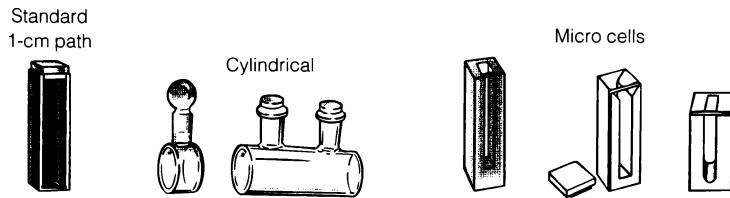


Figure 18-8 Common cuvets for ultraviolet and visible measurements. [From A. H. Thomas Co., Philadelphia, PA.]

as soon as you are finished using it. Let it dry upside down so that water drains out and no water marks are left on the walls. All vessels should be covered to protect them from dust, which scatters light and therefore makes it look as if the absorbance of the sample has increased. Another reason to cover a cuvet is to prevent evaporation of the sample.

Use *matched* cuvets manufactured to have identical pathlength. Any mismatch leads to systematic error. Place each cuvet in the spectrophotometer as reproducibly as possible. One side of the cuvet should be marked so that the cuvet is always oriented the same way. Slight misplacement of the cuvet in its holder, or turning a flat cuvet around by 180°, or rotation of a circular cuvet, can lead to random errors in absorbance. If matched cuvets are not available, use the same cuvet to read the absorbance of both sample and reference.

Modern spectrophotometers are most precise (reproducible) at intermediate levels of absorbance ($A \approx 0.3$ to 2). If too little light gets through the sample (high absorbance), intensity is hard to measure. If too much light gets through (low absorbance), it is hard to distinguish transmittance of the sample from that of the reference.

Figure 18-9 shows the relative standard deviation of replicate measurements made at 350 nm with a diode array spectrometer. The two curves show measurements made with a new cuvet holder (solid line) or a 10-year-old cuvet holder (dashed, colored line). For the new cuvet holder, removing the sample cuvet and replacing it in the holder between measurements had no significant effect. Relative standard deviation is below 0.1% in both cases for absorbance in the range 0.3 to 2. Results shown by squares were obtained when a 10-year-old cuvet holder was used and the sample cuvet was removed and replaced in the holder between measurements. Variability in the position of the cuvet more than doubles the relative standard deviation. The conclusion is that modern spectrometers have excellent precision and modern cell holders provide excellent reproducibility. Precision was degraded when an old cell holder was used and the sample was removed and inserted between measurements.

For spectrophotometric analysis, measurements are made at a wavelength (λ_{max}) corresponding to a peak in the absorbance spectrum. This wavelength gives the greatest sensitivity—maximum response for a given concentration of analyte. Errors due to wavelength drift and the finite width of wavelengths selected by the monochromator are minimized because the spectrum varies least with wavelength at the absorbance maximum.

In measuring a spectrum, it is routine to first record a baseline with pure solvent or a reagent blank in *both* the sample and reference cuvets. Cuvets are sold in matched pairs that are as identical as possible to each other. In principle, the baseline absorbance should be 0. However, small mismatches between the two cuvets and instrumental imperfections lead to small positive or negative baseline absorbance. The absorbance of the sample is then recorded and the absorbance of the baseline is subtracted from that of the sample to obtain true absorbance.

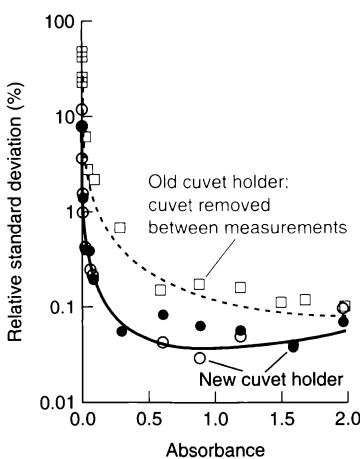


Figure 18-9 Precision of replicate absorbance measurements with a diode array spectrometer at 350 nm with a dichromate solution. Filled circles are from replicate measurements in which the sample was not removed from the cuvet holder between measurements. Open circles are from measurements in which the sample was removed and then replaced in the cuvet holder between measurements. Best reproducibility is observed at intermediate absorbance ($A \approx 0.3$ to 2). Note logarithmic ordinate. Lines are least-squares fit of data to theoretical equations. [Data from J. Galbán, S. de Marcos, I. Sanz, C. Ubide, and J. Zuriarrain, *Anal. Chem.* **2007**, 79, 4763].

Ask Yourself

- 18-C. (a) What precautions should you take in handling a cuvet and placing it in the spectrophotometer?
 (b) Why is it most accurate to measure absorbances in the range $A = 0.3$ to 2 ?

18-4 Using Beer's Law

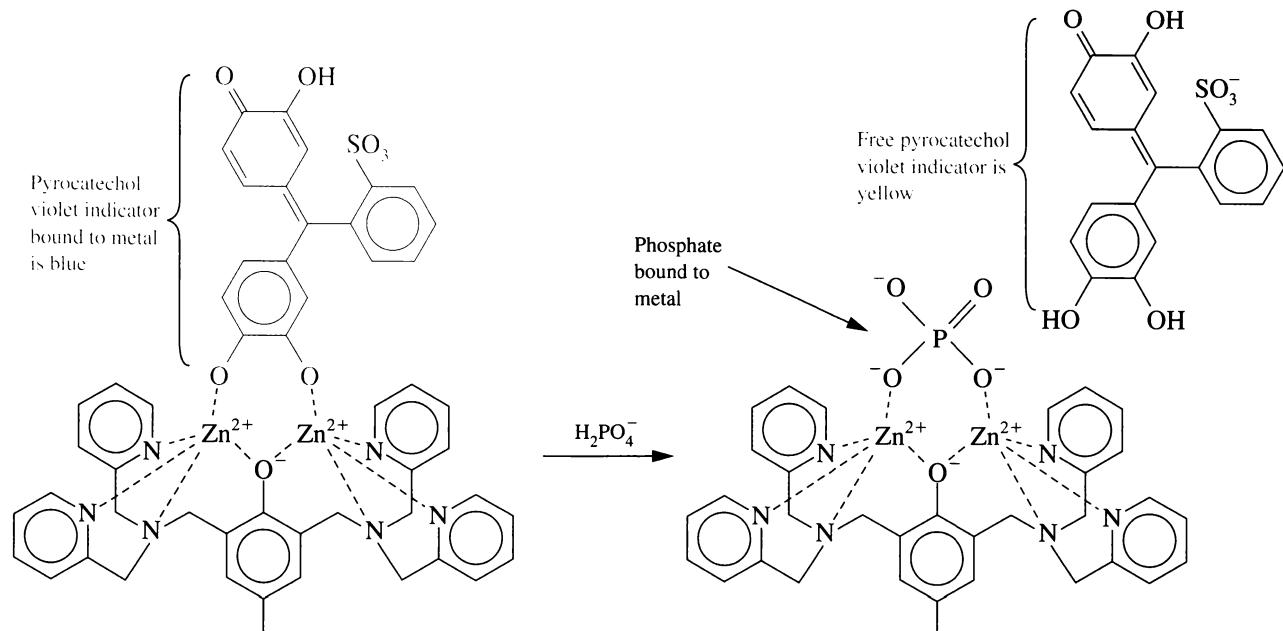
For a compound to be analyzed by spectrophotometry, it must absorb electromagnetic radiation, and this absorption should be distinguishable from that of other species in the sample. Biochemists assay protein solutions in the ultraviolet region at 280 nm, where the aromatic amino acids tyrosine, phenylalanine, and tryptophan (Table 11-1) have maximum absorbance. Common salts, buffers, and carbohydrates have little absorbance at this wavelength. In this section, we use Beer's law for a simple analysis and then discuss the measurement of nitrite in an aquarium.

Spectrophotometric analysis with visible radiation is called *colorimetric* analysis. Box 18-2 gives an example of the rational design of a colorimetric analysis.

Box 18-2 Designing a Colorimetric Reagent to Detect Phosphate⁵

Chemists in Korea demonstrated a clever, rational approach to designing a reagent for the spectrophotometric analysis of phosphate. A ligand containing six N atoms and one O atom that could bind two Zn²⁺ ions was selected. The distance between Zn²⁺ ions is just right for the metal ion indicator pyrocatechol violet to bind, as shown at the left below. Pyrocatechol violet is blue when bound to metal and yellow when free.

Near neutral pH, phosphate binds tightly to the two Zn²⁺ ions. When phosphate is added, indicator is displaced and the color changes from blue to yellow. The change in the absorption spectrum provides a quantitative measure of the amount of phosphate added. Color Plate 16 shows that common anions do not displace indicator from Zn²⁺ and therefore do not interfere in the analysis.





Benzene
 C_6H_6

Beer's law:

$$A = \epsilon bc$$

A = absorbance (dimensionless)

ϵ = molar absorptivity ($M^{-1} cm^{-1}$)

b = pathlength (cm)

c = concentration (M)

ϵ has funny units so that the product ϵbc will be dimensionless.



[Linda A. Hughes.]

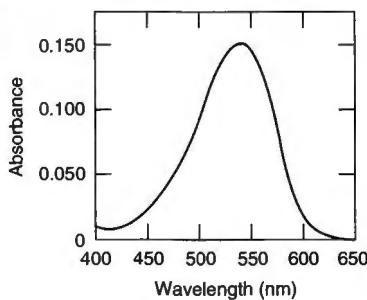


Figure 18-10 Spectrum of red-purple product of Reaction 18-7, beginning with a standard nitrite solution containing 0.915 ppm nitrogen. [From Kenneth Hughes, Georgia Institute of Technology.]

Example Measuring Benzene in Hexane

(a) A solution prepared by dissolving 25.8 mg of benzene (C_6H_6 , FM 78.11) in hexane and diluting to 250.0 mL has an absorption peak at 256 nm, with an absorbance of 0.266 in a 1.000-cm cell. Hexane does not absorb at 256 nm. Find the molar absorptivity of benzene at this wavelength.

SOLUTION The concentration of benzene is

$$[C_6H_6] = \frac{(0.025\ 8\ g)/(78.11\ g/mol)}{0.250\ 0\ L} = 1.32_1 \times 10^{-3}\ M$$

We find the molar absorptivity from Beer's law:

$$\text{molar absorptivity} = \epsilon = \frac{A}{bc} = \frac{0.266}{(1.000\ cm)(1.32_1 \times 10^{-3}\ M)} = 201.3\ M^{-1} cm^{-1}$$

(b) A sample of hexane contaminated with benzene has an absorbance of 0.070 at 256 nm in a cell with a 5.000-cm pathlength. Find the concentration of benzene.

SOLUTION Use the molar absorptivity from (a) in Beer's law:

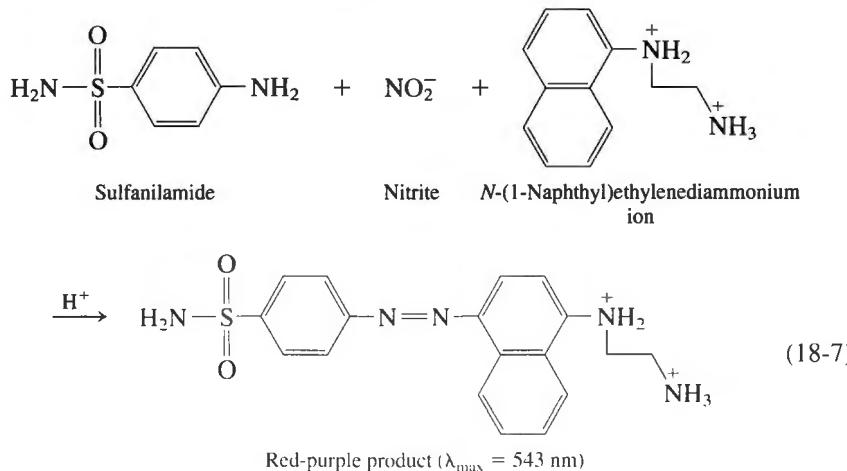
$$[C_6H_6] = \frac{A}{\epsilon b} = \frac{0.070}{(201.3\ M^{-1} cm^{-1})(5.000\ cm)} = 7.0 \times 10^{-5}\ M$$

Test Yourself Find the concentration of benzene in hexane if the absorbance is 0.188 in a 1.000-cm cell. (Answer: 0.934 mM)

Using a Standard Curve to Measure Nitrite

Box 6-1 stated that nitrogen compounds derived from animals and plants are broken down to ammonia by heterotrophic bacteria. Ammonia is oxidized first to nitrite (NO_2^-) and then to nitrate (NO_3^-) by nitrifying bacteria. In Section 6-3 we saw how a permanganate titration was used to standardize a nitrite stock solution. The nitrite solution is used here to prepare standards for a spectrophotometric analysis of nitrite in aquarium water.

The aquarium nitrite analysis is based on a reaction whose colored product has an absorbance maximum at 543 nm (Figure 18-10):



For quantitative analysis, we prepare a **standard curve** (also called a *calibration curve*) in which absorbance at 543 nm is plotted against nitrite concentration in a series of standards (Figure 18-11).

The procedure for measuring nitrite is to add color-forming reagent to an unknown or standard, wait 10 min for the reaction to be completed, and measure the absorbance. A *reagent blank* is prepared with nitrite-free, artificial seawater in place of unknown or standards. *The absorbance of the blank is subtracted from the absorbance of all other samples prior to any calculations.* The purpose of the blank is to subtract absorbance at 543 nm arising from starting materials or impurities. Here are the details:

Reagents

- Color-forming reagent* is prepared by mixing 1.0 g of sulfanilamide, 0.10 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride, and 10 mL of 85 wt% phosphoric acid and diluting to 100 mL. Store the solution in a dark bottle in the refrigerator to prevent thermal and photochemical degradation.
- Standard nitrite* ($\sim 0.02\text{ M}$) is prepared by dissolving NaNO_2 (FM 68.995) in water and standardizing (measuring the concentration) by the titration in Section 6-3. Dilute the concentrated standard with artificial seawater (containing no nitrite) to prepare standards containing 0.5–3 ppm nitrite nitrogen.

Procedure

For each analysis, dilute 10.00 mL of standard (containing 0.5–3 ppm nitrite nitrogen) or unknown up to 100.0 mL with water. Diluted solutions will contain 0.05–0.3 ppm nitrite nitrogen. Place 25.00 mL of diluted solution in a flask and add 1.00 mL of color-forming reagent. After 10 min, measure the absorbance in a 1.000-cm cuvet.

- Construct a *standard curve* from known nitrite solutions. Prepare a *reagent blank* by carrying artificial seawater through the same steps as a standard.
- Analyze duplicate samples of *unknown* aquarium water that has been filtered prior to dilution to remove suspended solids. Several trial dilutions may be required before the aquarium water is dilute enough to have a nitrite concentration that falls within the calibration range.

Table 18-2 and Figure 18-11 show typical results. The equation of the calibration line in Figure 18-11, determined by the method of least squares, is

$$\text{absorbance} = 0.176\ 9 [\text{ppm}] + 0.001\ 5 \quad (18-8)$$

Table 18-2 Aquarium nitrite analysis

Sample	Absorbance at 543 nm in 1.000-cm cuvet	Corrected absorbance (blank subtracted)
Blank	0.003	—
Standards		
0.457 5 ppm	0.085	0.082
0.915 0 ppm	0.167	0.164
1.830 ppm	0.328	0.325
Unknown	0.281	0.278
Unknown	0.277	0.274

The saltwater aquarium is filled with artificial seawater made by adding water to a mixture of salts.

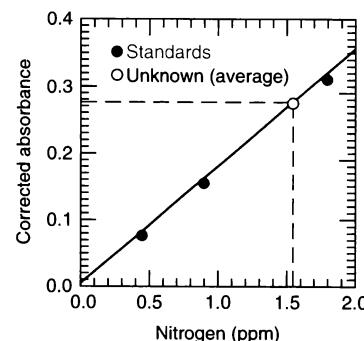


Figure 18-11 Calibration curve for nitrite analysis with corrected absorbance values from Table 18-2.

Unknown should be adjusted to fall within the calibration range, because you have not verified a linear response outside the calibration range.

where [ppm] represents micrograms of nitrite nitrogen per milliliter. In principle, the intercept should be 0, but we will use the observed intercept (0.001 5) in our calculations. By plugging the average absorbance of unknown into Equation 18-8, we can solve for the concentration of nitrite (ppm) in the unknown.

Example Preparing Nitrite Standards

How would you prepare a nitrite standard containing approximately 2 ppm nitrite nitrogen from a concentrated standard containing 0.018 74 M NaNO₂?

SOLUTION First, let's find out how many ppm of nitrogen are in 0.018 74 M NaNO₂. Because 1 mol nitrite contains 1 mol nitrogen, the concentration of nitrogen in the concentrated standard is 0.018 74 M. The mass of nitrogen in 1 mL is

$$\frac{\text{g of N}}{\text{mL}} = \left(0.018\ 74 \frac{\text{mol}}{\text{L}} \right) \left(14.007 \frac{\text{g N}}{\text{mol}} \right) \left(0.001 \frac{\text{L}}{\text{mL}} \right) = 2.625 \times 10^{-4} \frac{\text{g N}}{\text{mL}}$$

Assuming that 1.00 mL of solution has a mass of 1.00 g, we use the definition of parts per million to convert the mass of nitrogen into ppm:

$$\text{ppm} = \frac{\text{g N}}{\text{g solution}} \times 10^6 = \frac{2.625 \times 10^{-4} \text{ g N}}{1.00 \text{ g solution}} \times 10^6 = 262.5 \text{ ppm}$$

To prepare a standard containing ~2 ppm N, you could dilute the concentrated standard by a factor of 100 to give 2.625 ppm N. This dilution could be done by pipeting 10.00 mL of concentrated standard into a 1-L volumetric flask and diluting to the mark.

 **Test Yourself** How might you prepare a standard containing ~5 ppm N from 0.013 37 M NaNO₂? (Answer: stock concentration is 187.3 ppm N; dilute 25.00 mL to 1.000 L \Rightarrow 4.682 ppm N)

Example Using the Standard Curve

From the data in Table 18-2, find the molarity of nitrite in the aquarium.

SOLUTION The average corrected absorbance of unknowns in Table 18-2 is 0.276. Substituting this value into Equation 18-8 gives ppm of nitrite nitrogen in the aquarium:

$$0.276 = 0.176\ 9 [\text{ppm}] + 0.001\ 5$$

$$[\text{ppm}] = \frac{0.276 - 0.001\ 5}{0.176\ 9} = 1.55 \text{ ppm} = 1.55 \frac{\mu\text{g N}}{\text{mL}}$$

To find the molarity of nitrite nitrogen, we first find the mass of nitrogen in a liter, which is

$$1.55 \times 10^{-6} \frac{\text{g N}}{\text{mL}} \times 1\ 000 \frac{\text{mL}}{\text{L}} = 1.55 \times 10^{-3} \frac{\text{g N}}{\text{L}}$$

Then we convert mass of nitrogen into moles of nitrogen:

$$[\text{nitrite nitrogen}] = \frac{1.55 \times 10^{-3} \text{ g N/L}}{14.007 \text{ g N/mol}} = 1.11 \times 10^{-4} \text{ M}$$

Dilution formula 1-5:

$$M_{\text{conc}} \cdot V_{\text{conc}} = M_{\text{dil}} \cdot V_{\text{dil}}$$

Use any units you like for M and V, but use the same units on both sides of the equation. M could be ppm and V could be mL.



Figure 18-12 A stable form of nitrate reductase for NO₃⁻ analysis is made on a commercial scale from yeast in a 14-L fermentor. Recombinant DNA technology permits nitrate reductase genes from the flowering plant called thale cress to be expressed by yeast. [W. H. Campbell, P. Song, and G. G. Barbier, *Environ. Chem. Lett.* 2006, 4, 69. Photo courtesy W. H. Campbell, The Nitrate Elimination Co., Lake Linden, MI.]

Because one mole of nitrite (NO_2^-) contains one mole of nitrogen, the concentration of nitrite is also $1.11 \times 10^{-4} \text{ M}$.

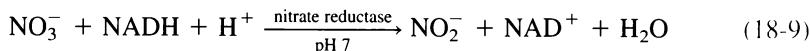


Test Yourself What is the molarity of nitrite if the observed (uncorrected) absorbance is 0.400? (Answer: $1.60 \times 10^{-4} \text{ M}$)

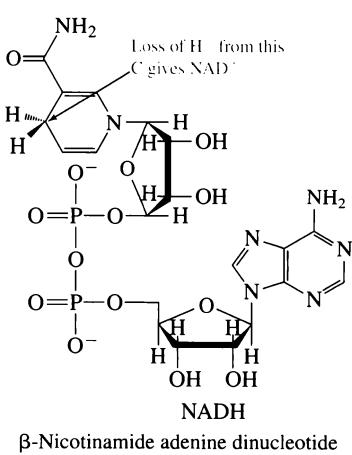
Enzyme-Based Nitrate Analysis—A Green Idea

Nitrate (NO_3^-) in natural waters is derived from sources such as fertilizers and under-treated animal and human waste. U.S. environmental regulations set a maximum of 10 ppm NO_3^- nitrogen in drinking water. Nitrate is commonly analyzed by reduction to nitrite (NO_2^-), followed by a colorimetric assay of NO_2^- . Metallic Cd has been the most common reducing agent for NO_3^- . However, the use of toxic Cd should be curtailed to protect the environment.

Therefore a field test for NO_3^- was developed with the biological reducing agent β -nicotinamide adenine dinucleotide (NADH, derived from the vitamin niacin) instead of Cd. The enzyme nitrate reductase (Figure 18-12) catalyzes the reduction:



Excess NADH is then oxidized to NAD^+ to eliminate interference with color development when NO_2^- from Reaction 18-9 is measured colorimetrically by reactions such as 18-7. For quantitative analysis in the field, a small, battery-operated spectrophotometer can be used with a set of nitrate standards. Alternatively, color can be compared visually with a chart showing colors from several standards. Commercial field kits allow analysis in the range 0.05–10 ppm nitrate nitrogen. Laboratory apparatus provides a precision of 2% when measuring 0.2 ppm NO_3^- nitrogen, with a detection limit of 3 ppb. Nitrate reductase has been applied to the measurement of nitrate in a classroom aquarium.⁶



Ask Yourself

18-D. You have been sent to India to investigate the occurrence of goiter disease attributed to iodine deficiency. As part of your investigation, you make field measurements of traces of iodide (I^-) in groundwater. The procedure is to oxidize I^- to I_2 and convert I_2 into a colored complex with the dye brilliant green in the solvent toluene.

(a) A $3.15 \times 10^{-6} \text{ M}$ solution of the colored complex exhibited an absorbance of 0.267 at 635 nm in a 1.000-cm cuvet. A blank solution made from distilled water in place of groundwater had an absorbance of 0.019. Find the molar absorptivity of the colored complex.

(b) The absorbance of an unknown solution prepared from groundwater was 0.175. Subtract the blank absorbance from the unknown absorbance and use Beer's law to find the concentration of the unknown.

Key Equations

Frequency-wavelength relation

$$\nu\lambda = c$$

ν = frequency λ = wavelength c = speed of light

Wavenumber

$$\tilde{\nu} = 1/\lambda$$

Photon energy

$$E = h\nu = hc/\lambda = hc\tilde{\nu}$$

h = Planck's constant

Transmittance

$$T = P/P_0$$

P_0 = radiant intensity of light incident on sample

P = radiant intensity of light emerging from sample

Absorbance

$$A = -\log T$$

Beer's law

$$A = \epsilon bc$$

ϵ = molar absorptivity of absorbing species ($M^{-1} \text{ cm}^{-1}$)

b = pathlength (cm)

c = concentration of absorbing species (M)

Important Terms

absorbance

excited state

monochromatic light

transmittance

absorption spectrum

frequency

photon

wavelength

Beer's law

ground state

spectrophotometer

wavenumber

cuvet

hertz

spectrophotometry

electromagnetic spectrum

molar absorptivity

standard curve

Problems

18-1. (a) When you double the frequency of electromagnetic radiation, you _____ the energy.

(b) When you double the wavelength, you _____ the energy.

(c) When you double the wavenumber, you _____ the energy.

18-2. How much energy (J) is carried by one photon of (a) red light with $\lambda = 650 \text{ nm}$? (b) violet light with $\lambda = 400 \text{ nm}$? After finding the energy of one photon of each wavelength, express the energy of a mole of each type of photons in kJ/mol.

18-3. One electron volt (eV) is the energy of an elementary charge accelerated through a potential difference of 1 V. X-rays emitted by peeling Scotch tape® (Figure 18-4) have an energy of $15 \times 10^3 \text{ eV}$ (15 keV). With the eV conversion factor in Table 1-4, find the wavelength of these X-rays in m and nm and locate them in Figure 18-2.

18-4. What color would you expect for light transmitted through a solution with an absorption maximum at (a) 450; (b) 550; (c) 650 nm?

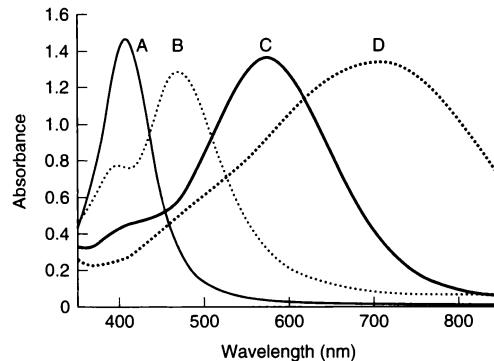
18-5. State the difference between transmittance, absorbance, and molar absorptivity. Which one is proportional to concentration?

18-6. An absorption spectrum is a graph of _____ or _____ versus _____.

18-7. Why does a compound that has a visible absorption maximum at 480 nm (blue-green) appear to be red?

18-8. Observed colors of four solutions whose spectra are shown here are violet, orange, blue, and yellow. Match the spectral traces A–D to the observed colors, bearing in mind

that Table 18-1 is only an *approximate* guide. Rationalize why solution D does not obey Table 18-1.



Absorption spectra of four solutions. [From A. J. Frank, N. Cathcart, K. E. Maly, and V. Kitaev, *J. Chem. Ed.* **2010**, 87, 1098.]

18-9. Calculate the frequency (Hz), wavenumber (cm^{-1}), and energy (J/photon and kJ/mole of photons) of (a) ultraviolet light with a wavelength of 250 nm and (b) infrared light with a wavelength of 2.50 μm .

18-10. Industrial CO₂ lasers with $\lambda = 10.6 \mu\text{m}$ are used in cutting and welding. How many photons/s are produced by a laser whose output is 5.0 kW? (1 watt = 1 joule/second.)

18-11. Convert transmittance (T) into absorbance (A):

$$T: 0.99 \quad 0.90 \quad 0.50 \quad 0.10 \quad 0.010 \quad 0.0010 \quad 0.00010$$

$$A: \qquad \qquad \qquad 1.0$$

18-12. Find the absorbance and percent transmittance of a 0.002 40 M solution of a substance with a molar absorptivity

of 1.00×10^2 or $2.00 \times 10^2 \text{ M}^{-1} \text{ cm}^{-1}$ in a cell with a 2.00-cm pathlength.

18-13. The “SPF number” of a sunscreen increases as the sunscreen absorbs more light and, therefore, provides more protection:⁷ $\text{SPF} = 1/T$, where T is transmittance of UV-B radiation (Figure 18-7) through a uniform layer of sunscreen at a concentration of 2 mg/cm^2 . What are the transmittance and absorbance when $\text{SPF} = 2, 10$, and 20 ? What fraction of UV-B radiation is absorbed by each sunscreen?

18-14. The absorbance of a $2.31 \times 10^{-5} \text{ M}$ solution is 0.822 at a wavelength of 266 nm in a 1.00-cm cell. Calculate the molar absorptivity at 266 nm .

18-15. The iron-transport protein in your blood is called transferrin. When its two iron-binding sites do not contain metal ions, the protein is called apotransferrin.

(a) Apotransferrin has a molar absorptivity of $8.83 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 280 nm . Find the concentration of apotransferrin in water if the absorbance is 0.244 in a 0.100-cm cell.

(b) The formula mass of apotransferrin is $81\,000$. Express the concentration from part (a) in g/L.

18-16. A 15.0-mg sample of a compound with a formula mass of 384.63 was dissolved in a 5-mL volumetric flask. A 1.00-mL aliquot was withdrawn, placed in a 10-mL volumetric flask, and diluted to the mark.

(a) Find the concentration of sample in the 5-mL flask.

(b) Find the concentration in the 10-mL flask.

(c) The 10-mL sample was placed in a 0.500-cm cuvet and gave an absorbance of 0.634 at 495 nm . Find the molar absorptivity at 495 nm .

18-17. (a) In Figure 18-7, estimate the absorbance of sunscreen at 350 nm .

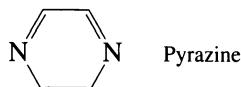
(b) What fraction of ultraviolet radiation is transmitted through the sunscreen at 350 nm ?

18-18. (a) What value of absorbance corresponds to $45.0\% T$?

(b) When the concentration of a solution is doubled, the *absorbance* is doubled. If a $0.010\,0 \text{ M}$ solution exhibits $45.0\% T$ at some wavelength, what will be the percent transmittance for a $0.020\,0 \text{ M}$ solution of the same substance?

18-19. A 0.267-g quantity of a compound with a formula mass of 337.69 was dissolved in 100.0 mL of ethanol. Then 2.000 mL were withdrawn and diluted to 100.0 mL . The spectrum of this solution exhibited a maximum absorbance of 0.728 at 438 nm in a 2.000-cm cell. Find the molar absorptivity of the compound.

18-20. Vapor at a pressure of $30.3 \mu\text{bar}$ from the solid compound pyrazine had a transmittance of 24.4% at a wavelength of 266 nm in a 3.00-cm cell at 298 K .



(a) Convert transmittance to absorbance.

(b) Convert pressure to concentration (mol/L) with the ideal gas law (Problem 16-16).

(c) Find the molar absorptivity of gaseous pyrazine at 266 nm .

18-21. (a) A $3.96 \times 10^{-4} \text{ M}$ solution of compound A exhibited an absorbance of 0.624 at 238 nm in a 1.000-cm cuvet. A blank solution containing only solvent had an absorbance of 0.029 at the same wavelength. Find the molar absorptivity of compound A.

(b) The absorbance of an unknown solution of compound A in the same solvent and cuvet was 0.375 at 238 nm . Subtract the blank absorbance from the unknown absorbance and use Beer’s law to find the concentration of the unknown.

(c) A concentrated solution of compound A in the same solvent was diluted from an initial volume of 2.00 mL to a final volume of 25.00 mL and then had an absorbance of 0.733 . What is the concentration of A in the 25.00-mL solution?

(d) Considering the dilution from 2.00 mL to 25.00 mL , what was the concentration of A in the 2.00-mL solution in (c)?

18-22. A compound with a formula mass of 292.16 was dissolved in solvent in a 5-mL volumetric flask and diluted to the mark. A 1.00-mL aliquot was withdrawn, placed in a 10-mL volumetric flask, and diluted to the mark. The absorbance measured at 340 nm was 0.427 in a 1.000-cm cuvet. The molar absorptivity for this compound at 340 nm is $6\,130 \text{ M}^{-1} \text{ cm}^{-1}$.

(a) Calculate the concentration of compound in the cuvet.

(b) What was the concentration of compound in the 5-mL flask?

(c) How many milligrams of compound were used to make the 5-mL solution?

18-23. When I was a boy, I watched Uncle Wilbur measure the iron content of runoff from his banana ranch. He acidified a 25.0-mL sample with HNO_3 and treated it with excess KSCN to form a red complex. (KSCN itself is colorless.) He then diluted the solution to 100.0 mL and put it in a variable-pathlength cell. For comparison, he treated a 10.0-mL reference sample of $6.80 \times 10^{-4} \text{ M Fe}^{3+}$ with HNO_3 and KSCN and diluted it to 50.0 mL . The reference was placed in a cell with a 1.00-cm pathlength. Runoff had the same absorbance as the reference when the pathlength of the runoff cell was 2.48 cm . What was the concentration of iron in Uncle Wilbur’s runoff?

18-24.  A nitrite analysis conducted by the procedure in Section 18-4 gave data in the table on the next page. Fill in corrected absorbance, which is measured absorbance minus the average blank absorbance (0.023). Construct a calibration line to find (a) ppm nitrite nitrogen \pm uncertainty and (b) molar concentration of nitrite in the aquarium. Use the average blank and the average unknown absorbances.

Sample	Absorbance	Corrected absorbance
Blank	0.022	—
Blank	0.024	—
Standards:		
0.538 ppm	0.121	0.098
1.076 ppm	0.219	
2.152 ppm	0.413	
3.228 ppm	0.600	
4.034 ppm	0.755	
Unknown	0.333	
Unknown	0.339	
Unknown	0.338	

18-25. Starting with 0.015 83 M NaNO₂ solution, explain how you would prepare standards containing *approximately* 0.5, 1, 2, and 3 ppm nitrogen (1 ppm = 1 µg/mL). Use any volumetric flasks and transfer pipets in Tables 2-2 and 2-3. What would be the exact concentrations of the standards prepared by your method?

18-26. (a) Nitrate in a classroom aquarium was determined by the nitrate reductase enzyme procedure described at the end of Section 18-4. Write the sequence of chemical reactions beginning with nitrate and ending with the colored product. Which step is catalyzed by the enzyme?

(b)  Each solution whose absorbance was measured contained 50.0 µL of standard or aquarium water mixed with reagents to give a total volume of 2.02 mL. Prepare a calibration curve showing absorbance versus nitrate nitrogen content in the 50-µL standards. No data are given for the blank, so do not subtract anything from the observed absorbance. Find the average concentration and uncertainty (Equation 4-19) of nitrate nitrogen (ppm) in the aquarium water.

Nitrate nitrogen (ppm) in 50-µL sample	Absorbance at 540 nm
0.250	0.062
0.500	0.069
1.00	0.108
1.50	0.126
2.50	0.209
5.00	0.423
7.50	0.592
10.00	0.761
aquarium	0.192
aquarium	0.201

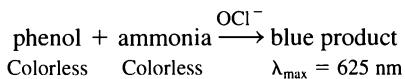
Data from H. Van Ryswyk, E. W. Hall, S. J. Petesch, and A. E. Wiedeman, *J. Chem. Ed.* **2007**, 84, 306.

(c) Nitrate nitrogen (ppm) means micrograms of nitrate N per gram of solution. The slope of the calibration line is (absorbance)/(ppm nitrate N in standard). Find the molarity of colored product if the standard contains 1.00 ppm nitrate

N. Estimate the molar absorptivity of colored product, assuming that the cuvet pathlength is 1.00 cm and the density of standard solutions is near 1.00 g/mL.

18-27. Starting with 28.6 wt% NH₃, explain how to prepare NH₃ standards containing exactly 1.00, 2.00, 4.00, and 8.00 ppm nitrogen (1 ppm = 1 µg/mL) for a spectrophotometric calibration curve. Use a known *mass* of concentrated reagent and any flasks and pipets from Tables 2-2 and 2-3.

18-28. Ammonia (NH₃) is determined spectrophotometrically by reaction with phenol in the presence of hypochlorite (OCl⁻):



1. A 4.37-mg sample of protein was chemically digested to convert its nitrogen into NH₃ and then diluted to 100.0 mL.
2. Then 10.0 mL of the solution were placed in a 50-mL volumetric flask and treated with 5 mL of phenol solution plus 2 mL of NaOCl solution. The sample was diluted to 50.0 mL, and the absorbance at 625 nm was measured in a 1.00-cm cuvet after 30 min.
3. A standard solution was prepared from 0.010 0 g of NH₄Cl (FM 53.49) dissolved in 1.00 L of water. A 10.0-mL aliquot of this standard was placed in a 50-mL volumetric flask and analyzed in the same manner as the unknown.
4. A reagent blank was prepared by using distilled water in place of unknown.

Sample	Absorbance at 625 nm
Blank	0.140
Standard	0.308
Unknown	0.592

(a) From step 3, calculate the molar absorptivity of the blue product.

(b) Using molar absorptivity, find [NH₃] in step 2.

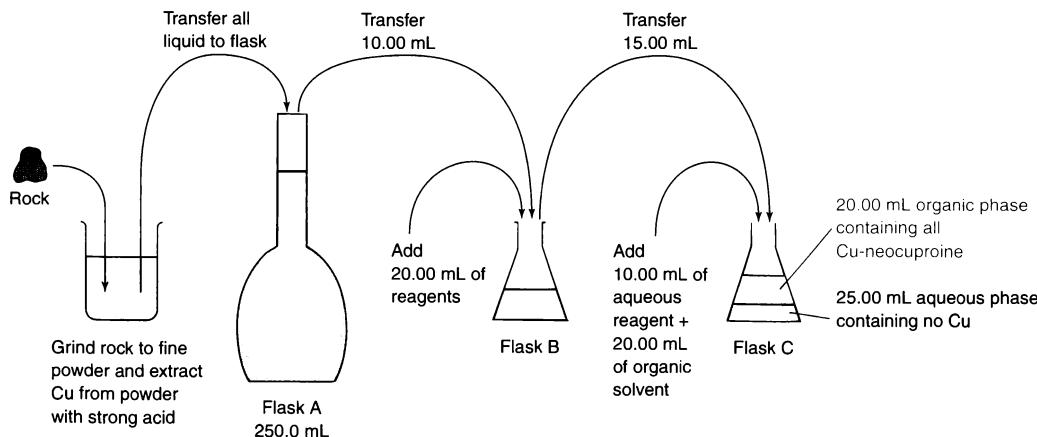
(c) From (b), find [NH₃] in the 100-mL solution in step 1.

(d) Find the weight percent of nitrogen in the protein.

18-29. Cu⁺ reacts with neocuproine to form (neocuproine)₂Cu⁺, with an absorption maximum at 454 nm. Neocuproine reacts with few other metals. The copper complex is soluble in isoamyl alcohol, an organic solvent that does not dissolve appreciably in water. When isoamyl alcohol is added to water, a two-layered mixture results, with the denser water layer at the bottom. If (neocuproine)₂Cu⁺ is present, virtually all of it goes into the organic phase. For the purpose of this problem, assume that no isoamyl alcohol dissolves in water and that the colored complex is only in the organic phase.

1. A rock containing copper is pulverized, and metals are extracted with strong acid. The acid is neutralized with base and made up to 250.0 mL in flask A.

2. 10.00 mL of solution are transferred to flask B and treated with 10.00 mL of reducing agent to reduce Cu²⁺ to Cu⁺. Then



10.00 mL of buffer are added to bring the pH to a value suitable for complex formation with neocuproine.

3. 15.00 mL of solution are withdrawn and placed in flask C. To the flask are added 10.00 mL of aqueous neocuproine and 20.00 mL of isoamyl alcohol. After the flask has been shaken well and the phases allowed to separate, all $(\text{neocuproine})_2\text{Cu}^+$ is in the organic phase.

4. A few milliliters of the upper layer are withdrawn, and absorbance is measured at 454 nm in a 1.00-cm cell. A blank carried through the same procedure gave an absorbance of 0.056.

(a) Suppose that the rock contained 1.00 mg of Cu. What will be the concentration of copper (moles per liter) in the isoamyl alcohol phase?

(b) If the molar absorptivity of $(\text{neocuproine})_2\text{Cu}^+$ is $7.90 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$, what will the observed absorbance be? Remember that a blank carried through the same procedure gave an absorbance of 0.056.

(c) A rock is analyzed and found to give a final absorbance of 0.874 (uncorrected for the blank). How many milligrams of copper are in the rock?

18-30. Spectrophotometric analysis of phosphate:

Standard solutions

A. KH_2PO_4 (potassium dihydrogen phosphate, FM 136.09): 81.37 mg dissolved in 500.0 mL H_2O

B. $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (sodium molybdate): 1.25 g in 50 mL of 5 M H_2SO_4

C. $\text{H}_3\text{NNH}_3^{2+}\text{SO}_4^{2-}$ (hydrazine sulfate): 0.15 g in 100 mL H_2O

Procedure

Place sample (unknown or standard phosphate solution, A) in a 5-mL volumetric flask and add 0.500 mL of B and 0.200 mL of C. Dilute to almost 5 mL with water and heat at 100°C for 10 min to form a blue product ($\text{H}_3\text{PO}_4(\text{MoO}_3)_{12}$, 12-molybdophosphoric acid). Cool the flask to room temperature, dilute to the mark with water, mix well, and measure absorbance at 830 nm in a 1.00-cm cell.

(a) When 0.140 mL of solution A was analyzed, an absorbance of 0.829 was recorded. A blank carried through the same procedure gave an absorbance of 0.017. Find the molar absorptivity of blue product.

(b) A solution of the phosphate-containing iron-storage protein ferritin was analyzed. Unknown containing 1.35 mg ferritin was digested in a total volume of 1.00 mL to release PO_4^{3-} from the protein. Then 0.300 mL of this solution was analyzed and gave an absorbance of 0.836. A blank carried through the procedure gave an absorbance of 0.038. Find wt% phosphorus in the ferritin.

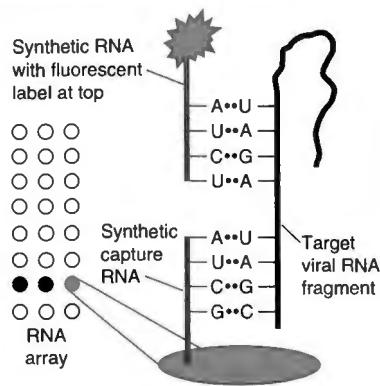
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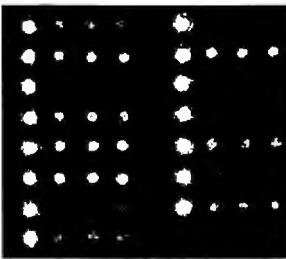
Flu Virus Identification with an RNA Array and Fluorescent Markers



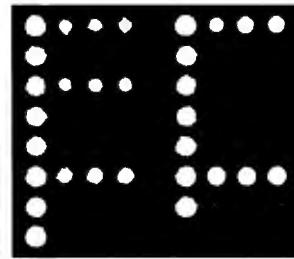
(a)

1	●	○	○	○	●	○	○	○	9
2	●	○	○	○	●	○	○	○	10
3	●	○	○	○	●	○	○	○	11
4	●	○	○	○	●	○	○	○	12
5	●	○	○	○	●	○	○	○	13
6	●	○	○	○	●	○	○	○	14
7	●	○	○	○	●	○	○	○	15
8	●	○	○	○					

(b)



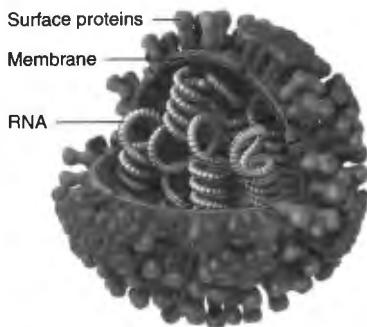
(c)



(d)



2009 H1N1 influenza virus. [Courtesy C. S. Goldsmith and A. Balish, CDC.]



Schematic structure of a generic influenza virus. [Courtesy Dan Higgins, CDC.]

Influenza virus is responsible for 36 000 deaths/yr in the U.S. The virus is classified into types A, B, and C, and subtypes (“strains”) according to differences in viral proteins. One particular strain of avian virus (“bird flu”) is of particular concern because it has the potential to cause widespread human disease. The World Health Organization identifies flu strains so that vaccines can be prepared. Conventional typing methods are expensive and require days or weeks. An RNA array has the potential to reduce cost and time.

The array above has 15 rows of spots containing synthetic “capture” RNA covalently attached to a glass slide. Three spots in each row contain identical capture RNA designed to bind to a short section of one strain of viral RNA. The spot at the left in each row is a control that will become fluorescent in every test and serves as an internal standard. Viral RNA extracted from patients is *amplified* (reproduced into many copies) and *digested* (cleaved into fragments). Capture RNA on the slide binds selected viral RNA fragments. Another synthetic RNA with a fluorescent tag is designed to bind to a different section of viral RNA. After allowing digested viral RNA to bind to capture RNA and to fluorescent RNA, excess fluorescent RNA is washed away. Fluorescence intensity in each spot is related to the amount of viral RNA bound at that spot.

Pattern recognition methods assign the relative brightness in different spots to a particular strain of flu. In its first trial with patients, 50 of 53 samples were correctly identified. There were also one *false positive* and two *false negatives*. A *false positive* states that the strain being sought is present when it is not. A *false negative* fails to find the strain when it is there. The success rate is higher than that of existing rapid diagnostic tests, which should give you pause when you receive results from your own medical tests.

Spectrophotometry: Instruments and Applications

In this chapter, we describe the components of a spectrophotometer, some of the physical processes that take place when light is absorbed by molecules, and a few important applications of spectrophotometry in analytical chemistry. New analytical instruments and procedures for medicine and biology, such as the RNA array, are being developed by combining sensitive optical methods with biologically specific recognition elements.

19-1 The Spectrophotometer

Minimum requirements for a *single-beam spectrophotometer* were shown in Figure 18-5. *Polychromatic light* from a lamp passes through a *monochromator* that separates different wavelengths from one another and selects one narrow band of wavelengths to pass through the sample. Transmittance is P/P_0 , where P_0 is the radiant power reaching the detector when the sample cell contains a blank solution with no analyte and P is the power reaching the detector when analyte is present in the sample cell. In a single-beam spectrophotometer, the sample and blank must be placed alternately in the beam. Error occurs if the source intensity or detector response drifts between the two measurements.

The *double-beam spectrophotometer* in Figure 19-1 features a rotating mirror (the *beam chopper*), which alternately directs light through the sample or reference

Polychromatic light contains many wavelengths (literally, “many colors”).

A refresher:

$$\text{transmittance} = T = \frac{P}{P_0}$$

$$\text{absorbance} = -\log T$$

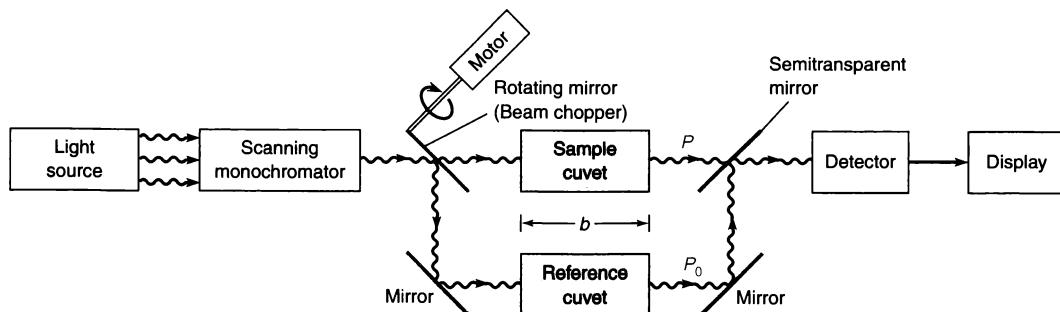
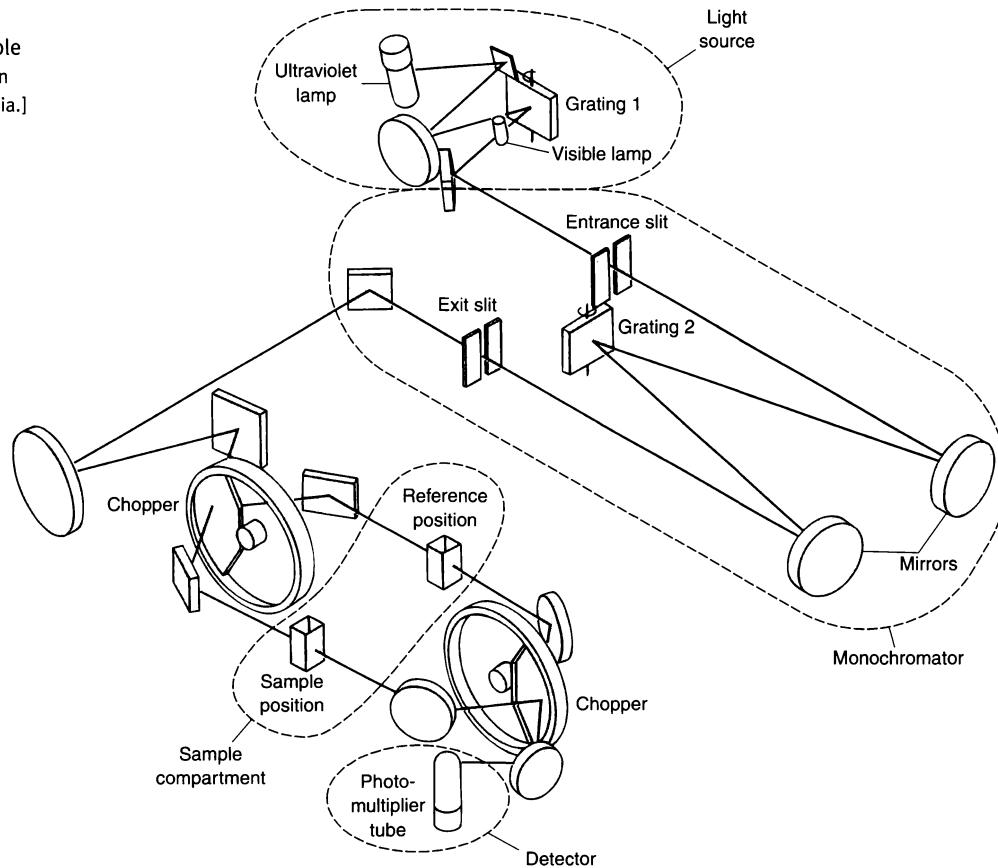


Figure 19-1 Double-beam scanning spectrophotometer. The incident beam is passed alternately through sample and reference cuvets by the rotating beam chopper.

Figure 19-2 Optical train of Varian Cary 3E Ultraviolet-Visible Spectrophotometer. [From Varian Australia Pty. Ltd., Victoria, Australia.]



cell several times per second. Radiant power emerging from the reference cell, which is filled with a blank solution or pure solvent, is P_0 . Light emerging from the sample cell has power P . By measuring P and P_0 many times per second, the instrument compensates for drift in source intensity or detector response. Figures 19-2 and 19-3 show the layouts of components in two double-beam instruments. Let's examine the principal components.

Light Source

The two lamps in the upper parts of Figures 19-2 and 19-3b provide visible or ultraviolet radiation. An ordinary *tungsten lamp*, whose filament glows at a temperature near 3 000 K, produces radiation in the visible and near-infrared regions at wavelengths of 320 to 2 500 nm (Figure 19-4). For ultraviolet spectroscopy, we normally employ a *deuterium arc lamp* in which a controlled electric discharge dissociates D₂ molecules, which then emit ultraviolet radiation from 200 to 400 nm (Figure 19-4). Typically, a switch is made between the deuterium and tungsten lamps when passing through 360 nm so that the source giving the most radiation is always employed. Other sources of visible and ultraviolet radiation are electric discharge (arc) lamps filled with mercury vapor or xenon. Infrared radiation ($5\ 000$ to $200\ \text{cm}^{-1}$) is commonly obtained from a silicon carbide rod called a *globar*, electrically heated to 1 500 K. Lasers are extremely bright sources of light at just one or a few wavelengths.

CAUTION Ultraviolet radiation is harmful to the naked eye. Do not view an ultraviolet source without protection.

Question What wavelengths in μm and nm correspond to $5\ 000$ and $200\ \text{cm}^{-1}$?

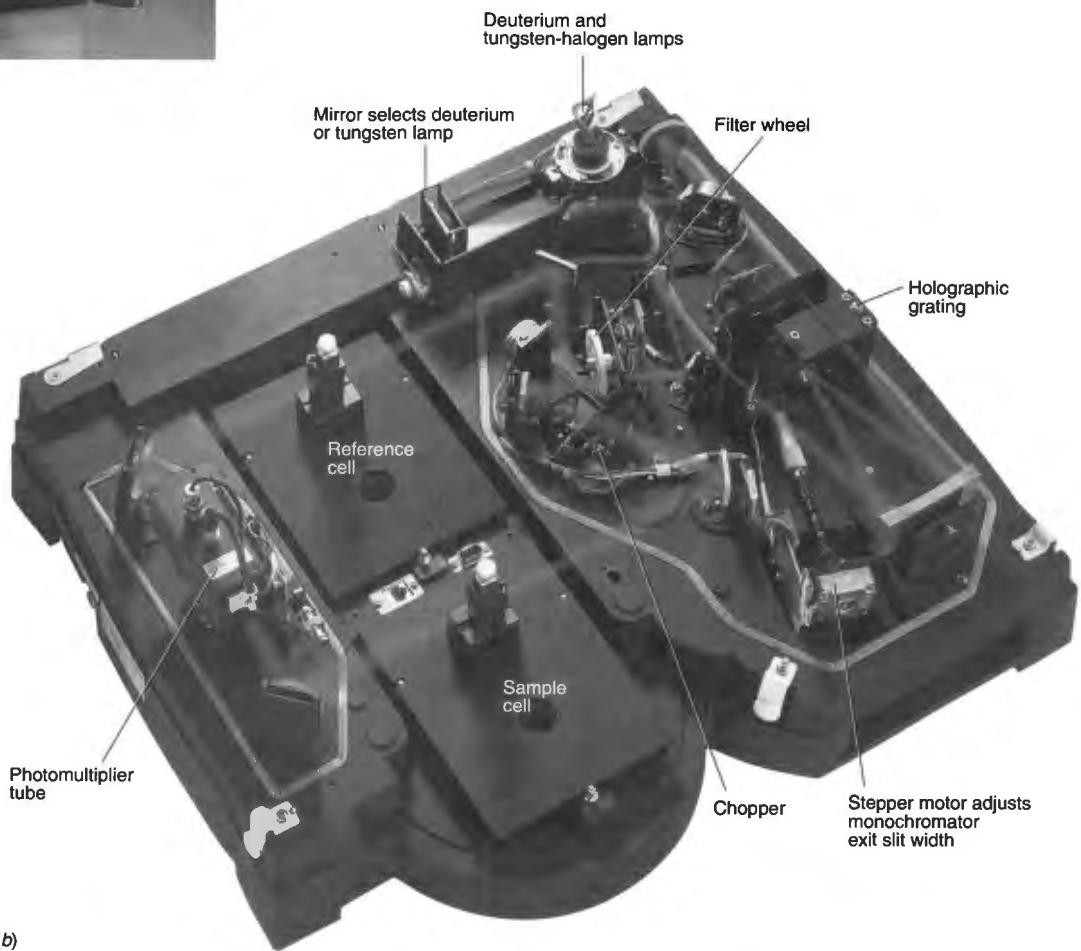
$$200\ \text{cm}^{-1} = 50\ \mu\text{m} = 50\ 000\ \text{nm}$$

$$5\ 000\ \text{cm}^{-1} = 2\ \mu\text{m} = 2\ 000\ \text{nm}$$

Answer:



(a)



(b)

Figure 19-3 (a) Thermo Scientific Evolution 600 ultraviolet-visible double-beam spectrophotometer. (b) Optical train of Evolution 600, showing layout of components. [Courtesy Thermo Fisher Scientific, Madison, WI.]

Monochromator

A **monochromator** disperses light into its component wavelengths and selects a narrow band of wavelengths to pass through the sample. The monochromator in Figure 19-2 consists of entrance and exit slits, mirrors, and a *grating* to disperse the light. *Prisms* were used in older instruments to disperse light.

A **grating** has a series of closely ruled lines. When light is reflected from or transmitted through the grating, each line behaves as a separate source of radiation. Different wavelengths are reflected or transmitted at different angles from the grating (Color Plate 17). The bending of light rays by a grating is called **diffraction**.

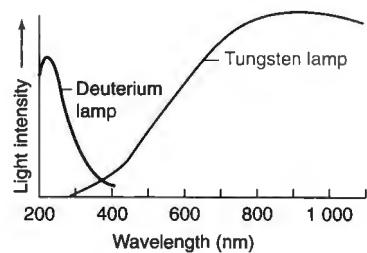


Figure 19-4 Intensities of a tungsten filament at 3200 K and of a deuterium arc lamp.

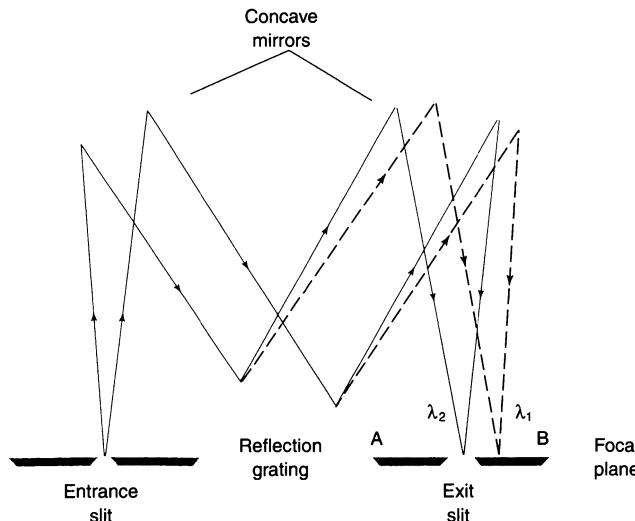


Figure 19-5 Czerny-Turner grating monochromator.

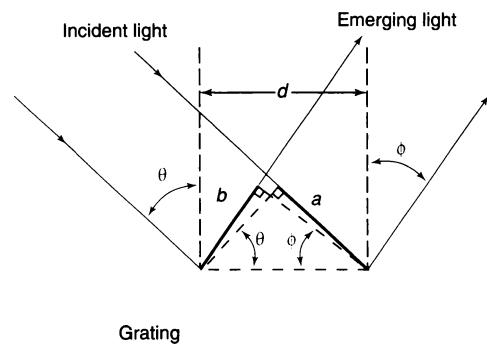


Figure 19-6 Principle of a reflection grating.

Grating: optical element with closely spaced lines

(In contrast, the bending of light rays by a prism or lens, which is called *refraction*, is shown in Color Plate 18.)

Diffraction: bending of light by a grating

In the grating monochromator in Figure 19-5, *polychromatic* radiation from the entrance slit is *collimated* (made into a beam of parallel rays) by a concave mirror. These rays fall on a reflection grating, whereupon different wavelengths are diffracted at different angles. The light strikes a second concave mirror, which focuses each wavelength at a different point. The grating directs a narrow band of wavelengths to the exit slit. Rotation of the grating allows different wavelengths to pass through the exit slit.

Refraction: bending of light by a lens or prism

Diffraction at a reflection grating is shown in Figure 19-6. The closely spaced parallel grooves of the grating have a repeat distance d . When light is reflected from the grating, each groove behaves as a source of radiation. If adjacent light rays are in phase, they reinforce one another. If they are out of phase, they cancel one another (Figure 19-7).

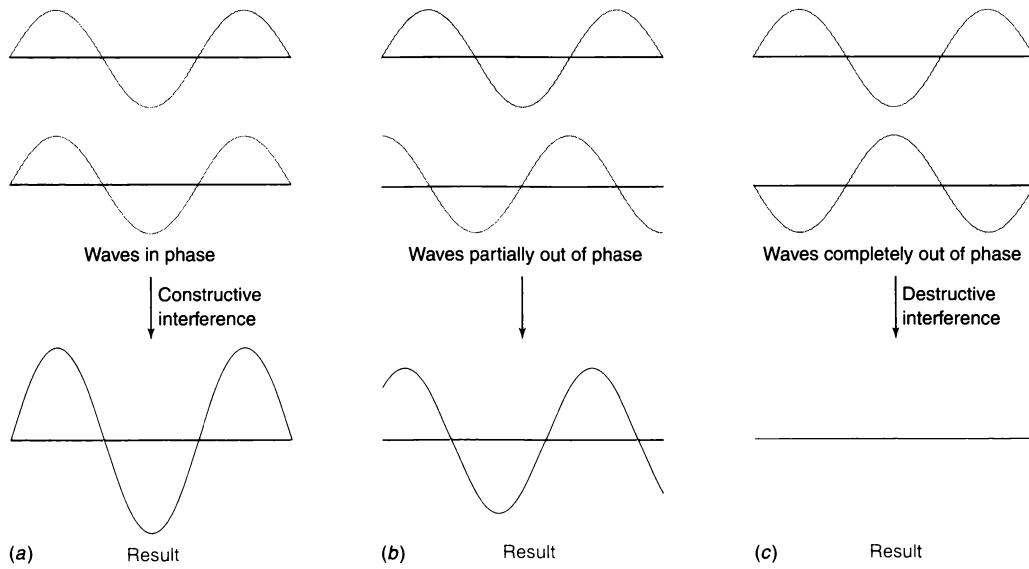


Figure 19-7 Interference of adjacent waves that are (a) 0° , (b) 90° , and (c) 180° out of phase.

Constructive interference occurs if the difference in pathlength ($a - b$) traveled by the two rays in Figure 19-6 is an integer multiple of the wavelength

$$n\lambda = a - b \quad (19-1)$$

where the diffraction order, n , is $\pm 1, \pm 2, \pm 3, \pm 4, \dots$. The maximum for which $n = \pm 1$ is called *first-order diffraction*. When $n = \pm 2$, we have *second-order diffraction*, and so on.

In Figure 19-6, the incident angle θ is defined to be positive. The diffraction angle ϕ in Figure 19-6 goes in the opposite direction from θ , so, by convention, ϕ is negative. It is possible for ϕ to be on the same side of the normal as θ , in which case ϕ would be positive. In Figure 19-6, $a = d \sin \theta$ and $b = -d \sin \phi$ (because ϕ is negative and $\sin \phi$ is negative). Substituting into Equation 19-1 gives the condition for constructive interference:

Grating equation: $n\lambda = d(\sin \theta + \sin \phi)$ (19-2)

For each incident angle θ , there are diffraction angles ϕ at which a given wavelength will produce maximum constructive interference, as shown in Color Plate 19.

In general, first-order diffraction of one wavelength overlaps higher-order diffraction of another wavelength. Therefore *filters* that reject many wavelengths are used to select a desired wavelength while rejecting other wavelengths at the same diffraction angle. High-quality spectrophotometers use several gratings with different line spacings optimized for different wavelengths. Spectrophotometers with two monochromators in series (a *double monochromator*) reduce unwanted radiation by orders of magnitude.

Decreasing the exit slit width in Figure 19-5 decreases the selected bandwidth and decreases the energy reaching the detector. Thus, *resolution of closely spaced bands, which requires a narrow slit width, can be achieved at the expense of decreased signal-to-noise ratio*. For quantitative analysis, a monochromator bandwidth that is $\lesssim \frac{1}{5}$ of the width of the absorption band is reasonable (Figure 19-8).

Trade-off between resolution and signal: The narrower the exit slit, the greater the ability to resolve closely spaced peaks and the noisier the spectrum.

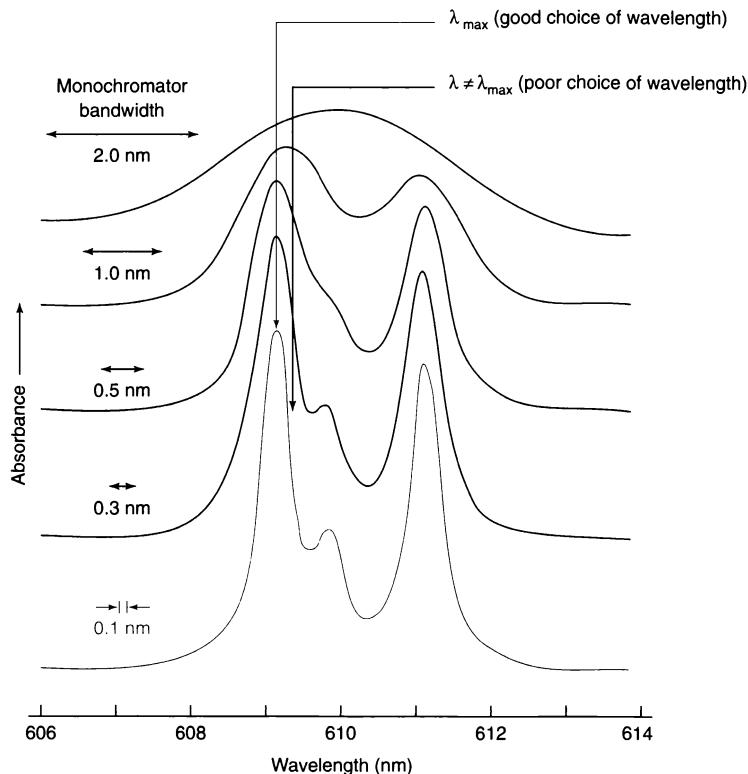
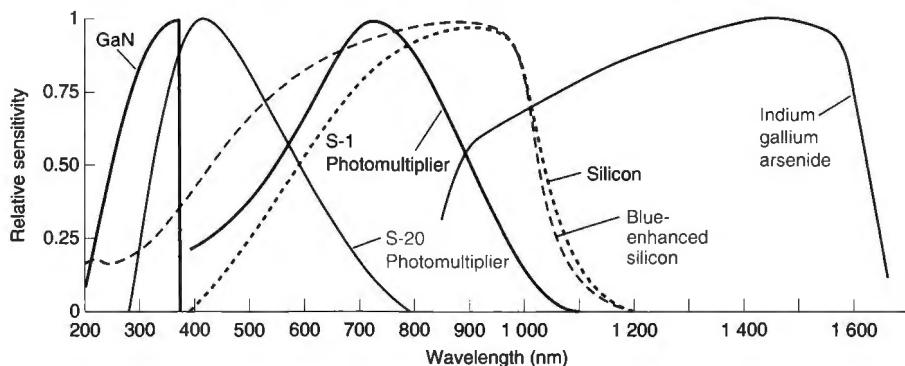


Figure 19-8 Choosing wavelength and monochromator bandwidth. For quantitative analysis, use a wavelength of maximum absorbance so that small errors in the wavelength do not change the absorbance very much. Choose a monochromator bandwidth (controlled by the exit slit width in Figure 19-5) small enough that it does not distort the band shape, but not so small that the spectrum is too noisy. Widening the slit width distorts the spectrum. In the lowest trace, a monochromator bandwidth that is one-fifth the width of the sharp absorption bands (measured at half the peak height) prevents distortion. [Courtesy M. D. Seltzer, Michelson Laboratory, China Lake, CA.]

Figure 19-9 Detector response. Each curve is normalized to a maximum value of 1. [Courtesy Barr Associates, Inc., Westford, MA. GaN data from APA Optics, Blaine, MN. InGaAs data from Shimadzu Corp., Tokyo.]



Detector

Detector response is a function of wavelength of incident light.

A detector produces an electric signal when it is struck by photons. Figure 19-9 shows that detector response depends on the wavelength of the incident photons. In a single-beam spectrophotometer, the 100% transmittance control must be re-adjusted each time the wavelength is changed because the maximum possible detector signal depends on the wavelength. Subsequent readings are scaled to the 100% reading.

A **photomultiplier tube** (Figure 19-10) is a very sensitive detector. When light of sufficient energy strikes a photosensitive cathode, electrons are emitted into the vacuum inside the tube. Emitted electrons strike a second surface, called a *dynode*, which is positive with respect to the cathode. Electrons strike the dynode with more than their original kinetic energy. Each energetic electron knocks more than one electron from the dynode. These new electrons are accelerated toward a second dynode, which is more positive than the first dynode. On striking the second dynode, even more electrons are knocked off and accelerated toward a third dynode. This process is repeated so that more than 10^6 electrons are finally collected for each photon striking the cathode. Extremely low light intensities are translated into measurable electric signals.

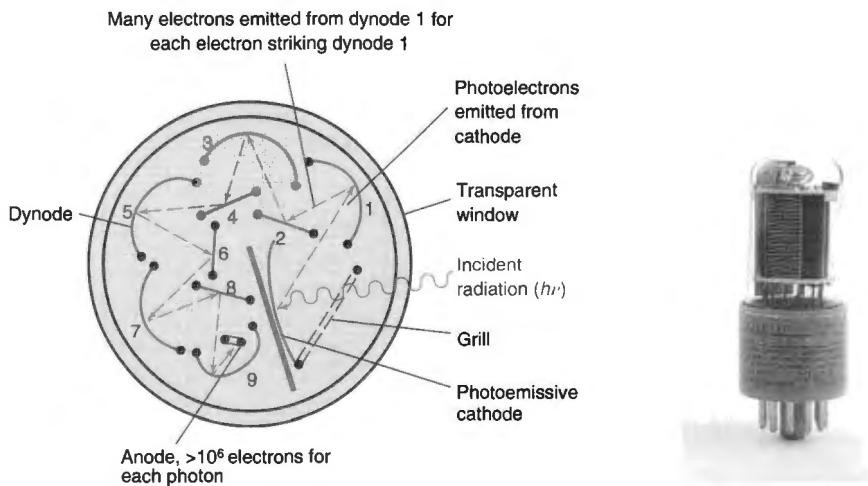


Figure 19-10 Left: Diagram of photomultiplier tube with nine dynodes. Amplification occurs at each dynode, which is approximately 90 volts more positive than the preceding dynode. Right: Photomultiplier tube. [Photograph by David J. Green/Alamy.]

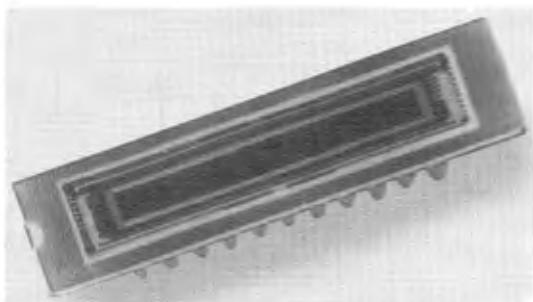


Figure 19-11 Photodiode array with 1 024 elements, each 25 μm wide and 2.5 mm high. The entire chip is 5 cm long. [Courtesy Oriel Corporation, Stratford, CT.]

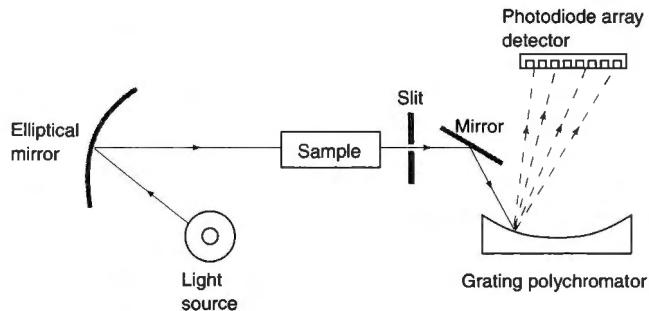


Figure 19-12 Diode array spectrophotometer. The spectrum in Figure 20-16 (in the next chapter) was produced with a photodiode array detector.

Photodiode Array Spectrophotometer

Dispersive spectrophotometers described so far scan through a spectrum one wavelength at a time. A **diode array spectrophotometer** records the entire spectrum at once. The entire spectrum of a compound emerging from a chromatography column can be recorded in a fraction of a second by a photodiode array spectrophotometer. At the heart of rapid spectroscopy is a **photodiode array** such as the one in Figure 19-11, which contains 1 024 individual semiconductor detector elements (diodes) in a row.

In the diode array spectrophotometer in Figure 19-12, *white light* (with all wavelengths) passes through the sample. The beam then enters a **polychromator**, which disperses light into its component wavelengths and directs the light to the diode array. *A different wavelength band strikes each diode*. The resolution, which is typically 1 to 3 nm, depends on how closely spaced the diodes are and how much dispersion is produced by the polychromator. By comparison, high-quality dispersive spectrophotometers can resolve features that are 0.1 nm apart. Diode array spectrophotometers are faster than dispersive spectrophotometers because the array measures all wavelengths at once, instead of one at a time. A diode array spectrophotometer is usually a single-beam instrument, subject to absorbance errors from drift in the source intensity and detector response between calibrations.



Ask Yourself

19-A. Explain what each component in the optical train in Figure 19-2 does, beginning with the lamp and ending with the detector. Locate the same components—as best you can—in Figure 19-3.

A *dispersive spectrophotometer* spreads light from the source into its component wavelengths and then measures the absorption of one narrow band of wavelengths at a time.

A typical photodiode array responds to visible and ultraviolet radiation, with a response curve similar to that of the blue-enhanced silicon in Figure 19-9.

Attributes of diode array spectrophotometer:

- speed (~1 s per spectrum)
- excellent wavelength repeatability (because the grating does not rotate)
- simultaneous measurements at multiple wavelengths
- relatively insensitive to errors from stray light
- relatively poor resolution (1 to 3 nm)

19-2 Analysis of a Mixture

When there is more than one absorbing species in a solution, *the absorbance at a particular wavelength is the sum of absorbances from all species at that wavelength*:

$$\text{Absorbance of a mixture: } A = \epsilon_X b[X] + \epsilon_Y b[Y] + \epsilon_Z b[Z] + \dots \quad (19-3)$$

Absorbance is additive.

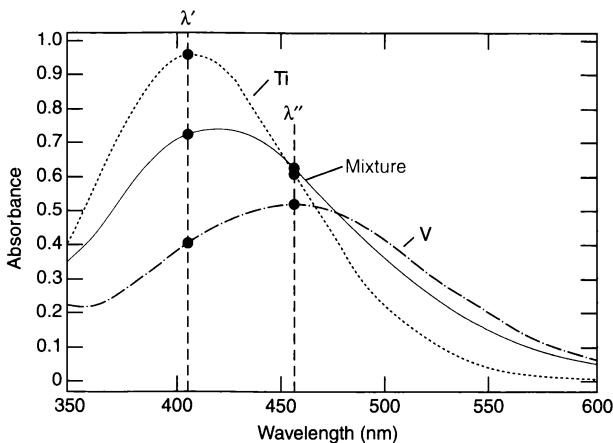


Figure 19-13 Visible spectra of hydrogen peroxide complexes of Ti(IV) (1.32 mM), V(V) (1.89 mM), and an unknown mixture containing both. All solutions contain 0.5 wt% H₂O₂ and ~0.01 M H₂SO₄ in a 1.00-cm-pathlength cell. [From M. Blanco, H. Iturriaga, S. MasPOCH, and P. Tarín, *J. Chem. Ed.* **1989**, 66, 178. Consult this article for a more accurate method for finding the composition of the mixture by using more than two wavelengths.]

where ϵ is the molar absorptivity of each species (X, Y, Z, and so on) and b is the pathlength. If we measure the spectra of the pure components in a separate experiment, we can mathematically disassemble the spectrum of the mixture into those of its components.

Figure 19-13 shows spectra of titanium and vanadium complexes and an unknown mixture of the two. Let's denote the titanium complex by X and the vanadium complex by Y. To analyze a mixture, we usually choose wavelengths of maximum absorption for the individual components. Accuracy is improved if compound Y absorbs weakly at the maximum for compound X and if compound X absorbs weakly at the maximum for compound Y. In Figure 19-13, the two spectra overlap badly, so there will be some loss of accuracy.

Choosing wavelengths λ' and λ'' as the absorbance maxima in Figure 19-13, we can write a Beer's law expression for each wavelength:

$$A' = \epsilon'_X b[X] + \epsilon'_Y b[Y] \quad A'' = \epsilon''_X b[X] + \epsilon''_Y b[Y] \quad (19-4)$$

Solving Equations 19-4 for [X] and [Y], we find

$$\begin{aligned} \text{Analysis of a mixture} \\ \text{when spectra are resolved:} \quad [X] &= \frac{1}{D}(A' \epsilon''_Y - A'' \epsilon'_Y) \\ & \qquad \qquad \qquad (19-5) \\ [Y] &= \frac{1}{D}(A'' \epsilon'_X - A' \epsilon''_X) \end{aligned}$$

where $D = b(\epsilon'_X \epsilon''_Y - \epsilon'_Y \epsilon''_X)$. To analyze the mixture, we measure absorbances at two wavelengths and must know ϵ at each wavelength for each compound.

Example Analysis of a Mixture with Equations 19-5

The molar absorptivities of X (the Ti complex) and Y (the V complex) in Figure 19-13 were measured with pure samples of each:

λ (nm)	ϵ ($M^{-1} \text{ cm}^{-1}$)	
	X	Y
$\lambda' = 406$	$\epsilon'_X = 720$	$\epsilon'_Y = 212$
$\lambda'' = 457$	$\epsilon''_X = 479$	$\epsilon''_Y = 274$

A mixture of X and Y in a 1.00-cm cell had an absorbance of $A' = 0.722$ at 406 nm and $A'' = 0.641$ at 457 nm. Find the concentrations of X and Y in the mixture.

SOLUTION Using Equations 19-5 and setting $b = 1.00$ cm, we find

$$D = b(\epsilon'_X \epsilon''_Y - \epsilon'_Y \epsilon''_X) = (1.00)[(720)(274) - (212)(479)] = 9.57_3 \times 10^4$$

$$[X] = \frac{1}{D}(A' \epsilon''_Y - A'' \epsilon'_Y) = \frac{(0.722)(274) - (0.641)(212)}{9.57_3 \times 10^4} = 6.47 \times 10^{-4} \text{ M}$$

$$[Y] = \frac{1}{D}(A'' \epsilon'_X - A' \epsilon''_X) = \frac{(0.641)(720) - (0.722)(479)}{9.57_3 \times 10^4} = 1.21 \times 10^{-3} \text{ M}$$

 **Test Yourself** The absorbance of the mixture is 0.600 at 406 nm and 0.500 at 457 nm. Find [X] and [Y]. (Answer: 0.610 mM, 0.758 mM)

Isosbestic Points

If species X is converted into species Y in the course of a chemical reaction, a spectrum of the mixture has an obvious, characteristic behavior, shown in Figure 19-14. If the spectra of pure X and pure Y cross each other at some wavelength, then every spectrum recorded during this chemical reaction crosses at the same point, called an **isosbestic point**. An *isosbestic point observed during a chemical reaction is good evidence that only two principal species are present*.

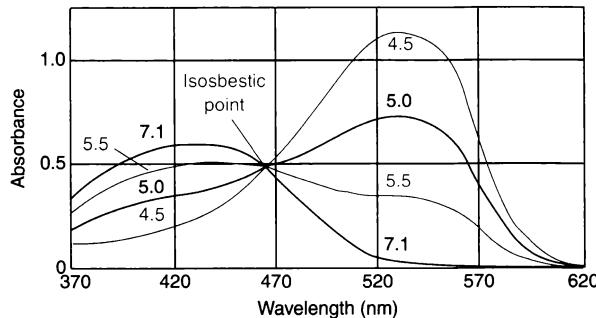
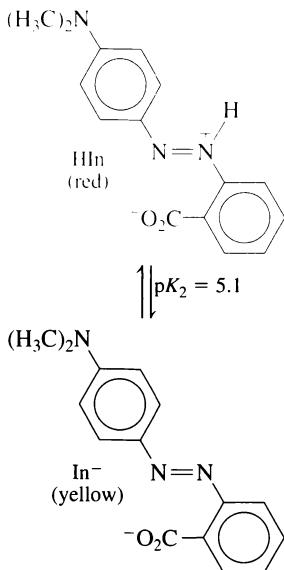


Figure 19-14 Absorption spectrum of $3.7 \times 10^{-4} \text{ M}$ methyl red as a function of pH between pH 4.5 and 7.1. [From E. J. King, *Acid-Base Equilibria* (Oxford: Pergamon Press, 1965).]



The acid-base indicator methyl red, shown in the margin, changes between red (HIn) and yellow (In^-) near pH 5.1. The spectra of HIn and In^- at equal concentration happen to cross at 465 nm in Figure 19-14, so *all* spectra cross at this point. (If the spectra of HIn and In^- crossed at several points, each would be an isosbestic point.)

To see why there is an isosbestic point, we write an equation for the absorbance of the solution at 465 nm:

$$A^{465} = \epsilon_{\text{HIn}}^{465} b[\text{HIn}] + \epsilon_{\text{In}^-}^{465} b[\text{In}^-] \quad (19-6)$$

But the spectra of pure HIn and pure In^- at equal concentration cross at 465 nm, so $\epsilon_{\text{HIn}}^{465}$ must be equal to $\epsilon_{\text{In}^-}^{465}$. Setting $\epsilon_{\text{HIn}}^{465} = \epsilon_{\text{In}^-}^{465} = \epsilon^{465}$, we rewrite Equation 19-6 in the form

$$A^{465} = \epsilon^{465} b([\text{HIn}] + [\text{In}^-]) \quad (19-7)$$

In Figure 19-14, all solutions contain the same total concentration of methyl red ($=[\text{HIn}] + [\text{In}^-]$). Only the pH varies. Therefore the sum of concentrations in Equation 19-7 is constant, and there is an isosbestic point because A^{465} is constant. An isosbestic point occurs when $\epsilon_X = \epsilon_Y$ and $[\text{X}] + [\text{Y}]$ is constant.

Ask Yourself

- 19-B. (a) In the example on page 417, a mixture of X and Y in a 0.100-cm cell had an absorbance of 0.233 at 406 nm and 0.200 at 457 nm. Find [X] and [Y].
 (b) If the total concentration of methyl red were increased by 37% from whatever concentration gave Figure 19-14, would there still be an isosbestic point at 465 nm? Why?
-

19-3 Spectrophotometric Titrations

In a **spectrophotometric titration**, we monitor changes in absorption or emission of electromagnetic radiation to detect the end point. We now consider an example from biochemistry.

Iron for biosynthesis is transported through the bloodstream by the protein *transferrin* (Figure 19-15). A solution of transferrin can be titrated with iron to measure its iron-binding capacity. Transferrin without iron, called *apotransferrin*, is colorless. Each protein molecule with a formula mass of 81 000 has two Fe^{3+} -binding sites. When Fe^{3+} binds to the protein, a red color with an absorbance maximum at 465 nm develops. The color intensity allows us to follow the course of the titration of an unknown amount of apotransferrin with standard Fe^{3+} .

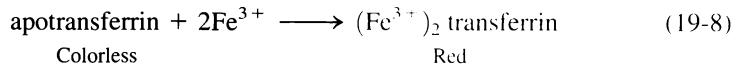
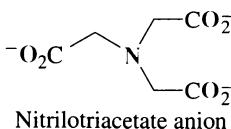


Figure 19-16 shows the titration of 2.000 mL of apotransferrin with 1.79×10^{-3} M ferric nitrilotriacetate. As iron is added to protein, red color develops and absorbance increases. When protein is saturated with iron, no more iron can bind and the curve levels off. The end point is the extrapolated intersection of the two straight lines at 203 μL . Absorbance rises slowly after the equivalence point because ferric nitrilotriacetate has some absorbance at 465 nm.

Ferric nitrilotriacetate is used because Fe^{3+} precipitates as $\text{Fe}(\text{OH})_3$ in neutral solution. Nitrilotriacetate binds Fe^{3+} through four **bold** atoms:



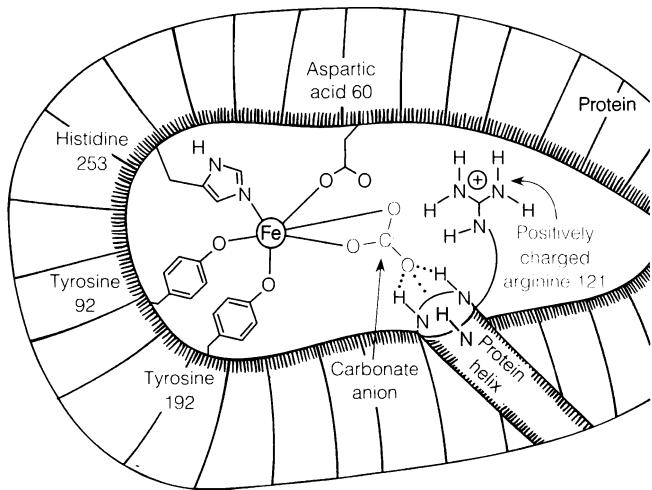


Figure 19-15 Each of the two Fe-binding sites of transferrin is located in a cleft in the protein. Each site has one nitrogen ligand from the amino acid histidine and three oxygen ligands from tyrosine and aspartic acid. Two oxygen ligands come from a carbonate anion (CO_3^{2-}) anchored by electrostatic attraction to positively charged arginine and by hydrogen bonding to the protein helix. When transferrin is taken up by a cell, it is brought into a vesicle (Box 1-1) whose pH is lowered to 5.5. H^+ reacts with carbonate to make HCO_3^- and H_2CO_3 , thereby releasing Fe^{3+} from the protein. [Adapted from E. N. Baker, B. F. Anderson, H. M. Baker, M. Haridas, G. E. Norris, S. V. Rumball, and C. A. Smith, *Pure Appl. Chem.* **1990**, 62, 1067.]

The quantity of Fe^{3+} required for complete reaction in Figure 19-16 is $(203 \times 10^{-6} \text{ L}) \times (1.79 \times 10^{-3} \text{ mol/L}) = 0.363 \mu\text{mol}$. Each protein molecule binds 2 Fe^{3+} ions, so the moles of protein in the sample must be $\frac{1}{2}(0.363 \mu\text{mol}) = 0.182 \mu\text{mol}$.

To construct the graph in Figure 19-16, we must account for the volume change as titrant is added. Each point plotted on the graph represents the absorbance that would be observed if the solution had not been diluted from its original volume of 2.000 mL.

$$\text{corrected absorbance} = \left(\frac{\text{total volume}}{\text{initial volume}} \right) (\text{observed absorbance}) \quad (19-9)$$

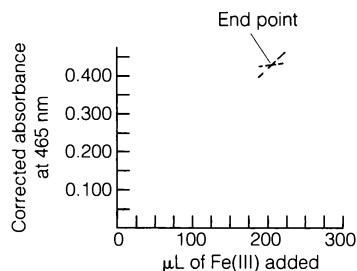


Figure 19-16 Spectrophotometric titration of apotransferrin with ferric nitrilotriacetate. Absorbance is corrected for dilution. The initial absorbance of the solution, before iron is added, is due to a colored impurity.

Example Correcting Absorbance for the Effect of Dilution

The absorbance measured after adding 125 μL (= 0.125 mL) of ferric nitrilotriacetate to 2.000 mL of apotransferrin was 0.260. Calculate the corrected absorbance that should be plotted in Figure 19-16.

SOLUTION The total volume was $2.000 + 0.125 = 2.125 \text{ mL}$. If the volume had been 2.000 mL, the absorbance would have been greater than 0.260 by a factor of $2.125/2.000$.

$$\text{corrected absorbance} = \left(\frac{2.125 \text{ mL}}{2.000 \text{ mL}} \right) (0.260) = 0.276$$

The absorbance plotted in the graph is 0.276.

Test Yourself The absorbance after adding 100 μL of ferric nitrilotriacetate was 0.210. Calculate the corrected absorbance to be plotted. (Answer: 0.221)

Ask Yourself

- 19-C. A 2.00-mL solution of apotransferrin, titrated as in Figure 19-16, required 163 μL of 1.43 mM ferric nitrilotriacetate to reach the end point.
- How many moles of Fe^{3+} were required to reach the end point?
 - Each apotransferrin molecule binds two Fe^{3+} ions. Find the concentration of apotransferrin in the 2.00-mL solution.
 - Why does the slope in Figure 19-16 change abruptly at the equivalence point?

19-4 What Happens When a Molecule Absorbs Light?

When a molecule absorbs a photon, the molecule is promoted to a more energetic *excited state* (Figure 18-3). Conversely, when a molecule emits a photon, the energy of the molecule falls by an amount equal to the energy of the photon that is given off. Figure 18-2 showed that molecules are promoted to excited electronic, vibrational, and rotational states by radiation in different regions of the electromagnetic spectrum.

As an example, we will discuss formaldehyde, whose ground state and one excited state are shown in Figure 19-17. The ground state is planar, with a double bond between carbon and oxygen. The double bond consists of a sigma bond between carbon and oxygen and a pi bond made from the $2p_y$ (out-of-plane) atomic orbitals of carbon and oxygen.

Electronic States of Formaldehyde

In *sigma* orbitals, electrons are localized between atoms. In *pi* orbitals, electrons are concentrated on either side of the plane of the formaldehyde molecule.

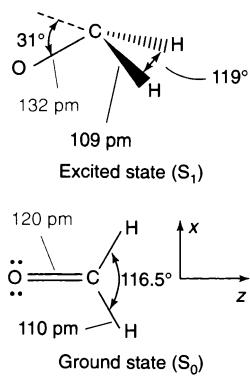


Figure 19-17 Geometry of formaldehyde in its ground state (S_0) and lowest excited singlet state (S_1).

Molecular orbitals describe the distribution of electrons in a molecule, just as *atomic orbitals* describe the distribution of electrons in an atom. In Figure 19-18, four low-lying orbitals of formaldehyde, labeled σ_1 through σ_4 , are each occupied by a pair of electrons with opposite spin (spin quantum numbers = $+\frac{1}{2}$ and $-\frac{1}{2}$ represented by \uparrow and \downarrow). At higher energy is a pi bonding orbital (π), made of the p_y atomic orbitals of carbon and oxygen. The highest energy occupied orbital is a nonbonding orbital (n), composed principally of the oxygen $2p_x$ atomic orbital. The lowest energy unoccupied orbital is a pi antibonding orbital (π^*). An electron in this orbital produces repulsion, rather than attraction, between the carbon and oxygen atoms.

In an **electronic transition**, an electron moves from one orbital to another. The lowest energy electronic transition of formaldehyde promotes a nonbonding (n) electron to the antibonding pi orbital (π^*). There are actually two possible transitions, depending on the spin quantum numbers in the excited state. The state in which spins are opposed in Figure 19-19 is called a **singlet state**. If spins are parallel, the excited state is a **triplet state**.

The lowest energy excited singlet and triplet states are called S_1 and T_1 . In general, T_1 has lower energy than S_1 . In formaldehyde, the weakly absorbing transition $n \rightarrow \pi^*(T_1)$ requires visible light with a wavelength of 397 nm. The more intense $n \rightarrow \pi^*(S_1)$ transition takes 355-nm ultraviolet radiation.

Although formaldehyde is planar in its ground state (S_0), it is pyramidal in both the S_1 (Figure 19-17) and T_1 excited states. Promotion of a nonbonding electron to an antibonding C—O orbital weakens and lengthens the C—O bond and changes the molecular geometry.

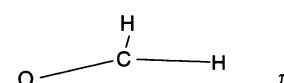
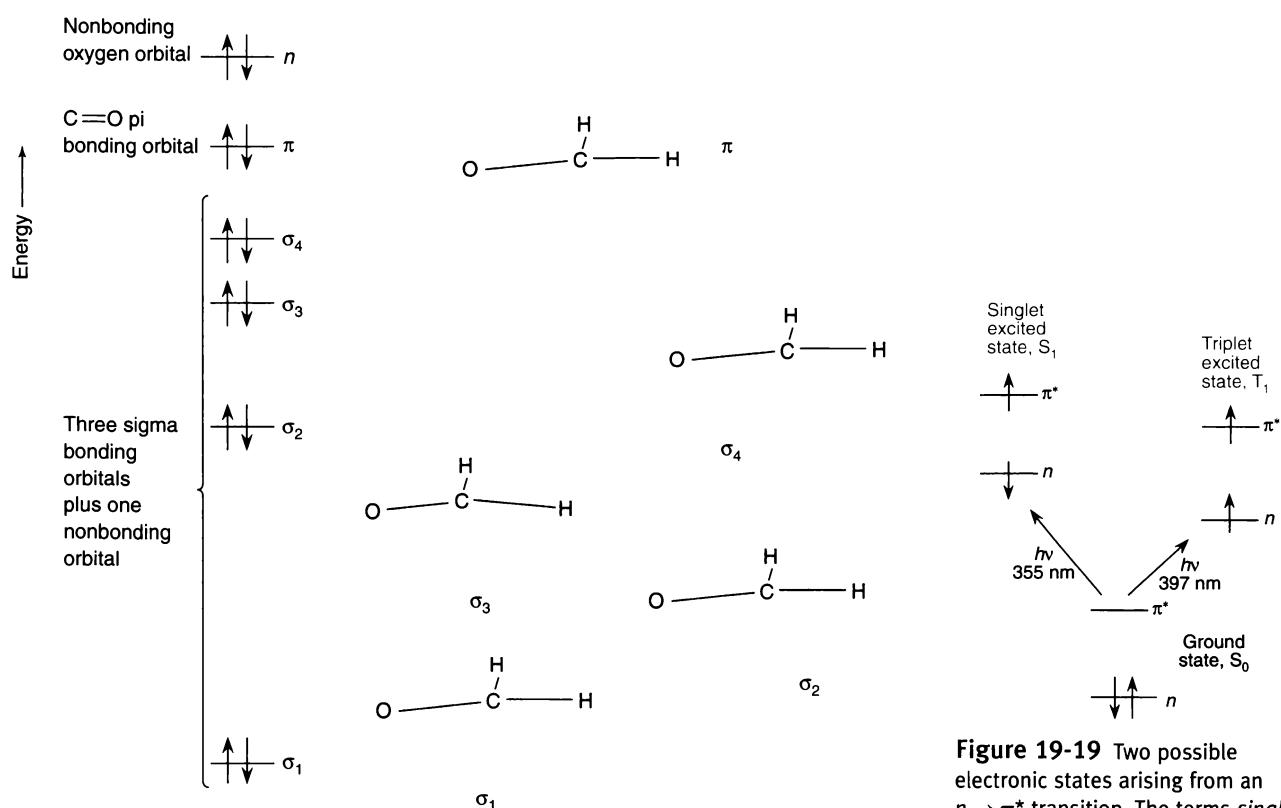


Figure 19-18 Molecular orbital diagram of formaldehyde, showing energy levels and orbital shapes. The coordinate system was shown in Figure 19-17. [From W. L. Jorgensen and L. Salem, *The Organic Chemist's Book of Orbitals* (New York: Academic Press, 1973).]



Vibrational and Rotational States of Formaldehyde

Infrared and microwave radiation are not energetic enough to induce electronic transitions, but they can change the vibrational or rotational motion of the molecule. The six modes of vibration of formaldehyde are shown in Figure 19-20. When formaldehyde absorbs an infrared photon with a wavenumber of 1746 cm^{-1} , for example, C—O stretching is stimulated: Oscillations of the atoms increase in amplitude and the energy of the molecule increases.

Rotation of a molecule involves less energy than vibration. Absorption of microwave radiation on the order of 10 cm^{-1} increases the rotational speed of formaldehyde about the x , y , and z axes in Figure 19-20.

C—O stretching is reduced from 1746 cm^{-1} in the S_0 state to 1183 cm^{-1} in the S_1 state because the strength of the C—O bond decreases when the antibonding π^* orbital is populated.

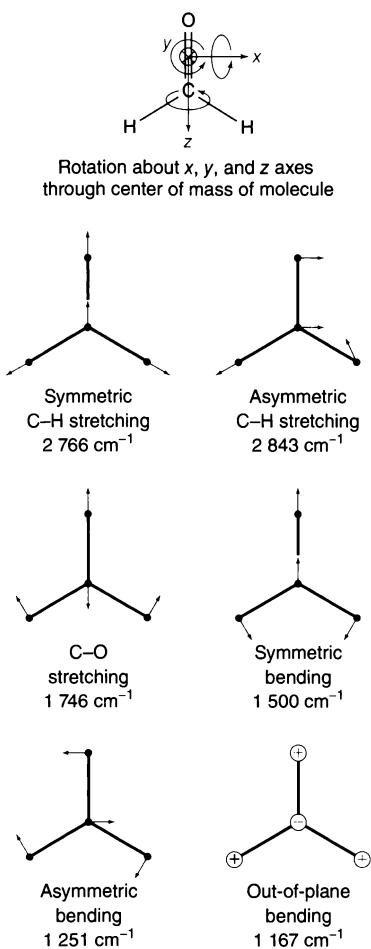


Figure 19-20 The six modes of vibration of formaldehyde. The wavenumber of infrared radiation needed to stimulate each vibration is given in units of reciprocal centimeters, cm^{-1} . The molecule rotates about x , y , and z axes located at the center of mass near the middle of the $\text{C}=\text{O}$ bond.

Internal conversion: radiationless transition between states with the same spin (e.g., $S_1 \rightarrow S_0$)

Intersystem crossing: radiationless transition between states with different spin (e.g., $T_1 \rightarrow S_0$)

Combined Electronic, Vibrational, and Rotational Transitions

In general, when a molecule absorbs light of sufficient energy to cause an electronic transition, **vibrational** and **rotational transitions**—changes in the vibrational and rotational states—occur as well. Formaldehyde can absorb one photon with just the right energy to (1) promote the molecule from S_0 to the S_1 electronic state; (2) increase the vibrational energy from the ground vibrational state of S_0 to an excited vibrational state of S_1 ; and (3) change from one rotational state of S_0 to a different rotational state of S_1 . Electronic absorption bands are usually very broad ($\sim 100 \text{ nm}$ in Figures 18-6 and 18-10) because many different vibrational and rotational levels are excited at slightly different energies.

What Happens to Absorbed Energy?

Suppose that absorption of a photon promotes a molecule from the ground electronic state, S_0 , to a vibrationally and rotationally excited level of the excited electronic state S_1 (Figure 19-21). Usually, the first event after absorption is *vibrational relaxation* to the lowest vibrational level of S_1 . In this process, labeled R_1 in Figure 19-21, energy is lost to other molecules (solvent, for example) through collisions. The net effect is to convert part of the energy of the absorbed photon into heat spread throughout the entire medium.

From S_1 , the molecule could enter a highly excited vibrational level of S_0 having the same energy as S_1 . This process is called *internal conversion*. Then the molecule can relax back to the ground vibrational state, transferring energy to neighboring molecules through collisions. If a molecule follows the path absorption $\rightarrow R_1 \rightarrow$ internal conversion $\rightarrow R_2$ in Figure 19-21, the entire energy of the photon will have been converted into heat.

Alternatively, the molecule could cross from S_1 into an excited vibrational level of T_1 . Such an event is known as *intersystem crossing*. Following relaxation R_3 , the molecule finds itself at the lowest vibrational level of T_1 . From here, the molecule might undergo a second intersystem crossing to S_0 , followed by relaxation R_4 , which liberates heat.

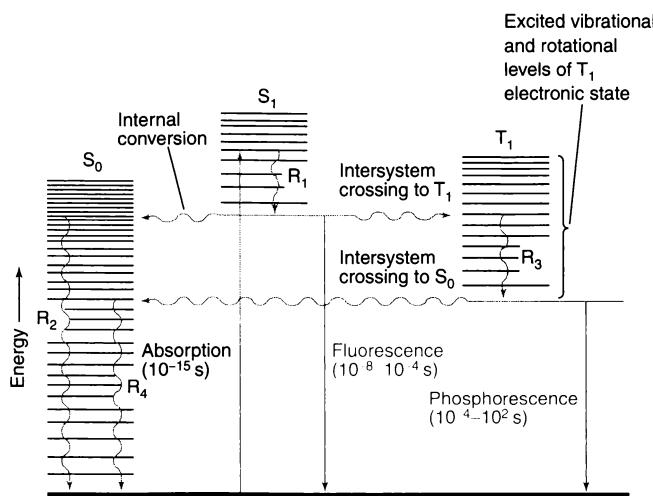
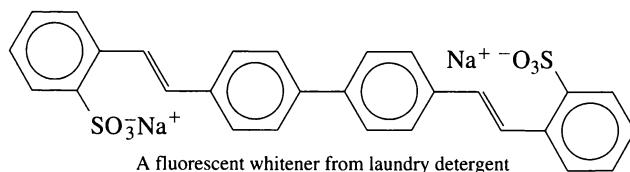


Figure 19-21 Physical processes that can occur after a molecule absorbs an ultraviolet or visible photon. S_0 is the ground electronic state. S_1 and T_1 are the lowest excited singlet and triplet states, respectively. Straight arrows represent processes involving photons, and wavy arrows are radiationless transitions. R is vibrational relaxation.



Demonstration 19-1 In Which Your Class Really Shines^{1,2,3}

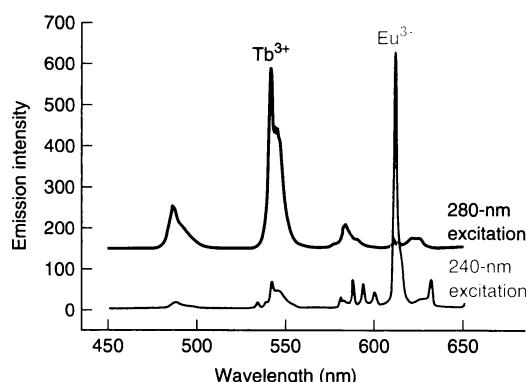


White fabrics are sometimes made “whiter” with a fluorescent dye. Turn on an ultraviolet lamp in a darkened classroom and illuminate people standing at the front of the room. (*The victims should not look directly at the lamp*, because ultraviolet light is harmful to eyes.) You will discover emission from white fabrics, including shirts, pants, shoelaces, and unmentionables. You may also be surprised to see fluorescence from teeth and from recently bruised areas of skin that show no surface damage.

A fluorescent lamp is a glass tube filled with Hg vapor. The inner walls are coated with a blend of red and green *phosphors* (luminescent substances). The red phosphor is Eu³⁺ doped into Y₂O₃. The green phosphor

is Tb³⁺ doped into CeMgAl₁₁O₁₉. Hg vapor in the lamp is excited by an electric current and emits ultraviolet radiation at 185 and 254 nm, and a series of visible lines. Hg emission by itself looks blue to our eyes. When the ultraviolet radiation is absorbed by the phosphors, Eu³⁺ emits red light at 612 nm and Tb³⁺ emits green light at 542 nm. The combination of blue, red, and green emissions appears white to us.

Fluorescent lamps are more efficient than incandescent lamps in converting electricity into light. In the near future, even more efficient LED (light-emitting diode) lamps will replace fluorescent lamps. Replacing a 75 W incandescent bulb with an 18 W compact fluorescent bulb saves 57 W. Over the 10 000-h lifetime of the fluorescent bulb, you will reduce CO₂ emission by ~600 kg and will put 10 kg less SO₂ into the atmosphere (see Problem 19-22). Alas, fluorescent bulbs contain Hg and should be recycled at a collection center where Hg will be captured from the bulbs. They should not be discarded as ordinary waste.



Emission spectra of phosphors scraped from the inside of a compact fluorescent lamp. Tb³⁺ is selectively excited at 280 nm, and Eu³⁺ is selectively excited at 240 nm. [C. Degli Esposti and L. Bizzochi, *J. Chem. Ed.* **2008**, 85, 839.]

Lamp	Approximate efficiency (lumens per watt) ^{a,b}
Flashlight (incandescent)	<6
Incandescent light bulb	15
Long fluorescent tube	80
Compact fluorescent lamp ^c	60
White light-emitting diode (LED) ^d	100–150
High-pressure sodium street lamp	130

a. From C. J. Humphreys, *Mater. Res. Bull.*, April 2008, vol. 33, p. 459.

b. Lumen (lm) is a measure of luminous flux. 1 lm = radiant energy emitted in a solid angle of 1 steradian (sr) from a source that radiates 1/683 W/sr uniformly in all directions at a frequency of 540 THz (near the middle of the visible spectrum).

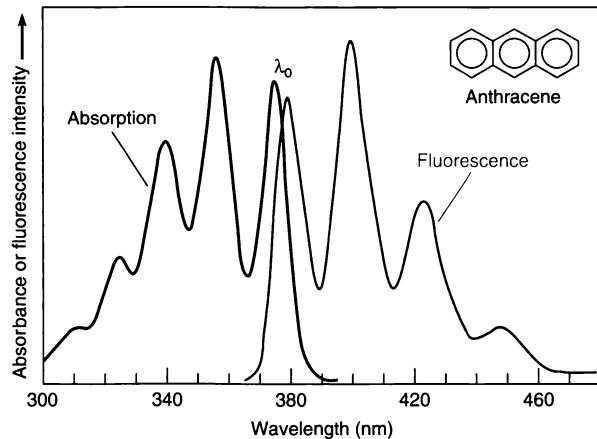
c. Lifetime of compact fluorescent lamp is reduced by a factor of 10 if it is frequently turned on and then off after just a few minutes.

d. LED lamp lifetime ≈10⁵ h; fluorescent lamp lifetime ≈10⁴ h; incandescent lamp lifetime ≈10³ h.

Alternatively a molecule could relax from S₁ or T₁ to S₀ by emitting a photon. The transition S₁ → S₀ is called **fluorescence** (Demonstration 19-1), and T₁ → S₀ is called **phosphorescence**. (Fluorescence and phosphorescence can terminate in any of the vibrational levels of S₀, not just the ground state shown in Figure 19-21.) The rates of internal conversion, intersystem crossing, fluorescence, and phosphorescence depend on the solvent and conditions such as temperature and pressure. We see in Figure 19-21 that phosphorescence occurs at lower energy (longer wavelength) than does fluorescence.

Fluorescence: emission of a photon from a transition between states with the same spin (e.g., S₁ → S₀)
Phosphorescence: emission of a photon from a transition between states with different spin (e.g., T₁ → S₀)

Figure 19-22 Spectra of anthracene show typical approximate mirror image relationship between absorption and fluorescence. Fluorescence comes at lower energy (longer wavelength) than absorption. [From C. M. Byron and T. C. Werner, *J. Chem. Ed.* 1991, 68, 433.]



An example of emission at lower energy (longer wavelength) than absorption is seen in Color Plate 20, in which blue light absorbed by a crystal gives rise to red emission.

Molecules generally decay from the excited state through collisions—not by emitting light. The *lifetime* of fluorescence is always very short (10^{-8} to 10^{-4} s). The lifetime of phosphorescence is much longer (10^{-4} to 10^2 s). Phosphorescence is rarer than fluorescence, because a molecule in the T_1 state has a good chance of collisional deactivation before phosphorescence can occur.

Figure 19-22 compares absorption and fluorescence spectra of anthracene. Fluorescence comes at lower energy and is roughly the mirror image of absorption. To understand the mirror image relation, consider the energy levels in Figure 19-23. In the absorption spectrum, wavelength λ_0 corresponds to a transition from the ground vibrational level of S_0 to the lowest vibrational level of S_1 . Absorption

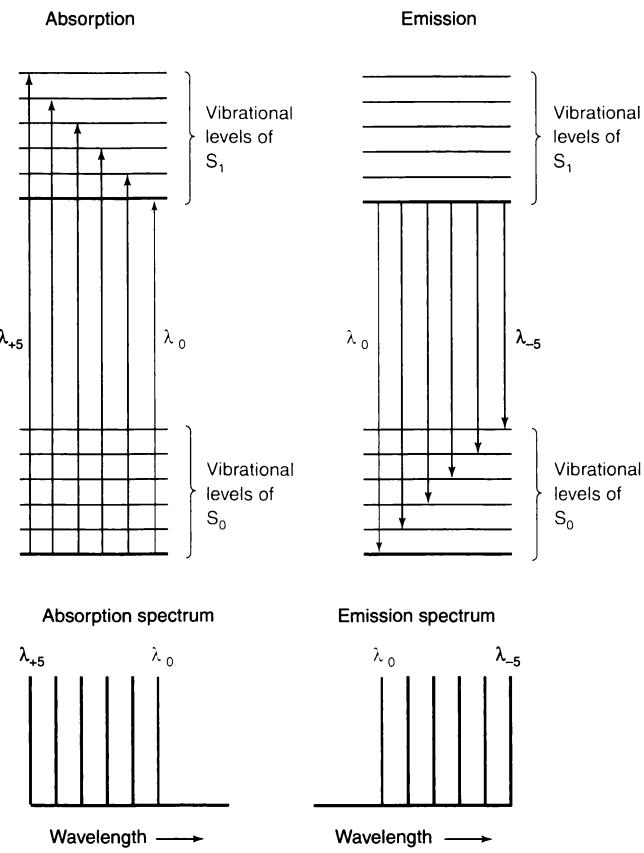


Figure 19-23 Energy-level diagram showing why structure is seen in the absorption and emission spectra and why the spectra are roughly mirror images. In absorption, wavelength λ_0 comes at lowest energy, and λ_{+5} is at highest energy. In emission, wavelength λ_0 comes at highest energy, and λ_{-5} is at lowest energy.

maxima at higher energy (shorter wavelength) correspond to the $S_0 \rightarrow S_1$ transition accompanied by absorption of one or more quanta of vibrational energy. In polar solvents, vibrational structure is often broadened beyond recognition, and only a broad envelope of absorption is observed. In Figure 19-22, the solvent is cyclohexane, which is nonpolar, and the vibrational structure is easily seen.

Following absorption, the vibrationally excited S_1 molecule relaxes back to the lowest vibrational level of S_1 prior to emitting any radiation. Emission from S_1 can go to any of the vibrational levels of S_0 in Figure 19-23. The highest energy transition comes at wavelength λ_0 , with a series of peaks following at longer wavelength. The absorption and emission spectra will have an approximate mirror image relation if spacings between vibrational levels are roughly equal and if transition probabilities are similar.

Another possible consequence of the absorption of light is the breaking of chemical bonds. **Photochemistry** is a chemical reaction initiated by absorption of light (as in the reaction $O_2 \xrightarrow{h\nu} 2O$ in the upper atmosphere mentioned at the opening of Chapter 18). Some chemical reactions release energy in the form of light, which is called **chemiluminescence**. The light from a firefly or a light stick⁴ is chemiluminescence.

Ask Yourself

- 19-D. (a) What is the difference between electronic, vibrational, and rotational transitions?
 (b) How can the energy of an absorbed photon be released without emission of light?
 (c) What processes lead to fluorescence and phosphorescence? Which comes at higher energy? Which is faster?
 (d) Why does fluorescence tend to be the mirror image of absorption?
 (e) What is the difference between photochemistry and chemiluminescence?

19-5 Luminescence in Analytical Chemistry

Luminescence is any emission of electromagnetic radiation and includes fluorescence, phosphorescence, and other possible processes. In Figure 19-24, luminescence is measured by exciting a sample at a wavelength that it absorbs ($\lambda_{\text{excitation}}$) and

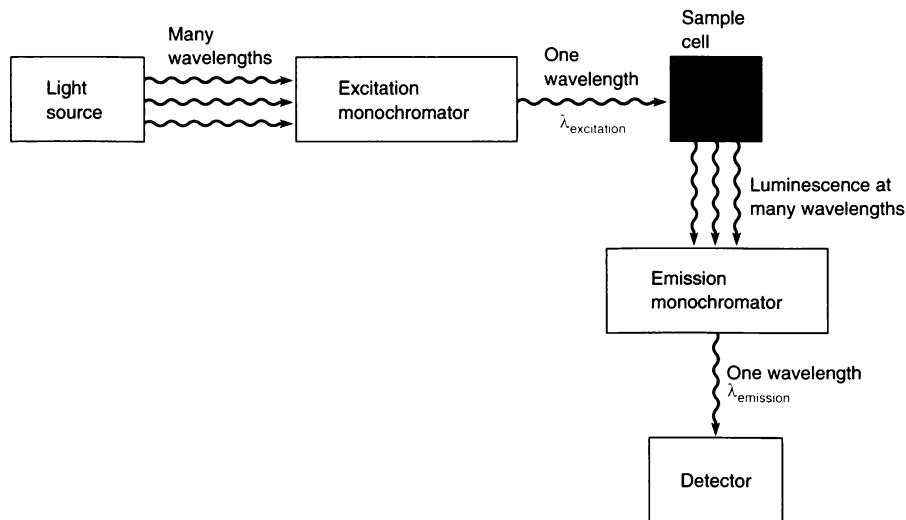


Figure 19-24 A luminescence experiment. Sample is irradiated at one wavelength and emission is observed over a range of wavelengths. The emission monochromator selects one emission wavelength at a time to measure the emission spectrum.

Luminescence is more sensitive than absorbance for detecting very low concentrations of analyte.

Luminescence can be increased by increasing the incident radiant power.

Derivatization is the chemical alteration of an analyte so that it can be detected conveniently or separated from other species easily.

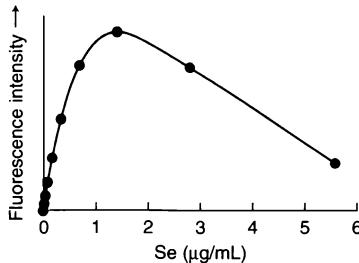


Figure 19-25 Fluorescence calibration curve for the selenium-containing product in Reaction 19-11. The curvature and maximum are due to self-absorption. [From M.-C. Sheffield and T. M. Nahir, *J. Chem. Ed.* 2002, 79, 1345.]

observing at the wavelength of maximum emission ($\lambda_{\text{emission}}$). Luminescence is observed perpendicular to the incident direction to minimize the detection of scattered radiation. Incident radiation is scattered to the side by particles or large molecules in the sample.

Luminescence is more sensitive than absorption. Imagine yourself in a stadium at night with the lights off, but each of the 50 000 raving fans is holding a lighted candle. If 500 people blow out their candles, you will hardly notice the difference. Now imagine that the stadium is completely dark, and then 500 people light their candles. The change would be dramatic. The first case is analogous to changing transmittance from 100% to 99%. It is hard to measure such a small change because the 50 000-candle background is so bright. The second case is analogous to observing luminescence from 1% of the molecules in a sample. Against the dark background, luminescence is easy to detect. Luminescence is so sensitive that scientists can observe emission from a *single molecule*.

For quantitative analysis, the intensity of luminescence (I) is proportional to (1) the concentration of the emitting species (c) over some limited concentration range and (2) the incident radiant power (P_0):

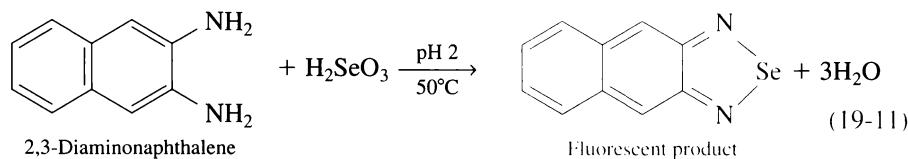
$$\text{Relation of emission intensity to concentration:} \quad I = kP_0c \quad (19-10)$$

where k is a constant that depends on the emitting molecule and all of the conditions of its environment. The point is that luminescence intensity is proportional to the concentration of the emitting molecule if all conditions are kept constant.

Fluorimetric Assay of Selenium in Brazil Nuts

Selenium is a trace element essential to life. For example, the selenium-containing enzyme glutathione peroxidase catalyzes the destruction of peroxides (ROOH) that are harmful to cells. Conversely, at high concentration, selenium can be toxic.

To measure selenium in Brazil nuts, 0.1 g of nuts is digested with 2.5 mL of 70 wt% HNO₃ in a Teflon bomb in a microwave oven (Figure 2-18). Hydrogen selenate (H₂SeO₄) in the digest is reduced to hydrogen selenite (H₂SeO₃) with hydroxylamine (NH₂OH). Selenite is then **derivatized** to form a fluorescent product that is extracted into cyclohexane.



Maximum response of the fluorescent product was observed with an excitation wavelength of 378 nm and an emission wavelength of 518 nm. The fluorescence calibration curve in Figure 19-25 is linear, obeying Equation 19-10, only up to ~0.1 μg Se/mL. Beyond 0.1 μg Se/mL, the response becomes curved, eventually reaches a maximum, and finally *decreases* with increasing selenium concentration.

What is going on here? Fluorescence is the emission of light from a molecule in an excited state. When the concentration becomes too great, neighboring unexcited molecules absorb light from the excited molecule before the light can escape from the cuvet. Absorption of excitation energy by neighboring molecules of the same substance is called **self-absorption**. Some fraction of excitation energy absorbed by the neighbors is converted to heat. The higher the concentration, the

more the analyte absorbs its own fluorescence and the less emission we observe. We say that the molecule *quenches* its own emission.

The behavior in Figure 19-25 is general. At low concentration, luminescence intensity is proportional to analyte concentration. At higher concentration, self-absorption becomes important and, eventually, luminescence reaches a maximum. Equation 19-10 applies only at low concentration.

Immunoassays

An important application of luminescence is in **immunoassays**, which employ antibodies to detect analyte. An **antibody** is a protein produced by the immune system of an animal in response to a foreign molecule, which is called an **antigen**. An antibody specifically recognizes and binds to the antigen that stimulated its synthesis.

Figure 19-26 illustrates the principle of an *enzyme-linked immunosorbent assay*, abbreviated ELISA in biochemical literature. Antibody 1, which is specific for the analyte of interest (the antigen), is bound to a polymer support. In steps 1 and 2, analyte is incubated with the polymer-bound antibody to form the antibody-antigen complex. The fraction of antibody sites that bind analyte is proportional to the concentration of analyte in the unknown. The surface is then washed to remove unbound substances. In steps 3 and 4, the antibody-antigen complex is treated with antibody 2, which recognizes a different region of the analyte. An enzyme that will be used later was covalently attached to antibody 2 (prior to step 3). Again, excess unbound substances are washed away.

Figure 19-27 shows two ways in which the enzyme attached to antibody 2 is used for quantitative analysis. In Figure 19-27a, the enzyme transforms a colorless reactant into a colored product. Because one enzyme molecule catalyzes the same reaction many times, many molecules of colored product are created for each molecule of antigen. The enzyme thereby *amplifies* the signal in chemical analysis. The higher the concentration of analyte in the unknown, the more enzyme is bound and the greater the extent of the enzyme-catalyzed reaction. In Figure 19-27b, the enzyme converts a nonfluorescent reactant into a fluorescent product. Enzyme-linked immunoassay assays are sensitive to <1 ng of analyte. Box 19-1 shows how an immunoassay is used in a home pregnancy test.

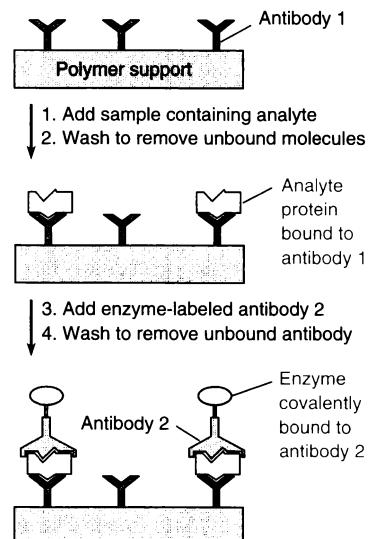


Figure 19-26 Enzyme-linked immunosorbent assay. Antibody 1, which is specific for the analyte of interest, is bound to a polymer support and treated with unknown. After excess, unbound molecules have been washed away, analyte remains bound to antibody 1. Bound analyte is then treated with antibody 2, which recognizes a different site on the analyte. An enzyme is covalently attached to antibody 2. After unbound material has been washed away, each molecule of analyte is coupled to an enzyme, which will be used in Figure 19-27.

Ask Yourself

- 19-E. (a) Why is Figure 19-25 curved and why does it reach a maximum?
 (b) How is signal amplification achieved in enzyme-linked immunosorbent assays?

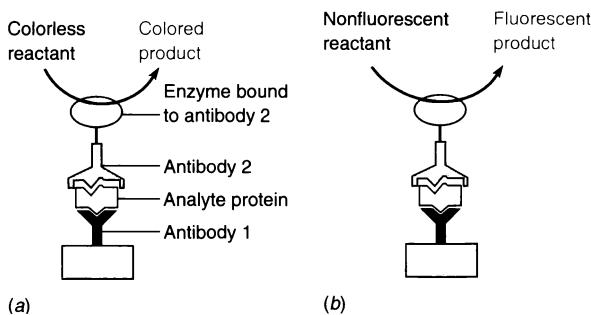


Figure 19-27 Enzyme bound to antibody 2 can catalyze reactions that produce (a) colored or (b) fluorescent products. Each molecule of analyte bound in the immunoassay leads to many molecules of colored or fluorescent product that are easily measured.

Box 19-1 How Does a Home Pregnancy Test Work?

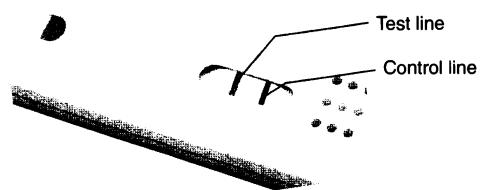
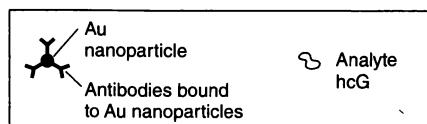
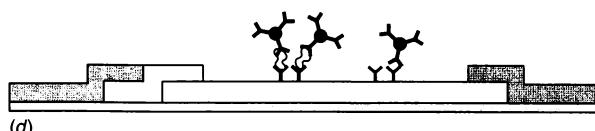
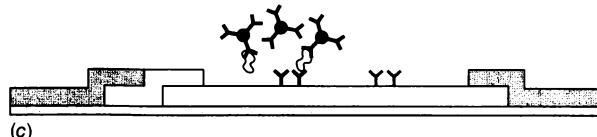
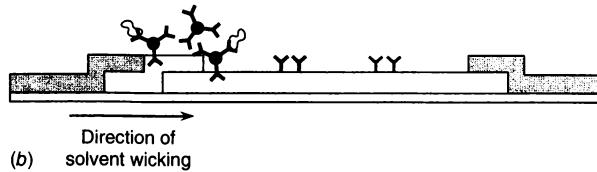
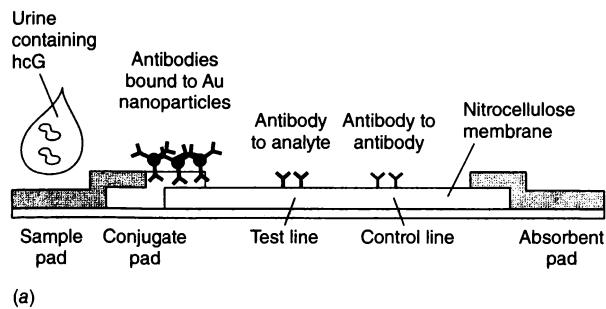
One of the most common home pregnancy tests is an immunoassay that detects the hormone human chorionic gonadotropin (hcG) in urine. HcG is a protein consisting of 244 amino acids in two subunits called α and β . The β subunit is unique to hcG, but the α subunit is found in some other proteins. The hormone begins to be secreted shortly after conception.

The *lateral flow home pregnancy immunoassay* shown schematically in the figure is a qualitative test for hcG. Urine is applied to the sample pad at the left end of a horizontal test strip made of nitrocellulose that serves as a wick. Liquid flows from left to right by capillary action. Liquid first encounters detection reagent on the “conjugate” pad. The reagent is called a conjugate because it consists of hcG monoclonal antibody

(page 280) attached to red-colored gold nanoparticles. The antibody binds to one site of the β chain of hcG.

As liquid flows to the right, hcG bound to the conjugate is trapped at the “test line,” which contains an antibody specific for another site on the β chain of hcG. Gold nanoparticles trapped at the test line create a visible red line. As liquid continues to the right, it encounters the “control line” with antibodies that bind to the conjugate reagent. A second red line forms at the control line. At the far right is an absorbent pad that soaks up liquid, excess reagent, and analyte that were not retained at the test or control lines.

In a positive pregnancy test, both lines turn red. The test is negative if only the control line turns red. If the control line fails to turn red, the test is invalid.



[Rob Byron/Fotolia.com]

Key Equations

Absorption of a mixture of species X and Y

$$A = \varepsilon_X b[X] + \varepsilon_Y b[Y]$$

A = absorbance at wavelength λ

ε_i = molar absorptivity of species i at wavelength λ

$$b = \text{pathlength}$$

You should be able to use Equations 19-5 to analyze the spectrum of a mixture.

Fluorescence intensity (at low concentration)

$$I = kP_0c$$

I = fluorescence intensity

k = constant

P_0 = radiant power of incident radiation

c = concentration of fluorescing species

Important Terms

antibody	fluorescence	monochromator	rotational transition
antigen	grating	phosphorescence	self-absorption
chemiluminescence	immunoassay	photochemistry	singlet state
derivatization	isosbestic point	photodiode array	spectrophotometric titration
diffraction	luminescence	photomultiplier tube	triplet state
electronic transition	molecular orbital	polychromator	vibrational transition

Problems

19-1. State the differences between single- and double-beam spectrophotometers and explain how each measures the transmittance of a sample. What source of error in a single-beam instrument is absent in the double-beam instrument?

19-2. Would you use a tungsten or a deuterium lamp as a source of 300-nm radiation?

19-3. What are the advantages and disadvantages of decreasing monochromator slit width?

19-4. Consider a reflection grating operating with an incident angle of 40° in Figure 19-6.

(a) How many lines per centimeter should be etched in the grating if the first-order diffraction angle for 600 nm (visible) light is to be -30° ?

(b) Answer the same question for $1\ 000\ \text{cm}^{-1}$ (infrared) light.

19-5. (a) In Color Plate 19a, red light with a wavelength of 633 nm strikes a grating at normal incidence ($\theta = 0$). The grating spacing is $d = 1.6\ \mu\text{m}$. At what angles are the $n = -1$, $n = +1$, and $n = +2$ diffracted beams expected?

(b) Explain why the $n = 3$ diffracted beam is not observed.

19-6. Why is a photomultiplier such a sensitive photo-detector?

19-7. What characteristic makes a photodiode array spectrophotometer suitable for measuring the spectrum of a compound as it emerges from a chromatography column and a dispersive spectrophotometer not suitable? What is the disadvantage of the photodiode array spectrophotometer?

19-8. When are isosbestic points observed and why?

19-9. Molar absorptivities of compounds X and Y were measured with pure samples of each:

λ (nm)	$\epsilon (\text{M}^{-1}\ \text{cm}^{-1})$	
	X	Y
$\lambda' = 272$	$\epsilon'_X = 16\ 440$	$\epsilon'_Y = 3\ 870$
$\lambda'' = 327$	$\epsilon''_X = 3\ 990$	$\epsilon''_Y = 6\ 420$

A mixture of X and Y in a 1.000-cm cell had an absorbance of $A' = 0.957$ at 272 nm and $A'' = 0.559$ at 327 nm. Find the concentrations of X and Y in the mixture.

19-10. Spreadsheet for simultaneous equations. Write a spreadsheet for the analysis of a mixture by using Equation 19-5. The input will be the sample pathlength, the observed absorbances at two wavelengths, and the molar absorptivities of the two pure compounds at two wavelengths. The output will be the concentration of each component of the mixture. Test your spreadsheet with numbers from Problem 19-9.

19-11. Ultraviolet absorbance for $1.00 \times 10^{-4}\ \text{M}$ MnO_4^- , $1.00 \times 10^{-4}\ \text{M}$ $\text{Cr}_2\text{O}_7^{2-}$, and an unknown mixture of both (all in a 1.000-cm cell) are given in the following table. Find the concentration of each species in the mixture.

Wavelength (nm)	MnO_4^- standard	$\text{Cr}_2\text{O}_7^{2-}$ standard	Mixture
266	0.042	0.410	0.766
320	0.168	0.158	0.422

19-12. Transferrin is the iron-transport protein found in blood. It has a molecular mass of 81 000 and carries two Fe^{3+} ions. Desferrioxamine B (Chapter 13 opening) is a potent iron chelator used to treat patients with iron overload. It has a

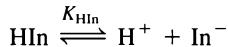
molecular mass of about 650 and can bind one Fe^{3+} . Desferrioxamine can take iron from many sites within the body and is excreted (with its iron) through the kidneys. The molar absorptivities of these compounds (saturated with iron) at two wavelengths are given in the following table. Both compounds are colorless (no visible absorption) in the absence of iron.

λ (nm)	$\epsilon (\text{M}^{-1} \text{cm}^{-1})$	
	Transferrin	Desferrioxamine
428	3 540	2 730
470	4 170	2 290

(a) A solution of transferrin has an absorbance of 0.463 at 470 nm in a 1.000-cm cell. Find the concentration of transferrin in mg/mL and the concentration of iron in $\mu\text{g}/\text{mL}$.

(b) After addition of desferrioxamine (which dilutes the sample), the absorbance at 470 nm was 0.424 and the absorbance at 428 nm was 0.401. Calculate the fraction of iron in transferrin. Remember that transferrin binds two Fe^{3+} ions and desferrioxamine binds only one.

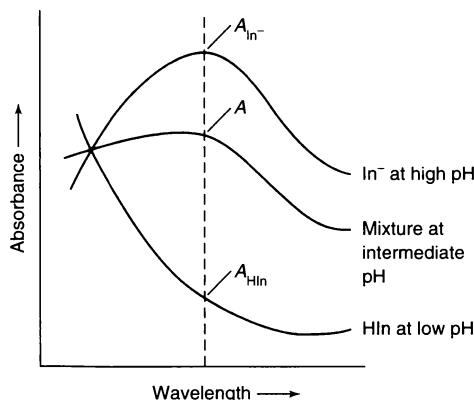
19-13. *Finding pK_a by spectrophotometry.* An indicator has a molar absorptivity of $2\ 080 \text{ M}^{-1} \text{cm}^{-1}$ for HIn and $14\ 200 \text{ M}^{-1} \text{cm}^{-1}$ for In^- , at a wavelength of 440 nm.



(a) Write a Beer's law expression for the absorbance at 440 nm of a solution in a 1.00-cm cuvet containing the concentrations $[\text{HIn}]$ and $[\text{In}^-]$.

(b) A solution of HIn adjusted to pH 6.23 contains a mixture of HIn and In^- with a total concentration of $1.84 \times 10^{-4} \text{ M}$. The absorbance at 440 nm is 0.868. From your expression from (a) and the mass balance $[\text{HIn}] + [\text{In}^-] = 1.84 \times 10^{-4} \text{ M}$, calculate pK_{HIn} .

19-14. *Graphical method to find pK_a by spectrophotometry.* This method requires a series of solutions containing a compound of unknown but constant concentration at different pH values. The figure shows that we choose a wavelength at which one of the species, say In^- , has maximum absorbance (A_{In^-}) and HIn has a different absorbance (A_{HIn}).



At intermediate pH, the absorbance (A) is between the two extremes. Let the total concentration be $c_0 = [\text{HIn}] + [\text{In}^-]$. At high pH, the absorbance is $A_{\text{In}^-} = \epsilon_{\text{In}^-} b c_0$, and at low pH, the absorbance is $A_{\text{HIn}} = \epsilon_{\text{HIn}} b c_0$, where ϵ is molar absorptivity and b is pathlength. At intermediate pH, both species are present and the absorbance is $A = \epsilon_{\text{HIn}} b [\text{HIn}] + \epsilon_{\text{In}^-} b [\text{In}^-]$. You can combine these expressions to show that

$$\frac{[\text{In}^-]}{[\text{HIn}]} = \frac{A - A_{\text{HIn}}}{A_{\text{In}^-} - A}$$

Placing this expression into the Henderson-Hasselbalch equation gives

$$\text{pH} = pK_{\text{HIn}} + \log\left(\frac{[\text{In}^-]}{[\text{HIn}]}\right) \Rightarrow \log\left(\frac{A - A_{\text{HIn}}}{A_{\text{In}^-} - A}\right) = \text{pH} - pK_{\text{HIn}}$$

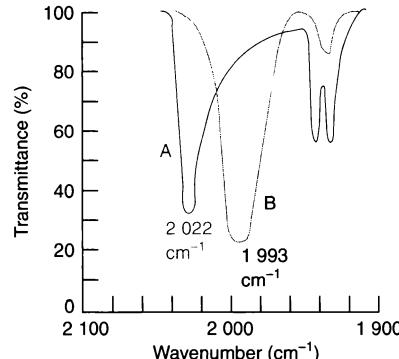
For several solutions of intermediate pH, a graph of $\log[(A - A_{\text{HIn}})/(A_{\text{In}^-} - A)]$ versus pH should be a straight line with a slope of 1 that crosses the x-axis at pK_{HIn} .

pH	Absorbance
~2	0.006 $\equiv A_{\text{HIn}}$
3.35	0.170
3.65	0.287
3.94	0.411
4.30	0.562
4.64	0.670
~12	0.818 $\equiv A_{\text{In}^-}$

Data at 590 nm for HIn = bromophenol blue from G. S. Patterson, *J. Chem. Ed.* **1999**, 76, 395.

Prepare a graph of $\log[(A - A_{\text{HIn}})/(A_{\text{In}^-} - A)]$ versus pH. Find the slope and intercept and pK_{HIn} .

19-15. Infrared spectra are customarily recorded on a transmittance scale so that weak and strong bands can be displayed on the same scale. The region near $2\ 000 \text{ cm}^{-1}$ in the infrared spectra of compounds A and B is shown in the figure. Absorption corresponds to a downward peak on this scale. Spectra of a $0.010\ 0 \text{ M}$ solution of each compound were obtained with a $0.005\ 00\text{-cm}$ -pathlength cell. A mixture of A and B in a $0.005\ 00\text{-cm}$ cell had a transmittance of 34.0% at $2\ 022 \text{ cm}^{-1}$ and 38.3% at $1\ 993 \text{ cm}^{-1}$. Find [A] and [B] with the spreadsheet from Problem 19-10.



Wavenumber	Pure A	Pure B
2 022 cm ⁻¹	31.0% T	97.4% T
1 993 cm ⁻¹	79.7% T	20.0% T

19-16. The iron-binding site of transferrin in Figure 19-15 accommodates certain other metal ions besides Fe³⁺ and certain other anions besides CO₃²⁻. Data are given in the table for the titration of transferrin (3.57 mg in 2.00 mL) with 6.64 mM Ga³⁺ in the presence of the anion oxalate, C₂O₄²⁻, and in the absence of a suitable anion. Prepare a graph similar to Figure 19-16, showing both sets of data. Mark the theoretical equivalence point for binding two Ga³⁺ ions per molecule of protein. How many Ga³⁺ ions are bound to transferrin in the presence and absence of oxalate?

Titration in presence of C ₂ O ₄ ²⁻		Titration in absence of anion	
Total μL Ga ³⁺ added	Absorbance at 241 nm	Total μL Ga ³⁺ added	Absorbance at 241 nm
0.0	0.044	0.0	0.000
2.0	0.143	2.0	0.007
4.0	0.222	6.0	0.012
6.0	0.306	10.0	0.019
8.0	0.381	14.0	0.024
10.0	0.452	18.0	0.030
12.0	0.508	22.0	0.035
14.0	0.541	26.0	0.037
16.0	0.558		
18.0	0.562		
21.0	0.569		
24.0	0.576		

19-17. The metal-binding compound semi-xylenol orange is yellow at pH 5.9 but turns red ($\lambda_{\text{max}} = 490 \text{ nm}$) when it reacts with Pb²⁺. A 2.025-mL sample of semi-xylenol orange was titrated with $7.515 \times 10^{-4} \text{ M Pb}(\text{NO}_3)_2$, with the following results:

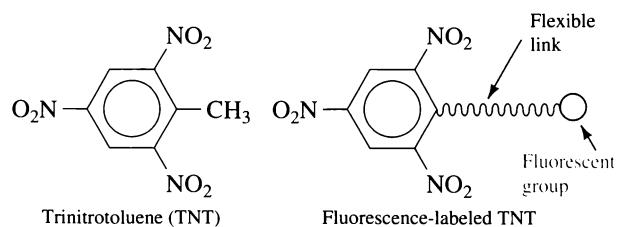
Total μL Pb ²⁺ added	Absorbance at 490 nm in 1-cm cell		Total μL Pb ²⁺ added	Absorbance at 490 nm in 1-cm cell
	in 1-cm cell	in 1-cm cell		
0.0	0.227		42.0	0.425
6.0	0.256		48.0	0.445
12.0	0.286		54.0	0.448
18.0	0.316		60.0	0.449
24.0	0.345		70.0	0.450
30.0	0.370		80.0	0.447
36.0	0.399			

Make a graph of corrected absorbance versus microliters of Pb²⁺ added. Corrected absorbance is what would be observed if the volume were not changed from its initial value of 2.025 mL.

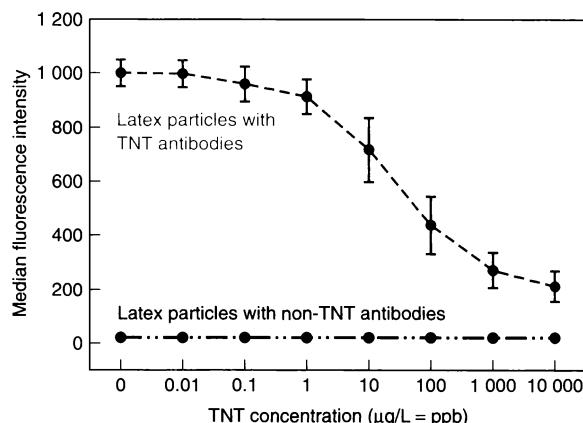
Assuming that the reaction of semi-xylenol orange with Pb²⁺ has a 1:1 stoichiometry, find the molarity of semi-xylenol orange in the original solution.

19-18. An *immunoassay* to measure explosives such as trinitrotoluene (TNT) in organic solvent extracts of soil employs a *flow cytometer*, which counts small particles (such as living cells) flowing through a narrow tube past a detector. The cytometer in this experiment irradiates the particles with a green laser and measures fluorescence from each particle as it flows past the detector.

1. Antibodies that bind TNT are chemically attached to 5-μm-diameter latex beads.
2. The beads are incubated with a fluorescent derivative of TNT to saturate the antibodies, and excess TNT derivative is removed.



3. 5 μL of a suspension of beads are added to 100 μL of sample. TNT in the sample displaces some derivatized TNT from the antibodies. The higher the concentration of TNT, the more derivatized TNT is displaced.
4. Sample/bead suspension is injected into the flow cytometer, which measures fluorescence of individual beads as they pass the detector. The figure shows median fluorescence intensity ± standard deviation. TNT can be quantified in the ppb to ppm range.



Fluorescence of TNT-antibody-beads versus TNT concentration.
[From G. P. Anderson, S. C. Moreira, P. T. Charles, I. L. Medintz, E. R. Goldman, M. Zeinali, and C. R. Taitt, *Anal. Chem.* **2006**, 78, 2279.]

Draw pictures showing the state of the beads in steps 1, 2, and 3 and explain how this method works.

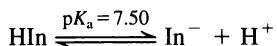
19-19. Standard addition. Selenium from 0.108 g of Brazil nuts was converted into the fluorescent product in Reaction 19-11, which was extracted into 10.0 mL of cyclohexane. Then 2.00 mL of the cyclohexane solution were placed in a cuvet for fluorescence measurements.

(a) Standard additions of fluorescent product containing 1.40 μg Se/mL are given in the table. Construct a standard addition graph like Figure 5-6 to find the concentration of Se in the 2.00-mL unknown solution. Find wt% Se in the nuts.

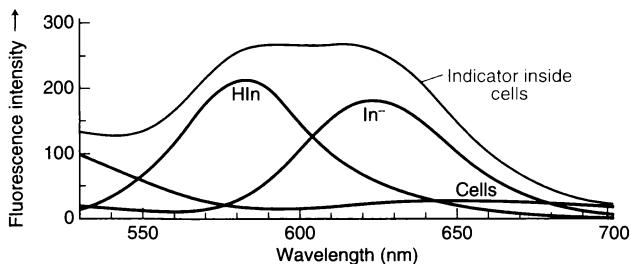
Volume of standard added (μL)	Fluorescence intensity (arbitrary units)
0	41.4
10.0	49.2
20.0	56.4
30.0	63.8
40.0	70.3

(b) With the formula in Problem 5-19, find the uncertainty in the x -intercept and the uncertainty in wt% Se.

19-20. pH measurement in living cells. A fluorescent indicator called C-SNARF-1 has acidic (HIn) and basic (In^-) forms with different emission spectra.



The figure shows emission from the indicator inside human lymphoblastoid cells and emission from cells in the absence of indicator. In a separate experiment, emission from pure HIn (in acidic solution) and pure In^- (in basic solution) at equal concentrations was measured. Results are shown in the following table.



Fluorescence of C-SNARF-1 dissolved in human lymphoblastoid cells and from cells that lack indicator. Emission spectra of HIn and In^- are superimposed with the correct intensities so that intensities from HIn , In^- , and pure cells add up to emission from cells + indicator. [From A.-C. Ribou, J. Vigo, and J.-M. Salmon, *J. Chem. Ed.* **2002**, 79, 1471.]

Emitting substance	Fluorescence intensity (arbitrary units)		
	590 nm	625 nm	Intensity ratio (I_{590}/I_{625})
Cells + indicator	269	258	
Cells only	13	21	
Difference	256	237	1.08 ₀ ≡ R
HIn	14 780	4 700	3.14 ₅ ≡ R _{HIn}
In ⁻	3 130	9 440	0.332 ≡ R _{In^-}

Emission intensities are additive. At wavelengths $\lambda' = 590$ nm and $\lambda'' = 625$ nm, we can write

$$I' = a'_{\text{HIn}} [\text{HIn}] + a'_{\text{In}^-} [\text{In}^-] \quad (\text{A})$$

$$I'' = a''_{\text{HIn}} [\text{HIn}] + a''_{\text{In}^-} [\text{In}^-] \quad (\text{B})$$

where the coefficients a relate emission intensity to concentration. We designate the ratio of emission intensities from the unknown mixture of $\text{HIn} + \text{In}^-$ dissolved in the cells as $R = I'/I''$. Similarly, the ratios for HIn and In^- are designated R_{HIn} and R_{In^-} . Values are given in the table. We can rearrange the simultaneous equations A and B to find

$$\frac{[\text{In}^-]}{[\text{HIn}]} = \left(\frac{R - R_{\text{HIn}}}{R_{\text{In}^-} - R} \right) \frac{a''_{\text{HIn}}}{a''_{\text{In}^-}} \quad (\text{C})$$

The quotient $a''_{\text{HIn}}/a''_{\text{In}^-}$ is the ratio of emission from HIn and In^- at wavelength $\lambda'' = 625$ nm when they are at equal concentration. From the table, this quotient is $a''_{\text{HIn}}/a''_{\text{In}^-} = 4 700/9 440 = 0.497_9$. The wavelengths 590 and 625 were chosen so that ratios in Equation C can be measured with some accuracy. Find the quotient $[\text{In}^-]/[\text{HIn}]$ inside the cells and from this quotient find the pH inside the cells.

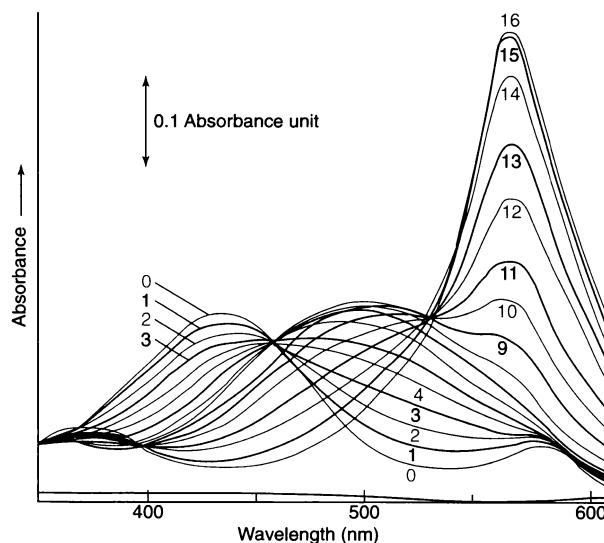
19-21. Explain how an enzyme-linked immunoassay (ELISA) amplifies the response to analyte.

19-22. Greenhouse gas reduction. An 18-W compact fluorescent bulb produces approximately the same amount of light as a 75-W incandescent bulb that screws into the same socket. The fluorescent bulb lasts $\sim 10\ 000$ h and the incandescent bulb lasts ~ 750 h. Over the lifetime of the fluorescent bulb, the electricity savings is $(75 - 18\text{ W})(10^4\text{ h}) = 570\text{ kW} \cdot \text{h}$. One kilogram of coal produces $\sim 2\text{ kW} \cdot \text{h}$ of electricity. If coal contains 60 wt% carbon, how many more kilograms of CO_2 are produced by running the incandescent bulb rather than the fluorescent bulb? If the coal contains 2 wt% sulfur, how many more kilograms of SO_2 are produced?

How Would You Do It?

19-23. The metal ion indicator xylenol orange (Table 13-2) is yellow at pH 6 ($\lambda_{\text{max}} = 439 \text{ nm}$). The spectral changes that occur as VO^{2+} (vanadyl ion) is added to the indicator at pH 6 are shown in the figure. The mole ratio $\text{VO}^{2+}/\text{xylene orange}$ at each point is shown in the following table. Suggest a sequence of chemical reactions to explain the spectral changes, especially the isosbestic points at 457 and 528 nm.

Trace	Mole ratio	Trace	Mole ratio	Trace	Mole ratio
0	0	6	0.60	12	1.3
1	0.10	7	0.70	13	1.5
2	0.20	8	0.80	14	2.0
3	0.30	9	0.90	15	3.1
4	0.40	10	1.0	16	4.1
5	0.50	11	1.1		

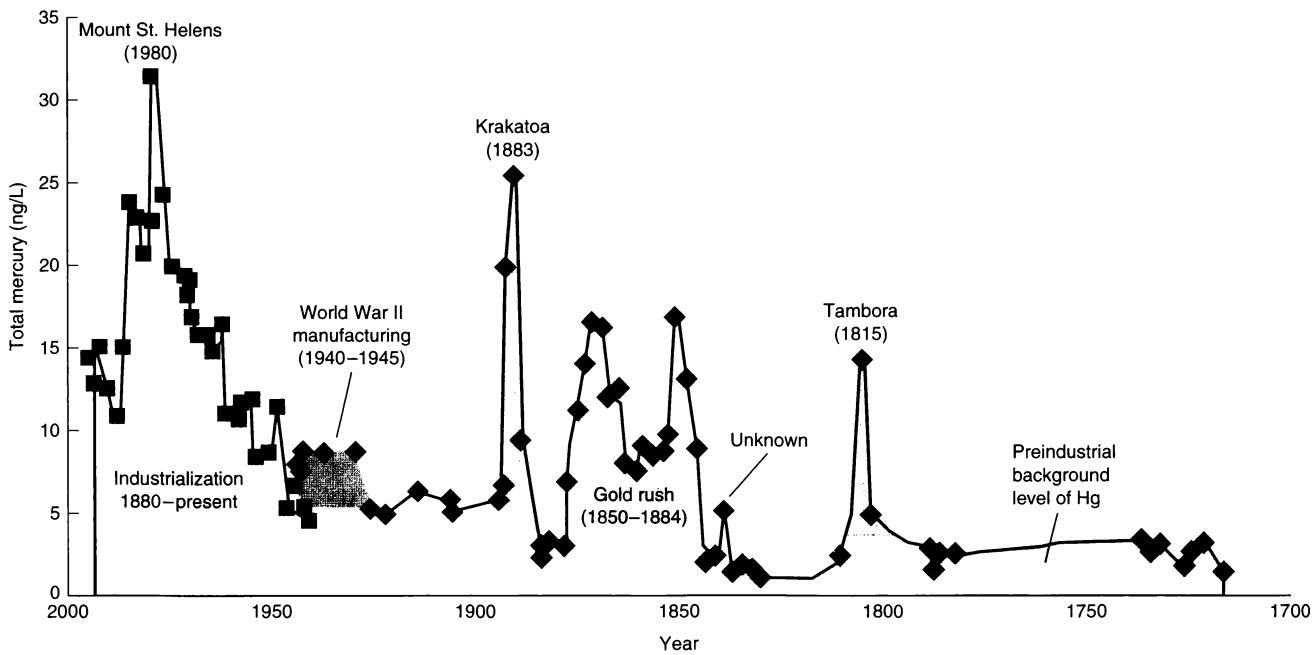


Titration of xylenol orange with VO^{2+} at pH 6.0. [From D. C. Harris and M. H. Gelb, *Biochim. Biophys. Acta*, **1980**, 623, 1.]

Notes and References

1. J. A. DeLuca, *J. Chem. Ed.* **1980**, 57, 541.
2. Demonstrations with a spectrophotometer and fiber-optic probe: J. P. Blitz, D. J. Sheeran, and T. L. Becker, "Classroom Demonstrations of Concepts in Molecular Fluorescence," *J. Chem. Ed.* **2006**, 83, 758. More fluorescent objects: A. MacCormac, E. O'Brien, and R. O'Kennedy, *J. Chem. Ed.* **2010**, 87, 685.
3. R. B. Weinberg, *J. Chem. Ed.* **2007**, 84, 797. Demonstration of fluorescence-quenching clock reaction with laundry detergent and household chemicals.
4. C. Salter, K. Range, and G. Salter, *J. Chem. Ed.* **1999**, 76, 84.

Historical Record of Mercury in the Snow Pack

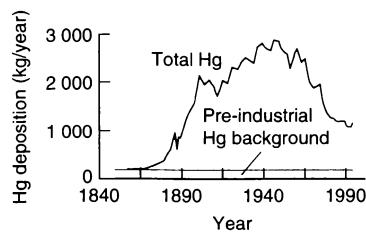


Mercury in Upper Fremont Glacier.
[From P. F. Schuster, D. P. Krabbenhoft, D. L. Naftz, L. D. Cecil, M. L. Olson, J. F. Dewild, D. D. Susong, J. R. Green, and M. L. Abbott, *Environ. Sci. Technol.* 2002, 36, 2303.]

Mercury at parts per trillion (ng/L) levels in glacial ice drilled to a depth of 160 m in Wyoming provides a record of events since the year 1720. Calibration of depths for the years 1958 and 1963 was determined by radioactive debris from nuclear bomb tests. Depths for 1815 and 1883 were found from peaks in electrical conductivity from acid produced by the volcanoes Krakatoa and Tambora.

Anthropogenic Hg is observed in 1850–1884 from the California gold rush when tons of Hg were used to extract Au from ore. Use of Hg for recovering Au was limited in 1884. High concentrations of Hg in the twentieth century are attributed to burning of coal, waste incineration, and use of Hg to manufacture Cl₂ in the chlor-alkali process. Atmospheric Hg has declined from its peak since Hg use was limited by international agreements late in the twentieth century. Coal burning remains a major source of Hg. Studies of “pristine” locations identify atmospheric deposition as the source of methylmercury (CH₃Hg⁺) in fish.^{1,2} High levels of mercury led to warnings to limit the quantity of fish in our diets.

Hg from melted ice was measured by reduction to Hg(0), which was purged from solution by bubbling Ar gas. Hg(g) was trapped by metallic Au coated on sand. (Mercury is soluble in gold.) For analysis, the trap was heated to liberate Hg, which passed into a cuvet. The cuvet was irradiated with a mercury lamp, and fluorescence from Hg vapor was observed. The detection limit was 0.04 ng/L. Blanks prepared by performing all steps with pure water in place of melted glacier had 0.66 ± 0.25 ng Hg/L, which was subtracted from glacier readings. All steps in trace analysis are carried out in a scrupulously clean environment.



Mercury flux to Lake Michigan estimated from sediment cores. Most Hg is deposited from the air and is due to anthropogenic sources such as burning of coal. [From R. Rossmann, *Environ. Sci. Technol.* 2010, 44, 935.]

Atomic Spectroscopy

Atomic spectroscopy is a principal tool for measuring metallic elements at major and trace levels in industrial and environmental laboratories. With an autosampler to feed in new samples automatically, each instrument can turn out hundreds of analyses per day.

20-1 What Is Atomic Spectroscopy?

In the atomic spectroscopy experiment in Figure 20-1, a liquid sample is *aspirated* (sucked) through a plastic tube into a flame that is hot enough to break molecules apart into atoms. The concentration of an element in the flame is measured by absorption or emission of radiation. For **atomic absorption spectroscopy**, radiation of the correct frequency is passed through the flame (Figure 20-2) and the intensity of transmitted radiation is measured. For **atomic emission spectroscopy**, no lamp is required. Radiation is emitted by hot atoms whose electrons have been promoted to excited states in the flame. For both experiments in Figure 20-2, a monochromator selects the wavelength that will reach the detector. Analyte concentrations at the parts per million level are measured with a precision of 2%. To analyze major constituents, a sample must be diluted to reduce concentrations to the ppm level. Box 20-1 describes an application of atomic emission for space exploration.

Molecules in solution typically have absorption and emission bands that are \sim 10 to 100 nm wide (Figure 19-22). In contrast, gaseous atoms in a flame have extremely sharp lines with widths of 10^{-3} to 10^{-2} nm (Figure 20-3). Because the lines are so sharp, there could be little overlap between spectra of different elements.

Atomic spectroscopy

- absorption (requires lamp with light that is absorbed by atoms)
- emission (luminescence from excited atoms—no lamp required)

Parts per million (ppm) means micrograms of solute per gram of solution. The density of dilute aqueous solutions is close to 1.00 g/mL, so ppm usually refers to $\mu\text{g}/\text{mL}$. $1 \text{ ppm Fe} = 1 \mu\text{g Fe/mL} \approx 2 \times 10^{-5} \text{ M}$.

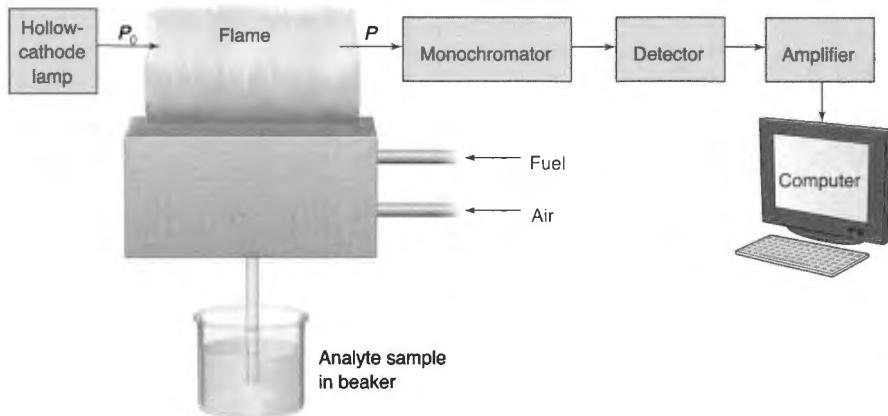


Figure 20-1 Atomic absorption experiment.

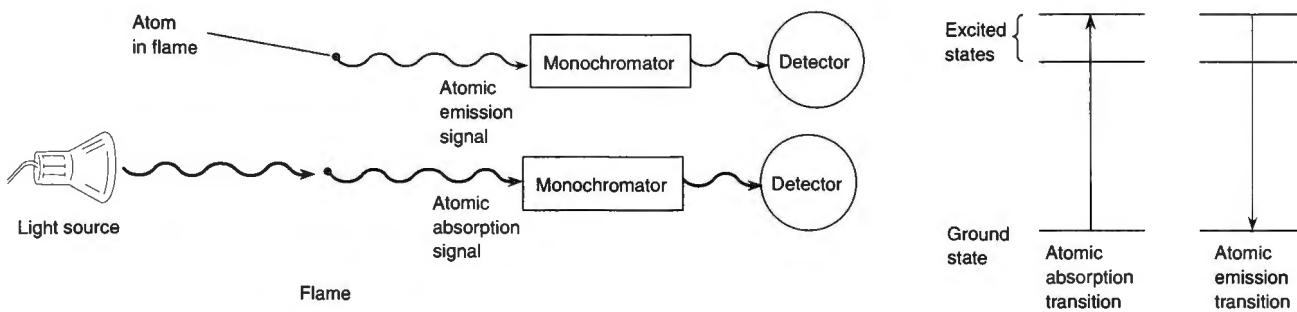


Figure 20-2 Absorption and emission of light by atoms in a flame. In atomic absorption, atoms absorb light from the lamp, decreasing the amount of light reaching the detector. In atomic emission, light is emitted by excited atoms in the flame.

in the sample. The lack of overlap allows some instruments to measure more than 70 elements simultaneously.

Ask Yourself

20-A. What is the difference between atomic absorption and atomic emission spectroscopy?

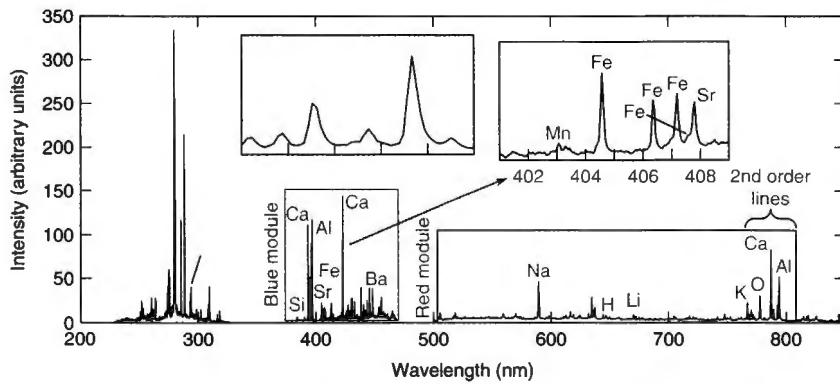
Box 20-1 Atomic Emission Spectroscopy on Mars



[Jean-luc LACOUR/2004.]

The *Mars Science Laboratory* rover, *Curiosity*, scheduled to begin exploring Mars in 2012, features a U.S./French *laser-induced breakdown spectroscopy* unit that

will measure the chemical composition of rocks and soil as far as seven meters away. Scientists on Earth select a target with the rover's high-resolution telescope. An infrared laser then fires a series of pulses through the telescope to vaporize a 0.5- to 1-mm-diameter area. Each pulse creates a luminous plasma of atoms from the irradiated surface. The first few pulses remove dust from the surface before subsequent pulses probe bulk material. Ultraviolet and visible emission from the plasma will be collected through the telescope and directed into three spectrographs that generate a profile of atomic composition of the irradiated area. Comparison with spectra of reference materials will enable scientists to identify minerals in the irradiated target.



Laser-induced breakdown spectrum of soil recorded at a distance of 5.3 m shows 15 major and trace elements.
[Courtesy Roger Weins, Los Alamos National Laboratory.]

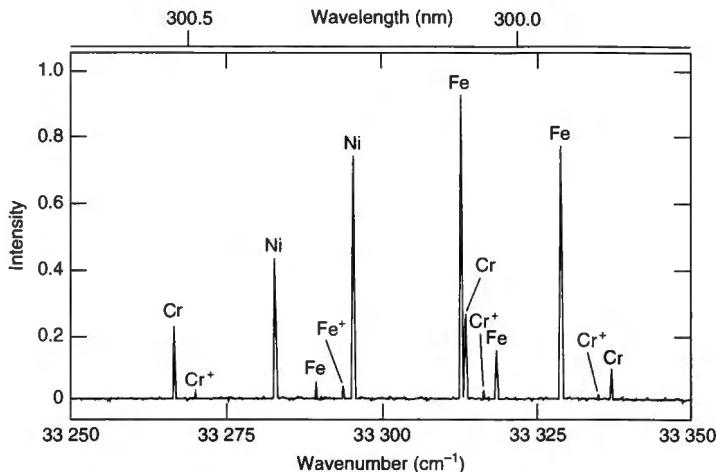


Figure 20-3 Part of the emission spectrum of a steel hollow-cathode lamp, showing sharp lines characteristic of gaseous Fe, Ni, and Cr atoms and weak lines from Cr⁺ and Fe⁺. Resolution is 0.001 nm, which is about half of the true linewidths of the signals. [From A. P. Thorne, *Anal. Chem.* **1991**, *63*, 57A.]

20-2 Atomization: Flames, Furnaces, and Plasmas

Atomization is the process of breaking analyte into gaseous atoms, which then are measured by their absorption or emission of radiation. Older atomic absorption spectrometers—and the apparatus often found in teaching laboratories—use a combustion flame to decompose analyte into atoms, as in Figure 20-1. Many instruments now employ an inductively coupled argon plasma or an electrically heated graphite tube (also called a graphite furnace) for atomization. The combustion flame and the graphite furnace are used for both atomic absorption and atomic emission measurements. The plasma is so hot that many atoms are in excited states and their emission is readily observed. Plasmas are used almost exclusively for atomic emission measurements. The newest technique found in industrial, environmental, and research laboratories is to atomize the sample with a plasma and measure the concentration of ions in the plasma with a *mass spectrometer*. This method does not involve absorption or emission of light and is not a form of spectroscopy.

Flame

Most flame spectrometers use a *premix burner*, such as that in Figure 20-4, in which the sample, oxidant, and fuel are mixed before being introduced into the flame. Sample solution is drawn in by rapid flow of oxidant and breaks into a fine mist when it leaves the tip of the *nebulizer* and strikes a glass bead. The formation of small droplets is termed *nebulization*. The mist flows past a series of baffles, which promote further mixing and block large droplets of liquid (which flow out to the drain). A fine mist containing about 5% of the initial sample reaches the flame. The remainder flows out the drain.

After solvent evaporates in the flame, the remaining sample vaporizes and decomposes to atoms. Many metal atoms (M) form oxides (MO) and hydroxides (MOH) as they rise through the flame. Molecules do not have the same spectra as atoms, so the atomic signal is lowered. If the flame is relatively rich in fuel (a “rich” flame), excess carbon species tend to reduce MO and MOH back to M and thereby increase sensitivity. The opposite of a rich flame is a “lean” flame, which has excess oxidant and is hotter. We choose lean or rich flames to provide optimum conditions for different elements.

Atomization method	Quantitation method
flame	absorption or emission
graphite furnace	absorption or emission
plasma	emission or mass spectrometry

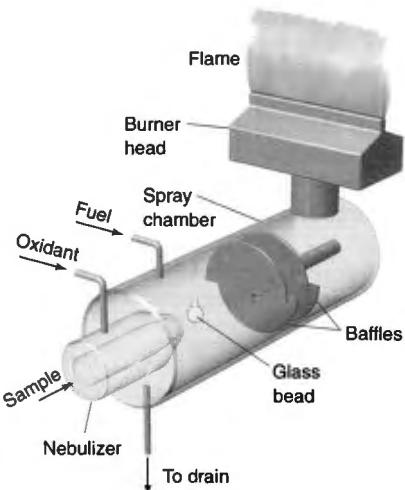


Figure 20-4 Premix burner with a pneumatic nebulizer. The slot in the burner head is typically 10 cm long and 0.5 mm wide.

Table 20-1 Maximum flame temperatures

Fuel	Oxidant	Temperature (K)
Acetylene	Air	2 400–2 700
Acetylene	Nitrous oxide	2 900–3 100
Acetylene	Oxygen	3 300–3 400
Hydrogen	Air	2 300–2 400
Hydrogen	Oxygen	2 800–3 000
Cyanogen	Oxygen	4 800

The most common fuel-oxidant combination is acetylene and air, which produces a flame temperature of 2 400–2 700 K (Table 20-1). When a hotter flame is required for *refractory* elements (those with high boiling points), acetylene and nitrous oxide is usually the mixture of choice. The height above the burner head at which maximum atomic absorption or emission is observed depends on the element being measured, as well as flow rates of sample, fuel, and oxidant. These parameters can be optimized for a given analysis.

Furnace

Furnaces offer increased sensitivity and require less sample than a flame.

Graphite is a form of carbon. It burns at high temperature in air:

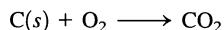
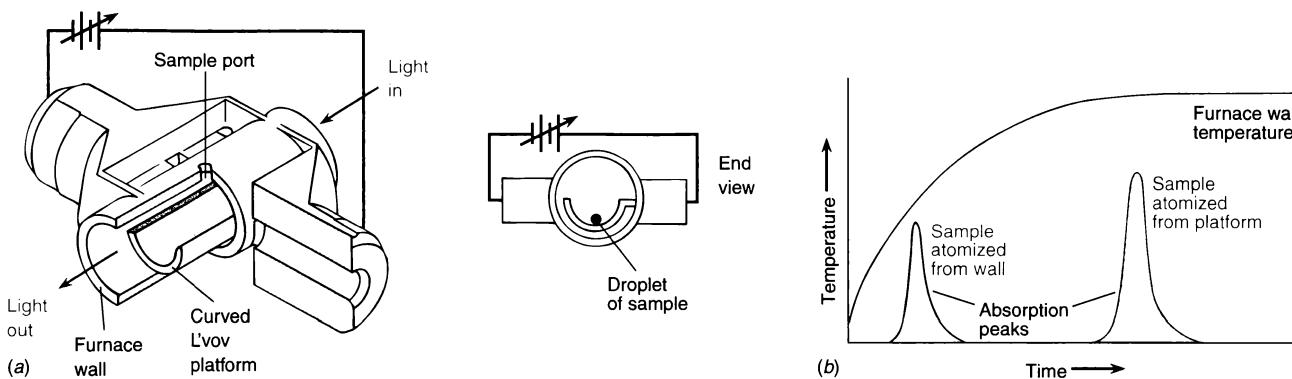


Figure 20-5 (a) Electrically heated graphite furnace for atomic spectroscopy. Sample is injected through the port at the top. L'vov platform inside the furnace is heated by radiation from the outer wall. Platform is attached to the wall by one small connection hidden from view. [Courtesy Perkin-Elmer Corp., Norwalk, CT.] (b) Heating profile comparing analyte evaporation from wall and from platform.

The electrically heated **graphite furnace** in Figure 20-5a provides greater sensitivity than a flame and requires less sample. A 1- to 100- μL sample is injected into the furnace through the hole at the center. The light beam travels through windows at each end of the tube. The maximum recommended temperature for a graphite furnace is 2 550°C for not more than 7 s. Surrounding the graphite with an atmosphere of Ar helps prevent oxidation of graphite.

A graphite furnace has high sensitivity because it confines atoms in the optical path for several seconds. In flame spectroscopy, the sample is diluted during nebulization, and its residence time in the optical path is only a fraction of a second. Flames require a sample volume of at least 10 mL, because sample is constantly flowing into the flame. The graphite furnace requires only tens of microliters. In an extreme case, when only nanoliters of kidney tubular fluid were available, a method was devised to reproducibly deliver 0.1 nL to a furnace for analysis of Na and K. Precision with a furnace is rarely better than 5–10% with manual sample injection, but automated injection improves reproducibility.

Sample is injected onto the *L'vov platform* inside the furnace in Figure 20-5a. Analyte does not vaporize until the furnace wall has reached constant temperature (Figure 20-5b). If sample were injected directly onto the inside wall of the furnace,



atomization would occur while the wall is heating up at a rate of 2 000 K/s (Figure 20-5b) and the signal would be less reproducible than with the platform.

A skilled operator must determine heating conditions for three or more steps to properly atomize a sample. To analyze Fe in the iron-storage protein ferritin, 10 μL of sample containing \sim 0.1 ppm Fe are injected into the cold graphite furnace. The furnace is programmed to *dry* the sample at 125°C for 20 s to remove solvent. Drying is followed by 60 s of *charring* (also called *pyrolysis*) at 1 400°C to destroy organic matter, which creates smoke that would interfere with the optical measurement. *Atomization* is then carried out at 2 100°C for 10 s, during which time absorbance reaches a maximum and then decreases as Fe evaporates from the furnace. The time-integrated absorbance (the peak area) is taken as the analytical signal. Finally, the furnace is heated to 2 500°C for 3 s to vaporize any residue.

The temperature needed to char the sample **matrix** (the medium containing the analyte) might also vaporize analyte. A *matrix modifier* is a chemical added to the sample to retard evaporation of analyte until the matrix has charred away. Alternatively, the matrix modifier might increase the evaporation of matrix and thereby reduce interference by the matrix during atomization. For example, the matrix modifier ammonium nitrate added to seawater reduces interference by NaCl. Figure 20-6a shows a graphite furnace heating profile used to analyze Mn in seawater. When 0.5 M NaCl is subjected to this profile, signals are observed in Figure 20-6b at the analytical wavelength of Mn. Much of the apparent absorbance is probably due to scattering of light by smoke created by heating NaCl. The NaCl signal at the start of atomization interferes with the measurement of Mn. Adding NH_4NO_3 to the sample in Figure 20-6c reduces the matrix signal by forming NH_4Cl and NaNO_3 , which cleanly evaporate instead of making smoke.

When using a graphite furnace, it is important to monitor the absorption signal as a function of time, as in Figure 20-6. Peak shapes help you to adjust time and temperature for each step to obtain a clean signal from analyte. Also, a graphite furnace has a finite lifetime. Degradation of peak shape, loss of precision, or a large change in the slope of the calibration curve tells you that it is time to change the furnace.

It is possible to *preconcentrate* a sample by injecting and evaporating multiple aliquots in the graphite furnace prior to charring and atomization. For example, to measure trace levels of arsenic in drinking water, a 30- μL aliquot of water plus matrix modifier was injected and evaporated. The procedure was repeated five more times so that the total sample was 180 μL . The detection limit for As was 0.3 $\mu\text{g/L}$ (parts per billion). Without preconcentration, the detection limit would have been approximately six times higher (1.8 $\mu\text{g/L}$). The increased capability with preconcentration is critical because As is a health hazard at concentrations of just a few parts per billion.

Inductively Coupled Plasma

The **inductively coupled plasma** in Figure 20-7 reaches a much higher temperature than that of combustion flames. Its high temperature and stability eliminate many problems encountered with conventional flames. The plasma's disadvantage is its expense to purchase and operate.

The plasma is energized by a radio-frequency induction coil wrapped around the quartz torch. High-purity argon gas plus analyte aerosol are fed into the torch. After a spark from a Tesla coil ionizes the Ar gas, free electrons accelerated by the radio-frequency field heat the gas to 6 000 to 10 000 K by colliding with atoms.

In a pneumatic nebulizer such as that in Figure 20-4, liquid sample is sucked in by the flow of gas and breaks into small drops. The concentration of analyte needed

The operator must determine reasonable time and temperature for each stage of the analysis. Once a program is established, it can be applied to similar samples.

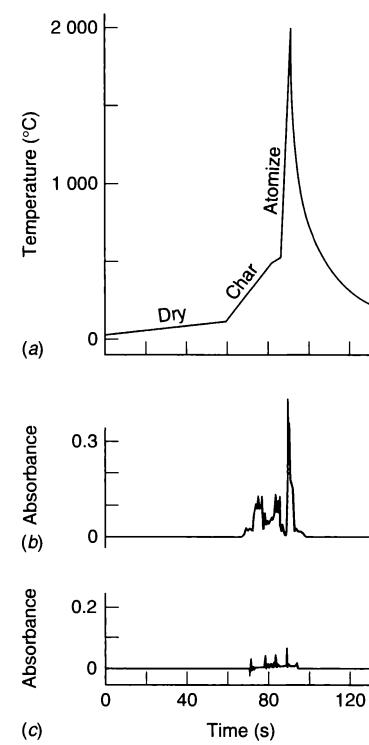


Figure 20-6 Reduction of interference by matrix modifier.
(a) Graphite furnace temperature profile for analysis of Mn in seawater.
(b) Absorbance profile when 10 μL of 0.5 M reagent grade NaCl are subjected to the temperature profile. Absorbance is monitored at the Mn wavelength of 279.5 nm with a bandwidth of 0.5 nm.
(c) Greatly reduced absorbance from 10 μL of 0.5 M NaCl plus 10 μL of 50 wt% NH_4NO_3 matrix modifier.
[From M. N. Quigley and F. Vernon, *J. Chem. Ed.* **1996**, 73, 980.]



Table 20-2 Comparison of detection limits for Ni^+ ion at 231 nm^a

Technique	Detection limits for different instruments (ng/mL)
Inductively coupled plasma–atomic emission (pneumatic nebulizer)	3–50
Inductively coupled plasma–atomic emission (ultrasonic nebulizer)	0.3–4
Graphite furnace–atomic absorption	0.02–0.06
Inductively coupled plasma–mass spectrometry	0.001–0.2

a. J. M. Mermet and E. Poussel, *Appl. Spectros.* 1995, 49, 12A.

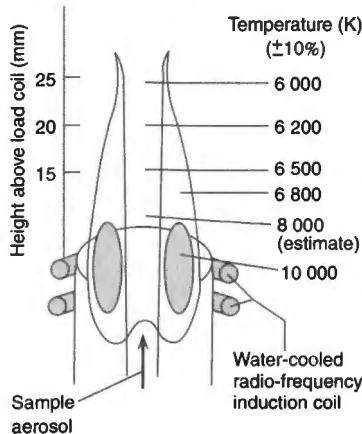


Figure 20-7 Temperature profile of a typical inductively coupled plasma used in analytical spectroscopy. [From V. A. Fassel, *Anal. Chem.* 1979, 51, 1290A.]

for adequate signal (Table 20-2) is reduced by an order of magnitude with the *ultrasonic nebulizer* in Figure 20-8, in which liquid sample is directed onto a quartz crystal oscillating at 1 MHz. The vibrating crystal creates a fine aerosol that is carried by a stream of Ar through a heated tube, where solvent evaporates. In the next cool zone, solvent condenses and is removed. Then the stream enters a desolvator containing a microporous polytetrafluoroethylene membrane in a chamber maintained at 160°C. Remaining solvent vapor diffuses through the membrane and is swept away by flowing Ar. Analyte reaches the plasma as an aerosol of dry, solid particles. Plasma energy is not wasted in evaporating solvent, so more energy is available for atomization. Also, a larger fraction of the original sample reaches the flame than with a conventional nebulizer. Detection limits for the inductively coupled

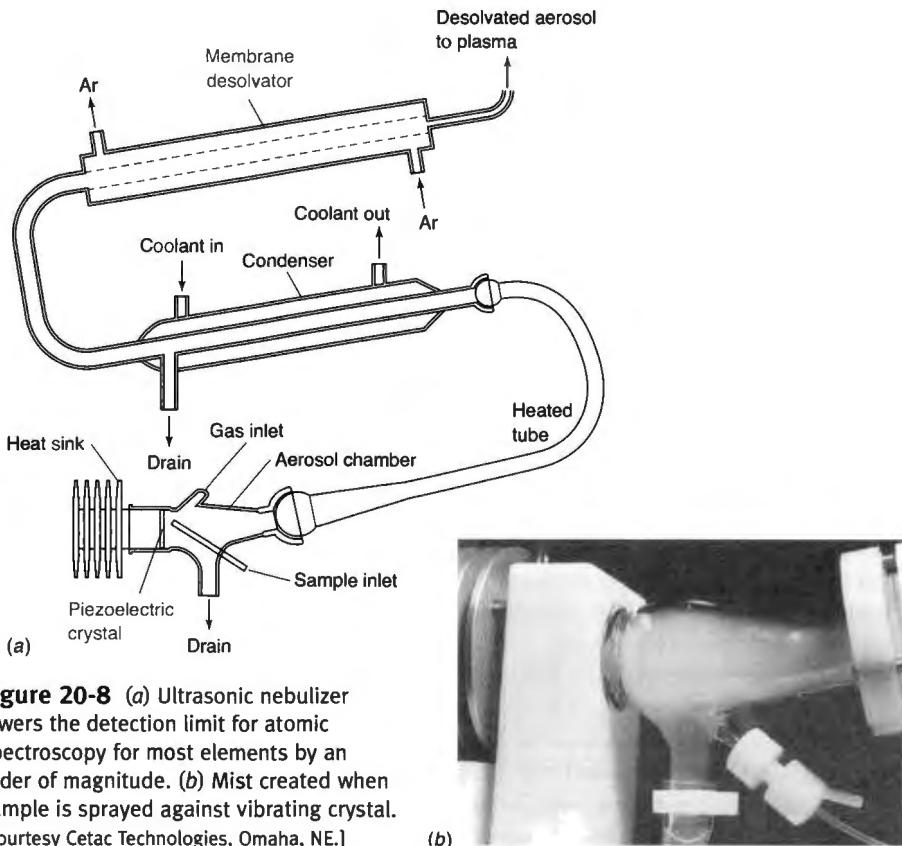


Figure 20-8 (a) Ultrasonic nebulizer lowers the detection limit for atomic spectroscopy for most elements by an order of magnitude. (b) Mist created when sample is sprayed against vibrating crystal. [Courtesy Cetac Technologies, Omaha, NE.]

Li	Be						B	C	N	O	F	Ne
0.7	0.07						I	10				
2	1						500	—				
0.1	0.02						15	—				
0.0002	0.0009						0.0008					
Na	Mg						Al	Si	P	S	Cl	Ar
3	0.08						2	5	7	3	60	
0.2	0.3						30	100	40 000	—	—	
0.005	0.004						0.01	0.1	30	—	—	
0.0002	0.0003						0.0002	<0.0001	0.0001			
K	Ca	Sc	Tl	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga
20	0.07	0.3	0.4	70	0.5	0.2	0.7	1	3	0.9	0.6	10
3	0.5	40	50	—	0.02	0.01	0.2	4	90	—	0.5	20
0.1	0.11	0.005	0.004	0.0003	0.0002	0.0002	0.008	0.002	11	0.02	0.001	60
0.0002	0.0002						0.0008	0.001	0.0005	0.003	0.006	200
Rb	Sr	V	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cr	In
7	1.2	0.6	5	20	—	—	20	10	2	0.4	20	40
7	2	200	1000	2000	—	20	60	10	2	0.4	20	40
0.05	0.1	0.003	0.003	0.0006	0.0008	0.002	1	—	0.3	0.005	0.003	40
0.0003	0.0003						0.001	0.0003	0.0007	0.0008	0.0003	30
Ca	Ba	La	Hf	Ts	W	Re	Os	Ir	Pt	Au	Hg	Tl
40	0.6	1	4	10	8	3	0.2	7	100	2	7	10
4	10	2000	2000	2000	1000	600	100	400	100	10	150	20
0.2	0.04	0.003	0.0003	0.0008	0.0005	0.002	0.0007	—	0.2	0.1	2	0.1
0.0003	0.0003						0.0004	0.001	0.0009	0.0009	0.0004	0.0006
Pb	Po						Bi					Rn
40	10	—					10	10	10	10	10	
4	10	—					7	—	—	—	—	
0.2	0.04	—					—	—	—	—	—	
0.0003	0.0003						—	—	—	—	—	

Ce 2 — 0.0003	Pr 9 6000	Nd 10 1000	Pm	Sm 10 1000	Eu 0.9 0.1	Gd 5 2000	Tb 6 500	Dy 2 30	Ho 2 40	Er 0.7 0.001	Tm 2 900	Yb 0.3 4	Lu 0.3 300
Th 7 — 0.0003	Pa U 60 40,000	Np — —	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	
0.0002	0.001			0.0004	0.001	0.0002	0.0009	0.0002	0.0007	0.0002	0.001	0.0002	

Detection limits (ng/mL)

– Inductively coupled plasma emission
Flame atomic absorption

- Flame atomic absorption
- Graphite furnace atomic absorption

Graphite furnace atomic absorption – Inductively coupled plasma–mass spectrometry

Requires N₂O/C₂H₂ flame and is therefore

Requires $\text{N}_2\text{O}/\text{C}_2\text{H}_2$ flame and is therefore better analyzed by inductively coupled plasma

Better analyzed by inductive methods.

plasma can be further improved by a factor of 3 to 10 by viewing emission along the length of the plasma, rather than at right angles to the plasma.

Detection Limits

The *detection limit* in Equation 5-4 is found by measuring the standard deviation of replicate samples whose analyte concentration is ~ 1 to 5 times the detection limit. Detection limits for a furnace are typically 100 times lower than those of a flame (Figure 20-9), because the sample is confined in a small volume for a relatively long time in the furnace.

$$\text{detection limit} \equiv \frac{3s}{m}$$

s = standard deviation of low concentration samples near the detection limit

m = slope of calibration curve



Ask Yourself

- 20-B.** **(a)** Why can we detect smaller samples with lower concentrations with a furnace than with a flame or a plasma?
(b) Tin can be leached (dissolved) from the tin-plated steel of a food can. A provisional tolerable weekly intake of tin in the diet is 14 mg Sn/kg body mass. How many kilograms of canned tomato juice will exceed the tolerable weekly intake for a 55-kg woman?

Tin in canned foods (mg Sn/kg food) measured by inductively coupled plasma

tomato juice	241	peach halves	58
grapefruit juice	182	chocolate drink	2
pineapple	114	chili con carne	2
fruit cocktail	73	Hungarian soup	2

From L. Perring and M. Basic-Dvorzak, *Anal. Bioanal. Chem.* **2002**, 374, 235.

20-3 How Temperature Affects Atomic Spectroscopy

Temperature determines the degree to which a sample breaks down to atoms and the extent to which an atom is found in its ground, excited, or ionized states. Each effect influences the strength of the observed signal.

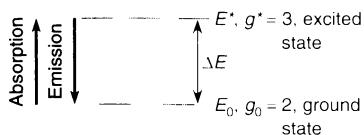


Figure 20-10 Two energy levels with degeneracies g_0 and g^* . Ground-state atoms can absorb light to be promoted to the excited state. Excited-state atoms can emit light to return to the ground state.

The Boltzmann Distribution

Consider a molecule with two energy levels (Figure 20-10) separated by energy ΔE . Call the lower level E_0 and the upper level E^* . An atom (or molecule) may have more than one state available at a given energy. In Figure 20-10, we show three states at E^* and two at E_0 . The number of states at each energy is called the *degeneracy*. Let the degeneracies be g_0 and g^* .

The **Boltzmann distribution** describes the relative populations of different states at thermal equilibrium. If equilibrium exists (which is not true in all parts of a flame), the relative population (N^*/N_0) of any two states is

$$\text{Boltzmann distribution: } \frac{N^*}{N_0} = \left(\frac{g^*}{g_0} \right) e^{-\Delta E/kT} \quad (20-1)$$

where T is temperature (K), k is Boltzmann's constant (1.381×10^{-23} J/K), and e is the base of the natural logarithm (2.718 ...).

The Effect of Temperature on the Excited-State Population

The lowest excited state of a sodium atom lies 3.371×10^{-19} J/atom above the ground state. The degeneracy of the excited state is 2, whereas that of the ground state is 1. Let's calculate the fraction of Na atoms in the excited state in an acetylene-air flame at 2 600 K:

$$\frac{N^*}{N_0} = \left(\frac{2}{1} \right) e^{-(3.371 \times 10^{-19} \text{ J}) / [(1.381 \times 10^{-23} \text{ J/K})(2 600 \text{ K})]} = 0.000 167$$

Fewer than 0.02% of the atoms are in the excited state.

How would the fraction of atoms in the excited state change if the temperature were 2 610 K instead?

$$\frac{N^*}{N_0} = \left(\frac{2}{1} \right) e^{-(3.371 \times 10^{-19} \text{ J}) / [(1.381 \times 10^{-23} \text{ J/K})(2 610 \text{ K})]} = 0.000 174$$

The fraction of atoms in the excited state is still less than 0.02%, but that fraction has increased by $[(1.74 - 1.67)/1.67] \times 100 = 4\%$.

The Effect of Temperature on Absorption and Emission

We see that 99.98% of the sodium atoms are in their ground state at 2 600 K. *Varying the temperature by 10 K hardly affects the ground-state population and would not noticeably affect the signal in atomic absorption.*

How would emission intensity be affected by a 10-K rise in temperature? In Figure 20-10, we see that absorption arises from ground-state atoms, but emission arises from excited-state atoms. Emission intensity is proportional to the population of the excited state. *Because the excited-state population changes by 4% when the temperature rises 10 K, the emission intensity rises by 4%.* It is critical in atomic emission spectroscopy that the flame temperature be very stable, or the emission intensity will vary. In atomic absorption spectroscopy, flame temperature variation is not as critical.

The inductively coupled plasma is almost always used for emission, not absorption, because it is so hot that a substantial fraction of atoms and ions are excited and

A 10-K temperature rise changes the excited-state population by 4% in this example.

Atomic absorption is not as sensitive as atomic emission to temperature variation.

Table 20-3 Effect of energy separation and temperature on population of excited states

Wavelength separation of states (nm)	Energy separation of states (J)	Excited-state fraction (N^*/N_0) ^a	
		2 500 K	6 000 K
250	7.95×10^{-19}	1.0×10^{-10}	6.8×10^{-5}
500	3.97×10^{-19}	1.0×10^{-5}	8.3×10^{-3}
750	2.65×10^{-19}	4.6×10^{-4}	4.1×10^{-2}

a. Based on the equation $N^*/N_0 = (g^*/g_0)e^{-\Delta E/kT}$ in which $g^* = g_0 = 1$.

because no lamp is required for emission measurements. Table 20-3 compares excited-state populations for a flame at 2 500 K and a plasma at 6 000 K. Although the fraction of excited atoms is small, each atom emits many photons per second because it is rapidly promoted back to the excited state by collisions.

Ask Yourself

- 20-C. (a) A ground-state atom absorbs light of wavelength 400 nm to be promoted to an excited state. Find the energy difference (in joules) between the two states.
 (b) If both states have a degeneracy of $g_0 = g^* = 1$, find the fraction of excited-state atoms (N^*/N_0) at thermal equilibrium at 2 500 K.

20-4 Instrumentation

Requirements for atomic absorption were shown in Figure 20-1. Principal differences between atomic and solution spectroscopy lie in the light source, the sample container (the flame, furnace, or plasma), and the need to subtract background emission from the observed signal.

The Linewidth Problem

For absorbance to be proportional to analyte concentration, the linewidth of radiation being measured must be substantially narrower than the linewidth of the absorbing atoms. Atomic absorption lines are very sharp, with an inherent width of $\sim 10^{-4}$ nm.

Two mechanisms broaden atomic spectra. One is the *Doppler effect*, in which an atom moving toward the lamp samples the oscillating electromagnetic wave more frequently than one moving away from the lamp (Figure 20-11). That is, an atom moving toward the source “sees” higher frequency light than that encountered by one moving away. Linewidth is also affected by *pressure broadening* from collisions between atoms. Colliding atoms absorb a broader range of frequencies than do isolated atoms. Broadening is proportional to pressure. The Doppler effect and *pressure broadening* are similar in magnitude and yield linewidths of 10^{-3} to 10^{-2} nm in atomic spectroscopy.

Hollow-Cathode Lamps

To produce narrow lines of the correct frequency for atomic absorption, we use a **hollow-cathode lamp** whose cathode is made from the element we want to observe.

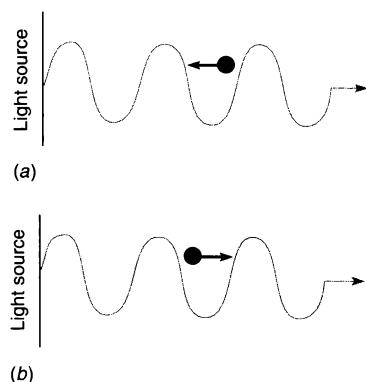


Figure 20-11 The Doppler effect. A molecule moving (a) toward the radiation source “feels” the electromagnetic field oscillate more often than one moving (b) away from the source.

The linewidth of the source must be narrower than the linewidth of the atomic vapor for Beer’s law (Section 18-2) to be obeyed. The terms “linewidth” and “bandwidth” are used interchangeably, but “lines” are narrower than “bands.”

Doppler and pressure effects broaden atomic lines by 1–2 orders of magnitude relative to their inherent linewidths.

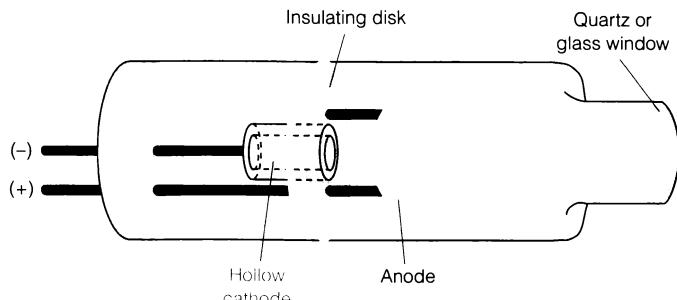


Figure 20-12 A hollow-cathode lamp.

The lamp in Figure 20-12 is filled with Ne or Ar at a pressure of 130–700 Pa. A high voltage between the anode and cathode ionizes the gas and accelerates cations toward the cathode. Ions striking the cathode “sputter” atoms from the metallic cathode into the gas phase. Gaseous metal atoms excited by collisions with high-energy electrons emit photons to return to the ground state. Atomic radiation shown in Figure 20-3 has the same frequency as that absorbed by atoms in the flame or furnace. The width of lamp emission in Figure 20-13 is sufficiently narrow for Beer’s law to hold. *A lamp with matching cathode material is required for each element.*

Background Correction

Background signal arises from absorption, emission, or scatter by the flame, plasma, or furnace, and from everything in the sample besides analyte (the *matrix*).

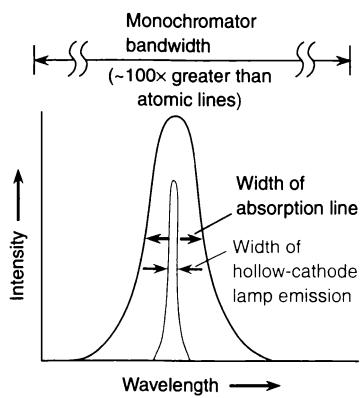


Figure 20-13 Relative linewidths of hollow-cathode emission, atomic absorption, and a monochromator. The linewidth from the hollow cathode is narrowest because gas temperature in the lamp is lower than flame temperature (so there is less Doppler broadening) and the pressure in the lamp is lower than flame pressure (so there is less pressure broadening).

Atomic spectroscopy requires **background correction** to distinguish analyte signal from absorption, emission, and optical scattering by the sample matrix, the flame, plasma, or white-hot graphite furnace. For example, Figure 20-14 shows the absorption spectrum of Fe, Cu, and Pb in a graphite furnace. Sharp atomic signals with maximum absorbance near 1.0 are superimposed on a flat background absorbance of 0.3. If we did not subtract background absorbance, significant errors would result. Background correction is most critical for graphite furnaces, which tend to be filled with smoke from the charring step. Optical scatter from smoke must somehow be distinguished from optical absorption by analyte.

For atomic absorption, **Zeeman background correction** (pronounced ZAY-man) is most commonly found in modern instruments for research and industrial laboratories. When a strong magnetic field is applied parallel to the light path through a flame or furnace, the absorption (or emission) lines of the atoms are split into

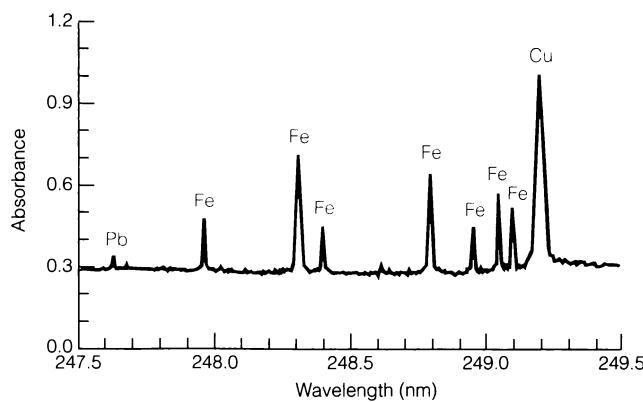
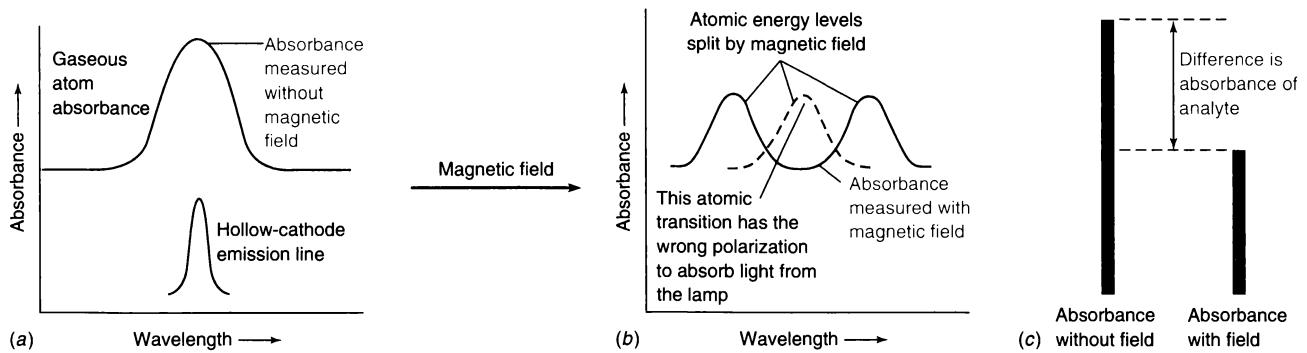


Figure 20-14 Graphite furnace absorption spectrum of bronze dissolved in HNO₃. Notice the high, constant background absorbance of 0.3 in this narrow region of the spectrum. [From B. T. Jones, B. W. Smith, and J. D. Winefordner, *Anal. Chem.* **1989**, 61, 1670.]



three components (Figure 20-15). Two are shifted to slightly lower and higher wavelengths. The third component is unshifted, but has the wrong electromagnetic polarization to absorb light from the lamp. Therefore analyte absorption decreases markedly in the presence of the magnetic field. For Zeeman background correction, the magnetic field is pulsed on and off. Absorption from sample plus background is observed when the field is off and absorption from background alone is observed when the field is on. The difference between the two absorbances is due to analyte.

Atomic emission spectrometers provide background correction by measuring the signal at the peak emission wavelength and then at wavelengths slightly above and slightly below the peak, where the signal has returned to background level. The mean signal on either side of the peak is subtracted from the peak signal.

Multielement Analysis with the Inductively Coupled Plasma

Inductively coupled plasma atomic emission is more versatile than atomic absorption because emission does not require a lamp for each element. An element emits light at many characteristic frequencies. As many as 70 elements can be measured with simultaneous measurement of emission from each different element. In Color Plate 21, atomic emission entering from the top right is dispersed in the vertical plane by a prism and in the horizontal plane by a grating. The radiation forms a two-dimensional pattern that lands on a semiconductor detector similar to that in a digital camera. Each pixel receives a different wavelength and therefore responds to a different element.

The spectrometer can be purged with N₂ or Ar to exclude O₂, thereby allowing ultraviolet wavelengths in the 100- to 200-nm range to be observed. This spectral region permits more sensitive detection of some elements that are normally detected at longer wavelengths and allows halogens, P, S, and N to be measured (with poor detection limits of tens of parts per million). These elements cannot be observed at wavelengths above 200 nm. In one application, N in fertilizers is measured along with other major elements. The plasma torch is specially designed to be purged with Ar to exclude N₂ from air. Unknowns are purged with He to remove dissolved air. Emission from N is observed near 174 nm.



Ask Yourself

20-D. How is background correction accomplished in atomic absorption and atomic emission spectroscopy?

Figure 20-15 Principle of Zeeman background correction for atomic absorption spectroscopy. (a) In the absence of a magnetic field, we observe the sum of the absorbances of analyte and background. (b) In the presence of a magnetic field, the analyte absorbance is split away from the hollow cathode wavelength and the absorbance is due to background only. (c) The desired signal is the difference between those observed without and with the magnetic field.

Comparison of methods:

Flame atomic absorption

- lowest cost equipment
- different lamp required for each element
- poor sensitivity

Furnace atomic absorption

- expensive equipment
- different lamp required for each element
- high background signals
- very high sensitivity

Plasma emission

- expensive equipment
- no lamps
- low background and low interference
- moderate sensitivity

Inductively coupled plasma-mass spectrometry

- very expensive equipment
- no lamps
- least background and interference
- highest sensitivity

20-5 Interference

Types of interference:

- **spectral:** unwanted signals overlap analyte signal
- **chemical:** chemical reactions decrease the concentration of analyte atoms
- **ionization:** ionization of analyte atoms decreases the concentration of neutral atoms

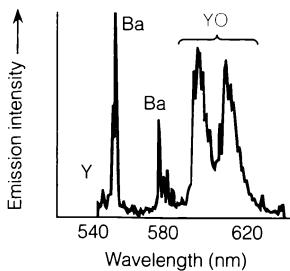


Figure 20-16 Emission from a plasma produced by laser irradiation of the high-temperature superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$. Solid is vaporized by the laser, and excited atoms and molecules in the gas phase emit light at characteristic wavelengths. [From W. A. Weimer, *Appl. Phys. Lett.* **1988**, 52, 2171.]

Interference is any effect that changes the signal when analyte concentration remains unchanged. Interference can be corrected by counteracting the source of interference or by preparing standards that exhibit the same interference.

Types of Interference

Spectral interference occurs when analyte signal overlaps signals from other species in the sample or signals due to the flame or furnace. Interference from the flame is subtracted by background correction. The best means of dealing with overlap between lines of different elements in the sample is to choose another wavelength for analysis. The spectrum of a molecule is much broader than that of an atom, so spectral interference can occur at many wavelengths. Figure 20-16 shows an example of a plasma containing Y and Ba atoms in addition to YO molecules. Elements that form stable oxides in the flame commonly give spectral interference.

Chemical interference is caused by any substance that decreases the extent of atomization of analyte. For example, SO_4^{2-} and PO_4^{3-} hinder the atomization of Ca^{2+} , perhaps by forming nonvolatile salts. *Releasing agents* can be added to a sample to decrease chemical interference. EDTA and 8-hydroxyquinoline protect Ca^{2+} from the interfering effects of SO_4^{2-} and PO_4^{3-} . La^{3+} is also a releasing agent, apparently because it preferentially reacts with PO_4^{3-} and frees the Ca^{2+} . A fuel-rich flame reduces oxidized analyte species that would otherwise hinder atomization. Higher flame temperatures eliminate many kinds of chemical interference.

Ionization interference is a problem in the analysis of alkali metals, which have the lowest ionization potentials. For any element, we can write a gas-phase ionization reaction:



At 2 450 K and a pressure of 0.1 Pa, Na is 5% ionized. With its lower ionization potential, K is 33% ionized under the same conditions. Ionized atoms have energy levels different from those of neutral atoms, so the desired signal is decreased.

An *ionization suppressor* is an element added to a sample to decrease the ionization of analyte. For example, 1 mg/mL of CsCl is added to the sample for the analysis of potassium, because cesium is more easily ionized than potassium. By producing a high concentration of electrons in the flame, ionization of Cs reverses Reaction 20-2 for K. This reversal is an example of Le Châtelier's principle.

The *method of standard addition*, described in Section 5-3, compensates for many types of interference by adding known quantities of analyte to the unknown in its complex matrix. For example, Figure 20-17 shows the analysis of strontium in aquarium water by standard addition. The slope of the standard addition curve is 0.018 8 absorbance units/ppm. If, instead, Sr is added to distilled water, the slope is 0.030 8 absorbance units/ppm. That is, in distilled water, the absorbance increases $0.030\ 8 / 0.018\ 8 = 1.64$ times more than it does in aquarium water for each addition of standard Sr. We attribute the lower response in aquarium water to interference by other species that are present. The negative *x*-intercept of the standard addition curve, 7.41 ppm, is a reliable measure of Sr in the aquarium. If we had just measured the atomic absorption of Sr in aquarium water and used the calibration curve for distilled water, we would have overestimated the Sr concentration by 64%.

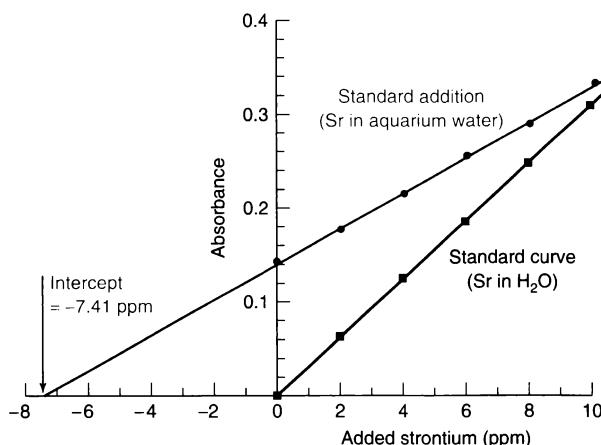


Figure 20-17 Atomic absorption calibration curve for Sr added to distilled water and standard addition of Sr to aquarium water. All solutions are made up to a constant volume, so the abscissa is the concentration of added Sr in the final solution, as in Figure 5-7. [Data from L. D. Gilles de Pelichy, C. Adams, and E. T. Smith, *J. Chem. Ed.* **1997**, 74, 1192.]

Virtues of the Inductively Coupled Plasma

An Ar plasma eliminates common interferences. The plasma is twice as hot as a conventional flame, and the residence time of analyte in the plasma is about twice as long. Atomization is more complete, and the signal is correspondingly enhanced. Formation of analyte oxides and hydroxides is negligible. The plasma is relatively free of background radiation.

In flame emission spectroscopy, the concentration of electronically excited atoms in the cooler, outer part of the flame is lower than in the warmer, central part of the flame. Emission from the central region is absorbed in the outer region. This *self-absorption* increases with increasing concentration of analyte and gives nonlinear calibration curves. In a plasma, the temperature is more uniform, and self-absorption is not nearly so important. Table 20-4 states that plasma emission calibration curves are linear over five orders of magnitude compared with just two orders of magnitude for flames and furnaces.

Self-absorption: ground-state atoms in cooler, outer part of flame absorb emission from excited atoms at center of flame, thereby decreasing overall emission; higher analyte concentration gives higher self-absorption and a nonlinear calibration curve

Table 20-4 Comparison of atomic analysis methods^a

	Flame absorption	Furnace absorption	Plasma emission	Plasma-mass spectrometry
Detection limits (ng/g)	10–1 000	0.01–1	0.1–10	0.000 01–0.000 1
Linear range	10 ²	10 ²	10 ⁵	10 ⁸
Precision				
Short term (5–10 min)	0.1–1%	0.5–5%	0.1–2%	0.5–2%
Long term (hours)	1–10%	1–10%	1–5%	<5%
Interferences				
Spectral	Very few	Very few	Many	Few
Chemical	Many	Very many	Very few	Some
Mass	—	—	—	Many
Sample throughput	10–15 s/element	3–4 min/element	6–60 elements/min	All elements in 2–5 min
Dissolved solid	0.5–5%	>20% slurries and solids	1–20%	0.1–0.4%
Sample volume	Large	Very small	Medium	Medium
Purchase cost	1	2	4–9	10–15

^a Adapted from TJA Solutions, Franklin, MA.

Ask Yourself

20-E. What is meant by (a) spectral, (b) chemical, and (c) ionization interference? (d) The Pb content of archaeological human skeletons measured by graphite furnace atomic absorption sheds light on customs and economic status of individuals in earlier times. For example, in colonial America, wealthy people used British pewter containing ~20 wt% lead for tableware, goblets, and beverage storage containers. Plantation owners absorbed lead from food in pewter vessels. Slaves and servants with little access to pewter absorbed much less lead.³ Bone consists of the protein collagen and the mineral hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Bone is prepared for analysis by heating in air to oxidize organic matter and leave behind inorganic oxides (called *ash*). Explain why La^{3+} is added to dissolved bone ash to suppress matrix interference in Pb analysis.

20-6 Inductively Coupled Plasma–Mass Spectrometry

Inductively coupled plasma–mass spectrometry is one of the most sensitive techniques available for trace analysis. Analyte ions produced in the plasma are directed into the inlet of a mass spectrometer, which separates ions by their mass-to-charge ratio. Ions are measured with a sensitive detector that is similar to a photomultiplier tube. The linear range listed in Table 20-4 extends over eight orders of magnitude, and the detection limit is 100–1 000 times lower than that of furnace atomic absorption.

Figure 20-18 shows an example in which coffee beans were extracted with trace-metal-grade nitric acid and the aqueous extract was analyzed by inductively coupled plasma–mass spectrometry. Coffee brewed from Cuban or Hawaiian beans contained ~15 ng Pb/mL. However, the Cuban beans also contained Hg at a concentration similar to that of Pb.

A problem that is unique to mass spectrometry is **isobaric interference** in which ions of similar mass-to-charge ratio cannot be distinguished from each other. For example, ${}^{40}\text{Ar}^{16}\text{O}^+$ found in an Ar plasma has nearly the same mass as ${}^{56}\text{Fe}^+$. Doubly

Ar is an “inert” gas with virtually no chemistry. However, Ar^+ has the electronic configuration of Cl, and its chemistry is similar to that of halogens. ${}^{40}\text{Ar}^{16}\text{O}^+$ and ${}^{56}\text{Fe}^+$ differ by 0.02 atomic mass units.

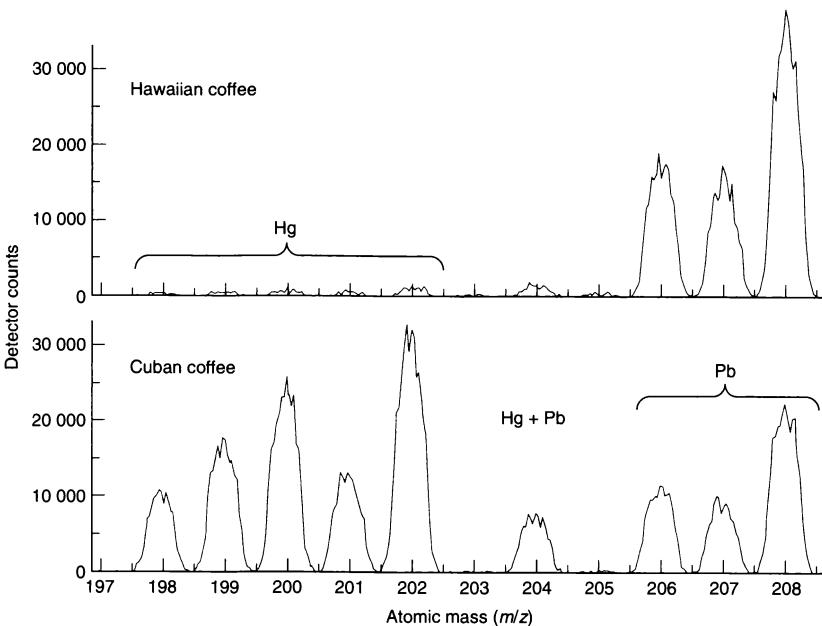


Figure 20-18 Partial elemental profile of coffee beans by inductively coupled plasma–mass spectrometry. Both beans had similar Pb content, but the Cuban beans have a much higher Hg content than the Hawaiian beans. A blank has not been subtracted from either spectrum, so the small amount of Hg in the upper spectrum could be in the blank. [Courtesy G. S. Ostrom and M. D. Seltzer, Michelson Laboratory, China Lake, CA.]

ionized $^{138}\text{Ba}^{2+}$ interferes with $^{69}\text{Ga}^+$ because each has nearly the same mass-to-charge ratio ($138/2 = 69/1$). If an element has multiple isotopes, you can check for isobaric interference by measuring isotope ratios. For example, if the ratio of Se isotopes agrees with those found in nature ($^{74}\text{Se}:^{76}\text{Se}:^{77}\text{Se}:^{78}\text{Se}:^{80}\text{Se}:^{82}\text{Se} = 0.008:7:0.090:0.078:0.235:0.498:0.092$), then it is unlikely that there is interference at any of these masses.

Detection limits are so low that solutions must be made from extremely pure water and trace-metal-grade HNO_3 in Teflon or polyethylene vessels protected from dust. HCl and H_2SO_4 are avoided because they create isobaric interferences. High concentrations of dissolved solids can clog the small orifice between the plasma and the mass spectrometer. The plasma reduces organic matter to carbon, which can also clog the orifice. Organic material can be analyzed if some O_2 is fed into the plasma to oxidize the carbon.

Matrix effects on the yield of ions in the plasma are important, so calibration standards should be in the same matrix as the unknown. Internal standards can be used if they have nearly the same ionization energy as that of analyte. For example, Tm can be used as an internal standard for U . The ionization energies of these two elements are 5.81 and 6.08 eV, respectively, so they ionize to nearly the same extent in different matrices. Internal standards with just one major isotope provide maximum response.

Archaeologists use inductively coupled plasma–mass spectrometry to help find the origin of artifacts containing lead. Different locations where lead is mined have different ratios of lead isotopes. Variation occurs because different isotopes of lead are produced by radioactive decay of ^{238}U , ^{235}U , and ^{232}Th , whose occurrence varies from place to place.

Fed by melting snow from the Himalayas, the Indus River and its tributaries sustain life in central Pakistan. South Asia's first urbanized civilization developed in the Indus River valley in the period 2600–1900 B.C. An archaeologist posed the question, “With whom were the residents of the Indus Civilization interacting when they acquired rock and mineral resources?”⁴ Many archaeological rock and mineral artifacts were studied, but we will focus on silver ornaments found at Allahdino and Mohenjo-daro (Figure 20-19).

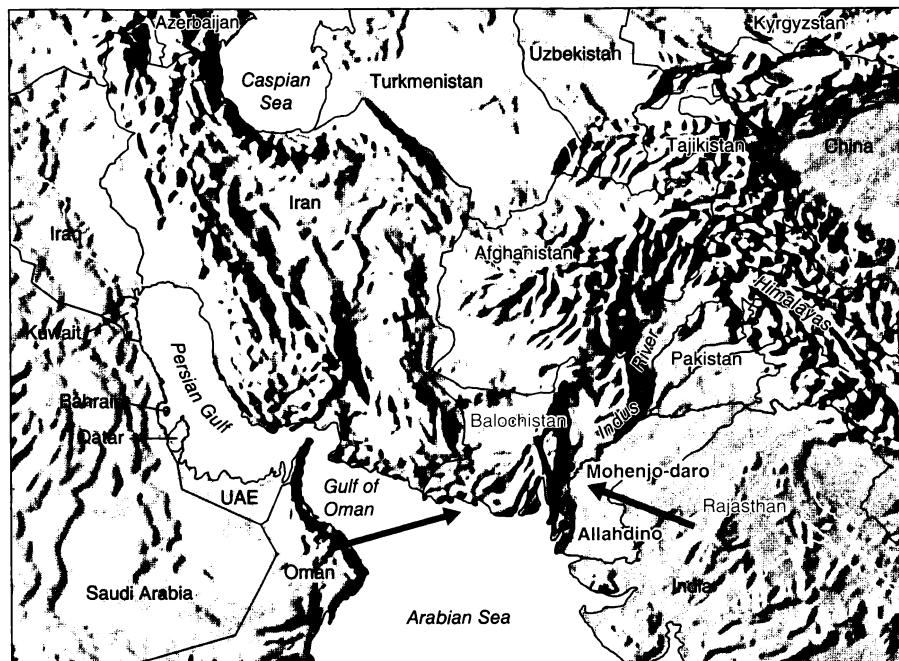
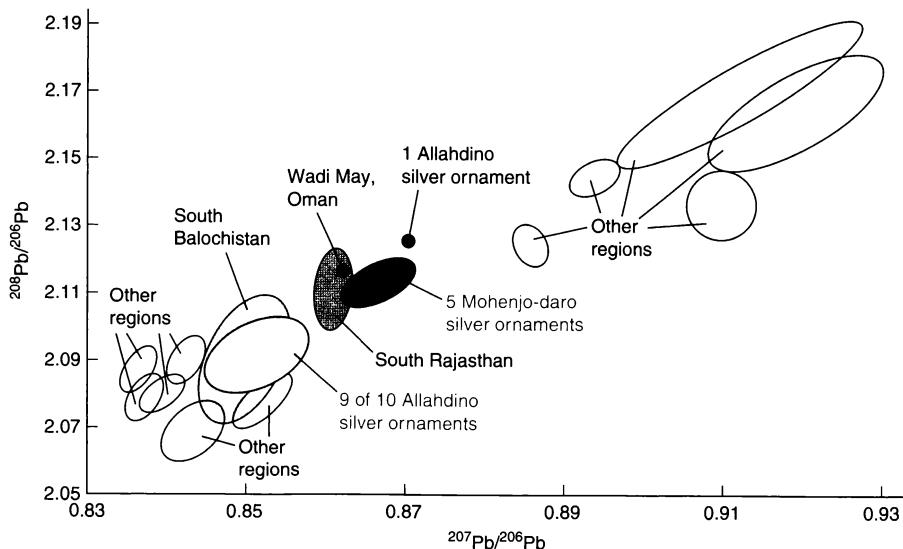


Figure 20-19 Map of South Asia. Arrows show regions that could be sources of silver found at the Indus Civilization sites of Mohenjo-daro and Allahdino. Balochistan is a region of present day Pakistan, and Rajasthan is a region of present day India. [Relief map adapted from Виктор Б, Wikipedia Commons, http://en.wikipedia.org/wiki/File:Relief_Map_of_Middle_East.jpg.]

Figure 20-20 Lead isotope ratios of silver ornaments compared with isotope ratios from known lead ore sources near the Indus River basin. Most isotope ratios from ornaments found near Allahdino match the ore composition from South Balochistan. Isotope ratios from ornaments found near Mohenjo-daro are similar to those from South Rajasthan and from one mine in Oman. [Adapted from R. W. Law and J. H. Burton, *Am. Lab. News Ed.*, September 2008, p. 14; R. W. Law, *Inter-Regional Interaction and Urbanism in the Ancient Indus Valley* (Kyoto: Research Institute for Humanity and Nature, 2010).]



Lead is an impurity in silver. The isotopic composition of the lead measured by inductively coupled plasma–mass spectrometry provides evidence for the origin of the silver because silver mined in each region retains the pattern of lead isotopes from that region.

Nine of ten ornaments found at Allahdino have a lead isotope composition similar to that of silver-containing lead ore from South Balochistan (Figure 20-20), which is near Allahdino. Five ornaments from Mohenjo-daro and one of ten from Allahdino do not match any known mining sites in Pakistan but are similar in isotopic composition to ore from South Rajasthan in India and a site across the Arabian Sea in Oman. It is possible that the silver came from these more distant sites. Archaeologists make increasing use of the most sophisticated analytical techniques to address question in anthropology.

Ask Yourself

20-F. Mercury has six isotopes with significant natural abundance: ^{198}Hg (10.0%), ^{199}Hg (16.9%), ^{200}Hg (23.1%), ^{201}Hg (13.2%), ^{202}Hg (29.9%), ^{204}Hg (6.9%). Lead has four isotopes: ^{204}Pb (1.4%), ^{206}Pb (24.1%), ^{207}Pb (22.1%), ^{208}Pb (52.4%). In Figure 20-18, why do we see six peaks for Hg and three for Pb? Why are there gaps at masses of 203 and 205?

Key Equation

Boltzmann distribution

$$\frac{N^*}{N_0} = \left(\frac{g^*}{g_0} \right) e^{-\Delta E/kT}$$

N^* = population of excited state

N_0 = population of ground state

ΔE = energy difference between excited and ground states

g^* = number of states with energy E^* (degeneracy of excited state)

g_0 = number of states with energy E_0 (degeneracy of ground state)

Important Terms

atomic absorption spectroscopy	chemical interference	ionization interference
atomic emission spectroscopy	graphite furnace	isobaric interference
atomization	hollow-cathode lamp	matrix
background correction	inductively coupled	spectral interference
Boltzmann distribution	plasma	

Problems

20-1. Compare the advantages and disadvantages of furnaces and flames in atomic absorption spectroscopy.

20-2. Compare the advantages and disadvantages of the inductively coupled plasma and flames in atomic spectroscopy.

20-3. In which technique, atomic absorption or atomic emission, is flame temperature stability more critical? Why?

20-4. Atomic *emission* spectrometers provide background correction by measuring the signal at the peak emission wavelength and then at wavelengths slightly above and slightly below the peak, where the signal has returned to background. The mean background is subtracted from the peak signal. Why can't we use the same technique for background correction in atomic *absorption*? (*Hint:* Think about the lamp.)

20-5. What is the purpose of a matrix modifier in atomic spectroscopy?

20-6. The first excited state of Ca is reached by absorption of 422.7-nm light.

(a) What is the energy difference (J) between the ground and excited states? (*Hint:* See Section 18-1.)

(b) The degeneracies are $g^*/g_0 = 3$ for Ca. Find N^*/N_0 at 2 500 K.

(c) By what percentage will the fraction in (b) be changed by a 15-K rise in temperature?

(d) Find N^*/N_0 at 6 000 K.

20-7. The first excited state of Cu is reached by absorption of 327-nm radiation.

(a) What is the energy difference (J) between the ground and excited states?

(b) The ratio of degeneracies is $g^*/g_0 = 3$ for Cu. Find N^*/N_0 at 2 400 K.

(c) By what percentage will the fraction in (b) be changed by a 15-K rise in temperature?

(d) What will the ratio N^*/N_0 be at 6 000 K?

20-8. *Detection limit.* (Refer to Section 5-2.) To estimate the detection limit for arsenic in tap water by furnace atomic absorption, eight tap water samples with a low concentration of As were measured. Then eight tap water samples spiked with 0.50 ppb (ng/mL) As were measured.

Tap water signal: 0.014, 0.005, 0.011, 0.001, -0.002, 0.002, 0.010, 0.008

Tap water + 0.50 ppb As: 0.046, 0.043, 0.036, 0.037, 0.041, 0.031, 0.039, 0.034

(a) A calibration curve is a graph of signal versus analyte concentration: signal = $m[\text{As}] + \text{blank signal}$, where [As] is the concentration in ppb and m is the slope. Spiked tap water has 0.50 ppb more As than the unspiked tap water. Find the slope of the calibration line by solving for m in the equation
 $(\text{mean spike signal} - \text{mean unspiked signal}) = m[0.50 \text{ ppb}]$

(b) From Equation 5-4, find the detection limit.

(c) From Equation 5-5, find the lower limit of quantitation.

20-9. (a) *Standard curve.* Subtract the blank from each emission intensity in the table and construct a calibration curve (Section 4-7) of corrected emission versus K^+ concentration ($\mu\text{g}/\text{mL}$). Find m , b , s_m , s_b , and s_y .

(b) Find $[\text{K}^+]$ ($\mu\text{g}/\text{mL}$) and its uncertainty in the unknown.

$[\text{K}^+]$ ($\mu\text{g K/mL}$)	Emission intensity	$[\text{K}^+]$ ($\mu\text{g K/mL}$)	Emission intensity
	at 404.3 nm		at 404.3 nm
Blank	6	20.0	492
5.00	130	30.0	718
10.0	249	Unknown	423

20-10. *Standard addition.* Suppose that 5.00 mL of blood serum containing an unknown concentration of potassium, $[\text{K}^+]_i$, gave an atomic emission signal of 3.00 mV. After the addition of 1.00 mL of 30.0 mM K^+ and dilution of the mixture to 10.00 mL, the emission signal increased to 4.00 mV.

(a) Find the concentration of added standard, $[\text{S}]_f$, in the mixture.

(b) The initial 5.00-mL sample was diluted to 10.00 mL. Therefore the serum potassium concentration decreased from $[\text{K}^+]_i$ to $[\text{K}^+]_f = \frac{1}{2}[\text{K}^+]_i$. Use Equation 5-6 to find the original K^+ content of the serum, $[\text{K}^+]_i$.

20-11. *Standard addition.* A blood serum sample containing Na^+ gave an emission signal of 4.27 mV. A small volume of concentrated standard Na^+ was then added to increase the

Na^+ concentration by 0.104 M, without significantly diluting the sample. This “spiked” serum sample gave a signal of 7.98 mV in atomic emission. Find the original concentration of Na^+ in the serum.

20-12. Standard addition. An unknown sample of Cu^{2+} gave an absorbance of 0.262 in an atomic absorption analysis. Then 1.00 mL of solution containing 100.0 ppm (= 100.0 $\mu\text{g/mL}$) Cu^{2+} was mixed with 95.0 mL of unknown, and the mixture was diluted to 100.0 mL in a volumetric flask. The absorbance of the new solution was 0.500. Find the original concentration of Cu^{2+} in the unknown.

20-13. Standard addition. An unknown containing element X was mixed with aliquots of a standard solution of element X for atomic absorption spectroscopy. The standard solution contained $1.000 \times 10^3 \mu\text{g}$ of X per milliliter.

Volume of unknown (mL)	Volume of standard (mL)	Total volume (mL)	Absorbance
10.00	0	100.0	0.163
10.00	1.00	100.0	0.240
10.00	2.00	100.0	0.319
10.00	3.00	100.0	0.402
10.00	4.00	100.0	0.478

(a) Calculate the concentration ($\mu\text{g X/mL}$) of added standard in each solution.

(b) Prepare a graph as in Figure 5-7 to find [X] in the unknown.

(c) Use the formula in Problem 5-19 to find the uncertainty in (b).

20-14. Standard addition. Li^+ was determined by atomic emission, using standard additions of solution containing 1.62 $\mu\text{g Li}^+/\text{mL}$. From the following data, prepare a standard addition graph to find the concentration of Li^+ in pure unknown. Use the formula in Problem 5-19 to find the uncertainty in concentration.

Unknown (mL)	Standard (mL)	Final volume (mL)	Emission intensity (arbitrary units)
10.00	0.00	100.0	309
10.00	5.00	100.0	452
10.00	10.00	100.0	600
10.00	15.00	100.0	765
10.00	20.00	100.0	906

20-15. Internal standard. A solution was prepared by mixing 10.00 mL of unknown (X) with 5.00 mL of standard (S) containing 8.24 $\mu\text{g S/mL}$ and diluting to 50.0 mL. The measured signal quotient was (signal due to X/signal due to S) = 1.69.

In a separate experiment, it was found that, for the concentration of X equal to 3.42 times the concentration of S, the signal due to X was 0.93 times as intense as the signal due to S. Find the concentration of X in the unknown.

20-16. Internal standard. Mn was used as an internal standard for measuring Fe by atomic absorption. A standard mixture containing 2.00 $\mu\text{g Mn/mL}$ and 2.50 $\mu\text{g Fe/mL}$ gave a quotient (Fe signal/Mn signal) = 1.05. A mixture with a volume of 6.00 mL was prepared by mixing 5.00 mL of unknown Fe solution with 1.00 mL containing 13.5 $\mu\text{g Mn/mL}$. The absorbance of this mixture at the Mn wavelength was 0.128, and the absorbance at the Fe wavelength was 0.185. Find the molarity of the unknown Fe solution.

20-17. (a) ${}^{40}\text{Ar}{}^{16}\text{O}^+$ in an Ar plasma causes *isobaric interference* in the measurement of ${}^{56}\text{Fe}^+$ at low concentrations by inductively coupled plasma–mass spectrometry. What is meant by isobaric interference?

(b) Which of the ions ${}^{51}\text{V}^+$, ${}^{52}\text{Cr}^+$, ${}^{53}\text{Cr}^+$, ${}^{54}\text{Fe}^+$, ${}^{55}\text{Mn}^+$, ${}^{56}\text{Fe}^+$, ${}^{57}\text{Fe}^+$, ${}^{58}\text{Ni}^+$, ${}^{59}\text{Co}^+$, ${}^{60}\text{Ni}^+$, ${}^{61}\text{Ni}^+$, ${}^{63}\text{Cu}^+$, ${}^{64}\text{Zn}^+$, ${}^{65}\text{Cu}^+$, and ${}^{66}\text{Zn}^+$ could be obscured by isobaric interference from ${}^{40}\text{Ar}{}^{16}\text{O}^+\text{H}^+$, ${}^{32}\text{S}{}^{16}\text{O}_2^+$, and ${}^{23}\text{Na}{}^{35}\text{Cl}^+$?

20-18. Quality assurance. Tin can be leached (dissolved) into canned foods from the tin-plated steel can.

(a) For analysis by inductively coupled plasma–atomic emission, food is digested by microwave heating in a Teflon bomb (Figure 2-18) in three steps with HNO_3 , H_2O_2 , and HCl . CsCl is added to the final solution at a concentration of 1 g/L. What is the purpose of the CsCl ?

(b)  Calibration data for the 189.927-nm emission line of Sn are shown in the following table. Use the Excel LINEST function (Section 4-8) to find the slope and intercept and their standard deviations and R^2 , which is a measure of the goodness of fit of the data to a line. Draw the calibration curve.

Sn ($\mu\text{g/mL}$)	Emission intensity (arbitrary units)	Sn ($\mu\text{g/mL}$)	Emission intensity (arbitrary units)
0	4.0	40.0	31.1
10.0	8.5	60.0	41.7
20.0	19.6	100.0	78.8
30.0	23.6	200.0	159.1

From L. Perring and M. Basic-Dvorzak, *Anal. Bioanal. Chem.* 2002, 374, 235.

(c) Interference by high concentrations of other elements was assessed at different emission lines of Sn. Foods containing little tin were digested and spiked with Sn at 100.0 $\mu\text{g/L}$. Then other elements were deliberately added. The following table shows selected results. Which elements interfere at each of the two wavelengths? Which wavelength is preferred for the analysis?

Element added at 50 mg/L	Sn found (μg/L) with 189.927-nm emission line	Sn found (μg/L) with 235.485-nm emission line
None	100.0	100.0
Ca	96.4	104.2
Mg	98.9	92.6
P	106.7	104.6
Si	105.7	102.9
Cu	100.9	116.2
Fe	103.3	intense emission
Mn	99.5	126.3
Zn	105.3	112.8
Cr	102.8	76.4

From L. Perring and M. Basic-Dvorzak, *Anal. Bioanal. Chem.* **2002**, 374, 235.

(d) *Limits of detection and quantitation.* The slope of the calibration curve in (b) is 0.782 units per (μg/L) of Sn. Food containing little Sn gave a mean signal of 5.1 units for seven replicates. Food spiked with 30.0 μg Sn/L gave a mean signal of 29.3 units with a standard deviation of 2.4 units for seven replicates. Use Equations 5-4 and 5-5 to estimate the limits of detection and quantitation.

(e) During sample preparation, 2.0 g of food are digested and eventually diluted to 50 mL for analysis. Express the limit of quantitation from (d) in terms of milligrams of Sn per kilogram of food.

20-19. Titanocene dichloride ($\pi\text{-C}_5\text{H}_5\right)_2\text{TiCl}_2$, is a potential antitumor drug thought to be carried to cancer cells by the protein transferrin (Figure 19-15). ($\pi\text{-C}_5\text{H}_5$ is the cyclopentadienyl group seen in ferrocene in Figure 17-5.) To measure the Ti(IV) binding capacity of transferrin, the protein was treated with excess titanocene dichloride. After allowing time for Ti(IV) to bind to the protein, excess small molecules were removed by dialysis (Demonstration 7-1). The protein was then digested with 2 M NH₃ and used to prepare a series of solutions with standard additions for chemical analysis. All solutions were made to the same total volume. Titanium and sulfur in each solution were measured by inductively coupled plasma–atomic emission spectrometry, with results in the

table. Each transferrin molecule contains 39 sulfur atoms. Find the molar ratio Ti/transferrin in the protein.

Added Ti (mg/L)	Signal	Added S (mg/L)	Signal
0	0.86	0	0.0174
3.00	1.10	37.0	0.0221
6.00	1.34	74.0	0.0268
12.0	1.82	148.0	0.0362

Data derived from A. Cardona and E. Meléndez, *Anal. Bioanal. Chem.* **2006**, 386, 1689.

How Would You Do It?

20-20. The measurement of Li⁺ in brine (saltwater) is used by geochemists to help determine the origin of this fluid in oil fields. Flame atomic emission and absorption of Li are subject to interference by scattering, ionization, and overlapping spectral emission from other elements. Atomic absorption analysis of replicate samples of a marine sediment gave the results in the following table.

Sample number and treatment	Li ⁺ found (μg/g)	Analytical method	Flame type
1. none	25.1	standard curve	air/C ₂ H ₂
2. dilute to 1/10 with H ₂ O	64.8	standard curve	air/C ₂ H ₂
3. dilute to 1/10 with H ₂ O	82.5	standard addition	air/C ₂ H ₂
4. none	77.3	standard curve	N ₂ O/C ₂ H ₂
5. dilute to 1/10 with H ₂ O	79.6	standard curve	N ₂ O/C ₂ H ₂
6. dilute to 1/10 with H ₂ O	80.4	standard addition	N ₂ O/C ₂ H ₂

From B. Baraj, L. F. H. Niencheski, R. D. Trapaga, R. G. França, V. Cocoli, and D. Robinson, *Fresenius J. Anal. Chem.* **1999**, 364, 678.

(a) Suggest a reason for the increasing apparent concentration of Li⁺ in samples 1 to 3.

(b) Why do samples 4 to 6 give an almost constant result?

(c) What value would you recommend for the real concentration of Li⁺ in the sample?

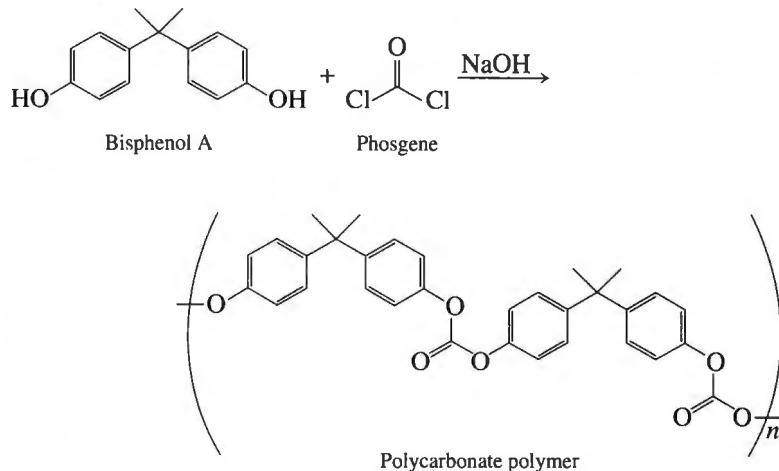
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Bisphenol A



Polycarbonate baby bottle. [Dani Cardona/Reuters.]



More than three million metric tons of bisphenol A (BPA) are produced each year to manufacture polycarbonate plastic and epoxy resins. Baby bottles and hikers' water bottles are made of polycarbonate. Metal beverage and food cans and metal lids for glass food jars are coated with epoxy resins. Traces of BPA leach out of bottles and cans into liquids and food.¹ BPA is an endocrine disruptor, binding to estrogen receptors that regulate ovulation and multiple metabolic pathways.² BPA has been classified as a carcinogen and a mutagen and is implicated in neurodevelopmental disorders. In 1988, the U.S Environmental Protection Agency set a tolerable daily intake of 0.05 mg of BPA per kg of body mass. For a 70-kg adult, this tolerable intake is 3.5 mg/day.

In 2010, the U.S. Food and Drug Administration expressed "some concern about the potential effects of BPA on the brain, behavior, and prostate gland in fetuses, infants, and young children." The Food and Drug Administration supports industry actions to stop producing BPA-containing baby bottles and infant feeding cups and is facilitating the development of alternatives for the linings of infant formula cans. In 2010, the European Union announced a ban on BPA in baby bottles. As a result of conflicting animal toxicity studies, there is no consensus at present to lower the allowed BPA intake for adults.³

A surprising source of BPA that became widely known in 2010 is cash register receipts. The heat-sensitive paper uses BPA as a color developer.⁴ In one study, 11 of 13 receipts contained 0.8–1.7 wt% BPA.⁵ When holding a receipt for 5 s, roughly 1 µg of BPA was transferred to fingers that were dry and ten times as much was transferred to wet or greasy fingers. BPA applied to fingers from ethanol solution disappears in 2 h, probably entering the skin.

Gas and liquid chromatography with mass spectrometric detection are methods of choice for measuring BPA. Chromatograms are shown in Box 21-3 and Figure 22-27.

VT 1% LOWFAT MILK*	2.59 F
POST SHRD WHEAT YOU JUST SAVED	2.50 1.99 F
POST SHRD WHEAT YOU JUST SAVED	2.50 1.99 F
POST SHRD WHEAT YOU JUST SAVED	2.50 1.99 F
FRENCH BREAD 2.72 lb 0 .69 / lb	1.49 F
BANANAS*	1.88 F
CUCUMBERS*	.99 F
LETTUCE-ICEBERG*	1.69 F
2.49 lb 0 1.99 / lb	
GRAPES-RED SDLS*	4.96 F
1.24 lb 0 .79 / lb	
JICAMA*	.98 F
2.31 lb 0 1.25 / lb	
APPLE-PINK LADY*	2.89 F
1.28 lb 0 1.50 / lb	
TOMATO-ROMA*	1.92 F
1 0 2 / 3.00	
BELL PEPPERS-RED*	1.50 F
SUBTOTAL	26.86
TOTAL TAX	.00
GIFT/SCRIPT	
TOTAL DUE	26.86
TENDER	26.86

Principles of Chromatography and Mass Spectrometry

Chromatography is the most powerful tool in an analytical chemist's arsenal for separating and measuring components of a complex mixture. With mass spectrometric detection, we can identify the components as well. This chapter discusses principles of chromatography and mass spectrometry, and the following chapters take up gas and liquid chromatography.

Chromatography is widely used for
Quantitative analysis: How much of a component is present?
Qualitative analysis: What is the identity of the component?

21-1 What Is Chromatography?

Chromatography is a process in which we separate compounds from one another by passing a mixture through a column that retains some compounds longer than others. In Figure 21-1, a solution containing compounds A and B is placed on top of

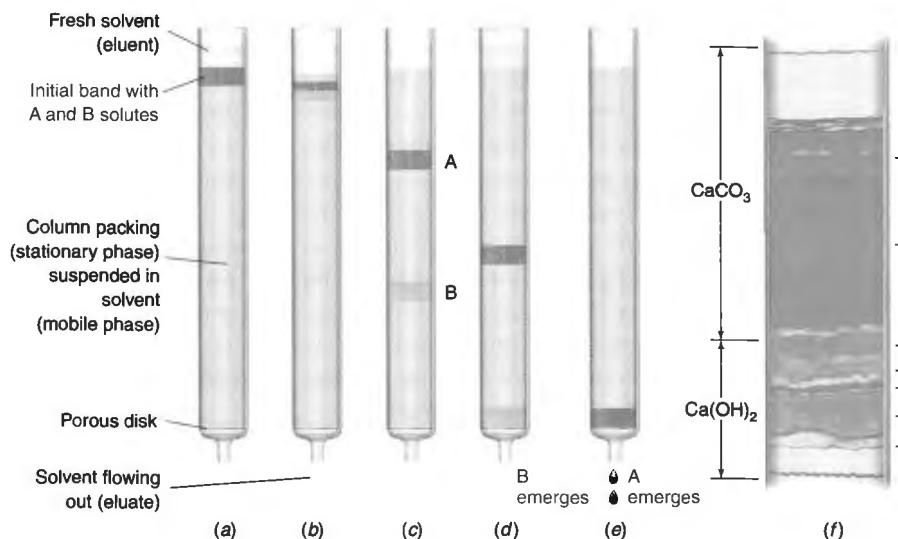
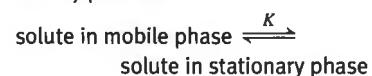


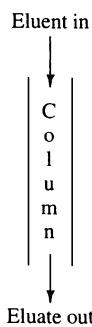
Figure 21-1 Separation by chromatography (panels *a–e*). Solute A has a greater affinity than solute B for the stationary phase, so A remains on the column longer than B. Panel *f* is a reconstruction of the separation of pigments from red paprika skin from work by L. Zechmeister in the 1930s with CaCO_3 and $\text{Ca}(\text{OH})_2$ stationary phases. [L. S. Ettre, *LCGC* 2007, 25, 640.]

Polish botanist M. Tswett invented chromatography in 1903 to separate plant pigments with a column containing solid CaCO_3 particles (the stationary phase) washed by hydrocarbon solvent (the mobile phase). Separation of colored bands led to the name *chromatography*, from the Greek *chromatos* ("color") and *graphein* ("to write")—“color writing.”

An excellent classroom activity introduces students to the *partition coefficient* to discover and understand the basis for chromatography. See M. J. Samide, *J. Chem. Ed.* 2008, 85, 1512. The partition coefficient (K) is the equilibrium constant for solute going between the mobile and stationary phases.



Adsorption means sticking to the surface of the solid particles. Color Plate 22 illustrates thin-layer chromatography, which is a form of adsorption chromatography.



For pioneering work on liquid-liquid partition chromatography in 1941, A. J. P. Martin and R. L. M. Synge received the Nobel Prize in 1952.

B. A. Adams and E. L. Holmes developed synthetic ion-exchange resins in 1935. *Resins* are relatively hard, amorphous (noncrystalline) organic solids. *Gels* are relatively soft.

In molecular exclusion chromatography, large molecules pass through the column *faster* than small molecules.

a column previously packed with solid particles and filled with solvent. When the outlet is opened, A and B flow down into the column and are washed through with fresh solvent applied to the top of the column. If solute A is more strongly *adsorbed* than solute B on the solid particles, then A spends a smaller fraction of the time free in solution. Solute A moves down the column more slowly than B and emerges at the bottom after B.

The **mobile phase** (solvent moving through the column) in chromatography is either a liquid or a gas. The **stationary phase** (the substance that stays fixed inside the column) is either a solid or a liquid that is usually covalently bonded to solid particles or to the inside wall of a hollow capillary column. Partitioning of solutes between the mobile and stationary phases gives rise to separation. In **gas chromatography**, the mobile phase is a gas; and, in **liquid chromatography**, the mobile phase is a liquid.

Fluid entering the column is called **eluent**. Fluid exiting the column is called **eluate**. The process of passing liquid or gas through a chromatography column is called **elution**.

Chromatography can be classified by the type of interaction of the solute with the stationary phase, as shown in Figure 21-2.

Adsorption chromatography uses a solid stationary phase and a liquid or gaseous mobile phase. Solute is adsorbed on the surface of the solid particles.

Partition chromatography involves a thin liquid stationary phase coated on the surface of a solid support. Solute equilibrates between the stationary liquid and the mobile phase.

Ion-exchange chromatography features ionic groups such as SO_3^- or $\text{N}(\text{CH}_3)_3^+$ covalently attached to the stationary solid phase, which is usually a *resin*. Solute ions are attracted to the stationary phase by electrostatic forces. The mobile phase is a liquid.

Molecular exclusion chromatography (also called *size exclusion, gel filtration*, or *gel permeation chromatography*) separates molecules by size, with larger molecules passing through fastest. There is no attractive interaction between the stationary phase and the solute. The stationary phase has pores small enough to exclude large molecules but not small ones. Large molecules stream past without entering the pores. Small molecules take longer to pass through the column because they enter the pores and therefore must flow through a larger volume before leaving the column.

Affinity chromatography, the most selective kind of chromatography, employs specific interactions between one kind of solute molecule and a second molecule that is covalently attached (immobilized) to the stationary phase. For example, the immobilized molecule might be an antibody to a particular protein. When a mixture containing a thousand different proteins is passed through the column, only that particular protein binds to the antibody on the column. After other proteins have been washed from the column, the desired protein is dislodged by changing the pH or ionic strength.

Ask Yourself

21-A. Match the terms with their definitions:

1. Adsorption chromatography
2. Partition chromatography

3. Ion-exchange chromatography
 4. Molecular exclusion chromatography
 5. Affinity chromatography
- A. Mobile-phase ions attracted to stationary phase ions.
 - B. Solute attracted to specific groups attached to stationary phase.
 - C. Solute equilibrates between mobile phase and surface of stationary phase.
 - D. Solute equilibrates between mobile phase and stationary liquid film.
 - E. Solute penetrates voids in stationary phase. Largest solutes eluted first.
-

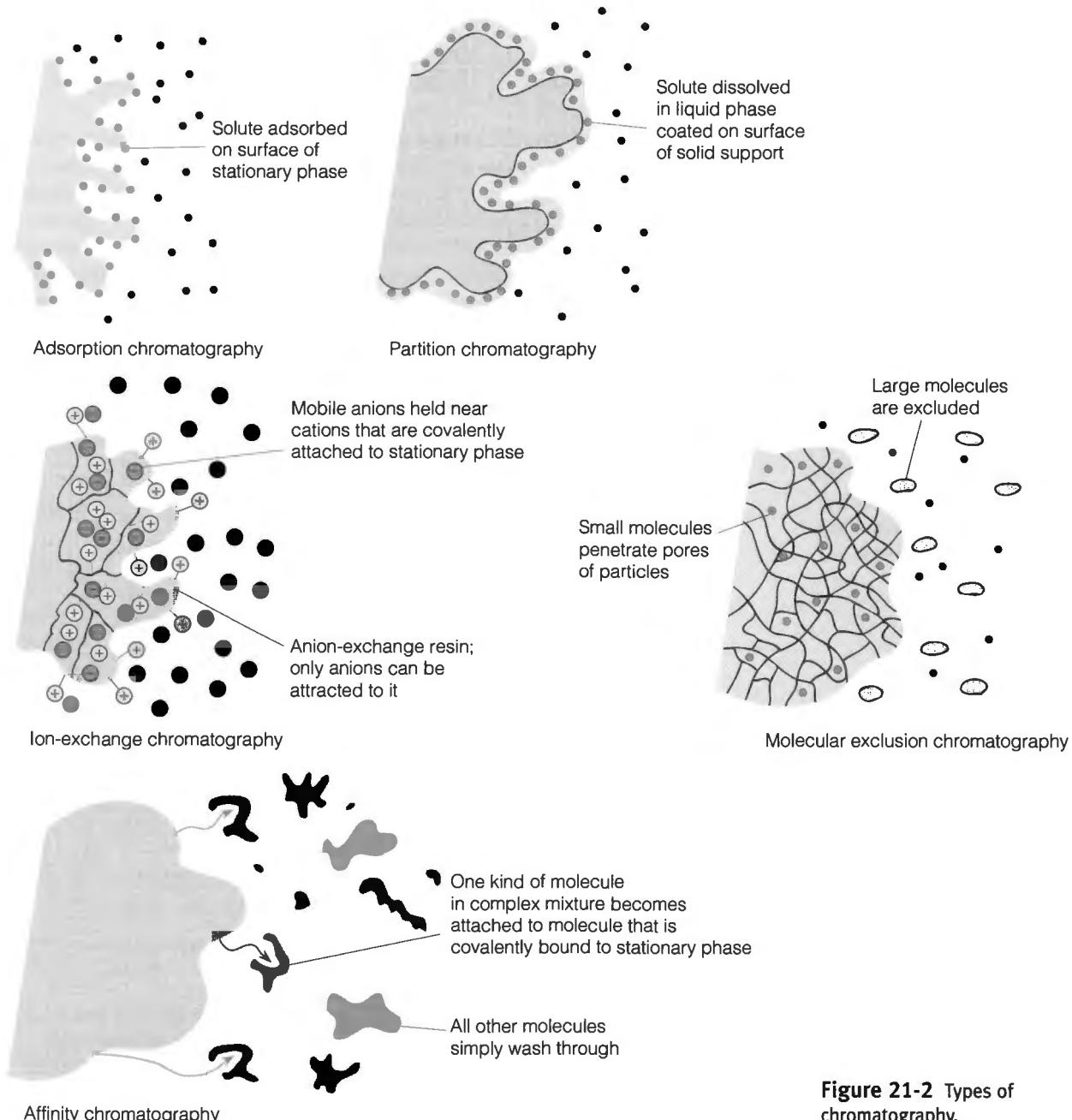


Figure 21-2 Types of chromatography.

21-2 How We Describe a Chromatogram

Detectors discussed in Chapter 22 respond to solutes as they exit the chromatography column. A **chromatogram** shows detector response as a function of time (or elution volume) in a chromatographic separation (Figure 21-3). Each peak corresponds to a different substance eluted from the column. **Retention time**, t_r , is the time needed after injection for an individual solute to reach the detector.

Theoretical Plates

An ideal chromatographic peak has a Gaussian shape, like that in Figure 21-3. If the height of the peak is h , the *width at half-height*, $w_{1/2}$, is measured at $\frac{1}{2}h$. For a Gaussian peak, $w_{1/2}$ is equal to 2.35σ , where σ is the standard deviation of the peak. The width of the peak at the baseline, as shown in Figure 21-3, is 4σ . The dashed lines that define the width at the baseline are drawn by eye to be tangent to the steepest part of the Gaussian peak.

In days of old, distillation was the most powerful means for separating volatile compounds. A distillation column was divided into sections (*plates*) in which liquid and vapor equilibrated with each other. The more plates on a column, the more equilibration steps and the better the separation between compounds with different boiling points.

Nomenclature from distillation carried over to chromatography. We speak of a chromatography column as if it were divided into discrete sections (called **theoretical plates**) in which a solute molecule equilibrates between the mobile and stationary phases. Retention of a compound on a column can be described by the number of theoretical equilibration steps between injection and elution. The more equilibration steps (the more theoretical plates), the narrower the bandwidth when the compound emerges.

For any peak in Figure 21-3, the number of theoretical plates is computed by measuring the retention time and the width at half-height:

$$\text{Number of plates on column: } N = \frac{5.55 t_r^2}{w_{1/2}^2} \quad (21-1)$$

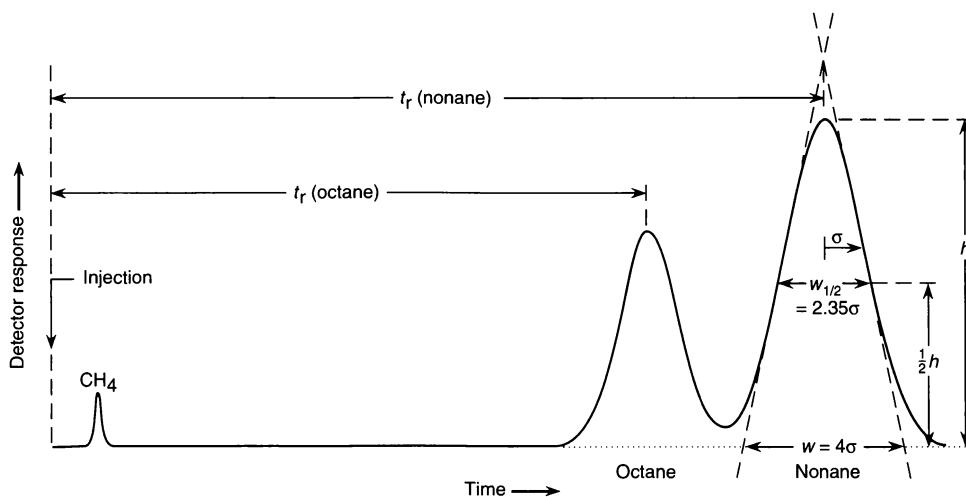


Figure 21-3 Schematic gas chromatogram showing measurement of retention time (t_r) and width at half-height ($w_{1/2}$). The width at the base (w) is found by drawing tangents to the steepest parts of the Gaussian curve and extrapolating down to the baseline. The standard deviation of the Gaussian curve is σ . In gas chromatography, a small volume of CH_4 injected with the 0.1- to 2- μL sample is usually the first component to be eluted.

Retention and width can be measured in units of time or volume (such as milliliters of eluate). Both t_r and $w_{1/2}$ must be expressed in the same units in Equation 21-1.

If a column is divided into N theoretical plates (only in our minds), then the **plate height**, H , is the height of one plate. H is the length of the column (L) divided by the number of theoretical plates:

Plate height:
$$H = L/N \quad (21-2)$$

The smaller the plate height, the narrower the peaks. The ability of a column to separate components of a mixture is improved by decreasing plate height. An efficient column has more theoretical plates than an inefficient column. Different solutes behave as if the column has somewhat different plate heights, because different compounds equilibrate between the mobile and stationary phases at different rates. Plate heights are ~ 100 to $1\,000\text{ }\mu\text{m}$ in gas chromatography, $\sim 10\text{ }\mu\text{m}$ in high-performance liquid chromatography, and $<1\text{ }\mu\text{m}$ in capillary electrophoresis.

small plate height \Rightarrow
narrow peaks \Rightarrow better separations

Example Measuring Plates

A solute with a retention time of 400.0 s has a width at half-height of 8.0 s on a column 12.2 m long. Find the number of plates and the plate height.

SOLUTION number of plates = $N = \frac{5.55 t_r^2}{w_{1/2}^2} = \frac{5.55 \cdot (400.0\text{ s})^2}{(8.0\text{ s})^2} = 1.39 \times 10^4$

$$\text{plate height} = H = \frac{L}{N} = \frac{12.2\text{ m}}{1.39 \times 10^4} = 0.88\text{ mm}$$

 **Test Yourself** If the number of plates is constant at 1.39×10^4 , what is the width of a compound eluted at 600 s? (Answer: 12.0 s)

Resolution

The **resolution** of neighboring peaks is the peak separation (Δt_r) divided by the average peak width (w_{av}) measured at the base, as in Figure 21-3:

Resolution:
$$\text{resolution} = \frac{\Delta t_r}{w_{av}} = \frac{0.589 \Delta t_r}{w_{1/2av}} \quad (21-3)$$

In the second equality, $w_{1/2av}$ is the average width at half-height, which is used more often than w_{av} because $w_{1/2av}$ is easier to measure. The better the resolution, the more complete the separation between neighboring peaks. Figure 21-4 shows peaks with a resolution of 0.50 and 1.00. For quantitative analysis, resolution ≥ 2 is desirable for negligible overlap. If you double the length of an ideal chromatography column, you will improve resolution by $\sqrt{2}$.

resolution $\propto \sqrt{\text{column length}}$
(The symbol \propto means "is proportional to.")

Qualitative and Quantitative Analysis

For *qualitative analysis*, the simplest way to identify a chromatographic peak is to compare its retention time with that of an authentic sample of the suspected compound. The most reliable way to do this is by **spiking**, also called *co-chromatography*, in which authentic sample is added to the unknown. If the added compound is identical to one component of unknown, the relative size of that one peak will increase. Two different

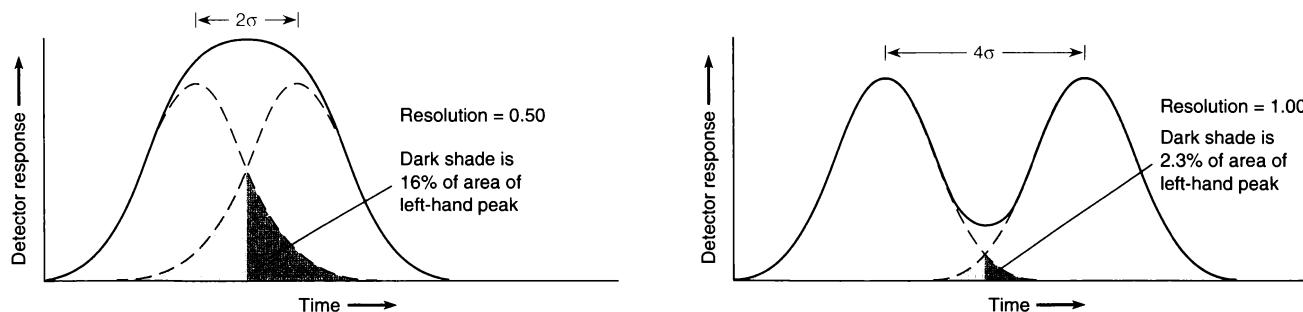


Figure 21-4 Resolution of Gaussian peaks of equal area and amplitude. Dashed lines show individual peaks; solid lines are the sum of two peaks. Overlapping area is shaded.

compounds might have the same retention time on a particular column. However, it is less likely that they will have the same retention time on different stationary phases.

For qualitative analysis, each chromatographic peak can be directed into a mass spectrometer to record a spectrum as the substance is eluted from the column. The compound might be identified by comparing its spectrum with a library of spectra in a computer.

For quantitative analysis, the area of a chromatographic peak is proportional to the quantity of analyte. Internal standards (Section 5-4) are frequently used in chromatography because the injected volume and exact chromatographic conditions vary somewhat from run to run. However, effects of variable conditions are usually the same for internal standard and analyte. By comparing the area of the analyte peak with that of the internal standard, we obtain a good measure of analyte concentration.

Scaling Up a Separation

Analytical chromatography: small-scale analysis

Preparative chromatography: large-scale separation

Analytical chromatography is conducted on a small scale to separate, identify, and measure components of a mixture. *Preparative chromatography* is carried out on a large scale to isolate a significant quantity of one or more components of a mixture. Analytical chromatography typically uses a long, thin column to obtain good resolution. Preparative chromatography usually employs a short, fat column that handles larger quantities of material but gives inferior resolution. (Long, fat columns may be prohibitively expensive to buy or operate.)

If you have developed a procedure to separate 2 mg of a mixture on a column with a diameter of 1.0 cm, what size column should you use to separate 20 mg of the mixture? The most straightforward way to scale up is to maintain column length and increase cross-sectional area to maintain a constant ratio of unknown to column volume. The cross-sectional area is proportional to the square of the column radius (r), so

$$\text{Scaling equation:} \quad \frac{\text{large load (g)}}{\text{small load (g)}} = \left(\frac{\text{large column radius}}{\text{small column radius}} \right)^2 \quad (21-4)$$

$$\frac{20 \text{ mg}}{2 \text{ mg}} = \left(\frac{\text{large column radius}}{0.50 \text{ cm}} \right)^2$$

large column radius = 1.58 cm

A column with a diameter near 3 cm would be appropriate.

To reproduce the conditions of the smaller column in the larger column, the *volume flow rate* (mL/min) should be increased in proportion to the cross-sectional

area of the column. If the area of the large column is 10 times greater than that of the small column, the volume flow rate should also be 10 times greater. If the volume of the small sample is V , the volume of the large sample can be $10V$.

Ask Yourself

- 21-B. (a) Use a ruler to measure retention times and widths at the half-height ($w_{1/2}$) of octane and nonane in Figure 21-3 to the nearest 0.1 mm.
 (b) Calculate the number of theoretical plates for octane and nonane.
 (c) If the column is 1.00 m long, find the plate height for octane and nonane.
 (d) Use your measurements from (a) to compute the resolution between octane and nonane.
 (e) Suppose that the sample size for Figure 21-3 was 3.0 mg, the column dimensions were 1.00 m length \times 4.0 mm diameter, and the flow rate was 7.0 mL/min. What size column and what flow rate should be used to obtain the same quality of separation of 27.0 mg of sample?

Rules for scaling up without losing resolution:

- cross-sectional area of column \propto mass of analyte
- keep column length constant
- volume flow rate \propto cross-sectional area of column
- sample volume \propto mass of analyte

21-3 Why Do Bands Spread?

If a solute is applied to a column in an ideal manner as an infinitesimally thin band, the band will broaden as it moves through a chromatography column (Figure 21-5a). Broadening occurs because of diffusion, slow equilibration of solute between the mobile and stationary phases, and irregular flow paths on the column.

Bands Diffuse

An infinitely narrow band of solute that is stationary inside the column slowly broadens because solute molecules diffuse away from the center of the band in both directions. This inescapable process, called *longitudinal diffusion*, begins at the moment solute is

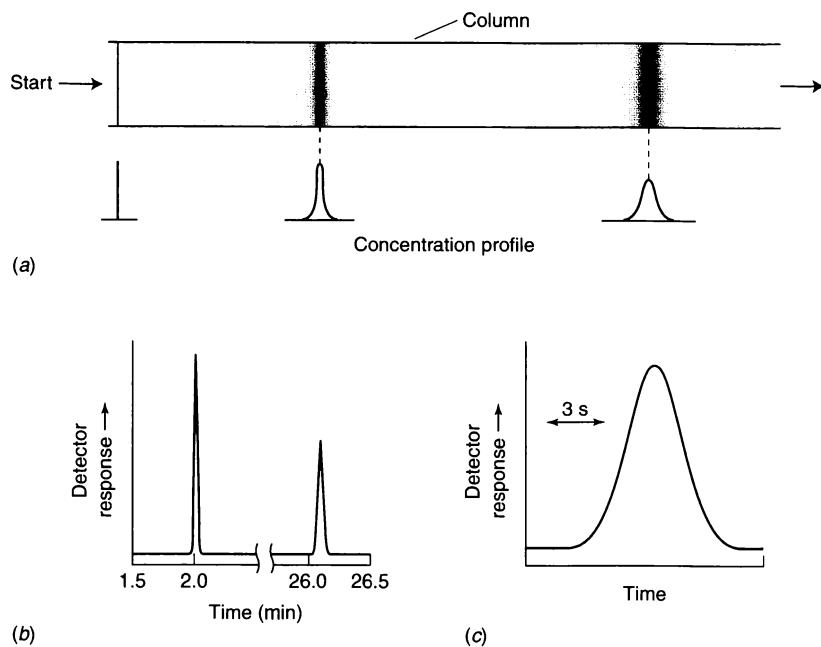


Figure 21-5 (a) Schematic representation of broadening of an initially sharp band of solute as it moves through a chromatography column. (b) Observed diffusional broadening of a band after 2 and 26 min in a capillary electrophoresis column. (c) Expanded view showing Gaussian bandshape near 26 min. [From M. U. Musheev, S. Javaherian, V. Okhonin, and S. N. Krylov, *Anal. Chem.* 2008, 80, 6752.]

Strictly speaking, flow rate in Equations 21-5 through 21-7 is *linear* flow rate (cm/min). This is the rate at which solvent goes past stationary phase. For a given column diameter, *volume* flow rate (mL/min) is proportional to linear flow rate.

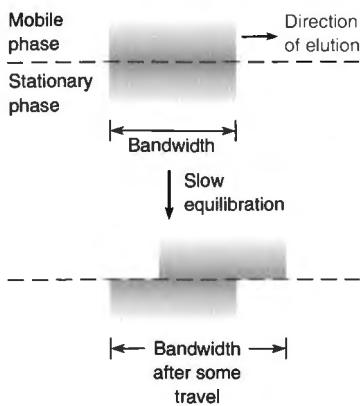


Figure 21-6 Solute requires a finite time to equilibrate between the mobile and stationary phases. If equilibration is slow, solute in the stationary phase lags behind that in the mobile phase, thereby causing the band to spread. The slower the flow rate, the less the zone broadening by this mechanism.

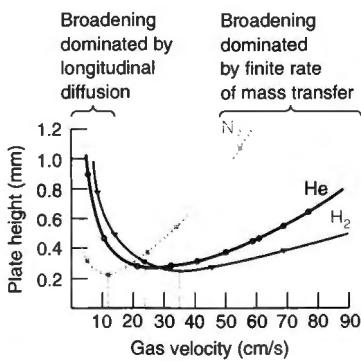


Figure 21-7 Optimum resolution (minimum plate height) occurs at an intermediate flow rate. Curves show measured plate height in gas chromatography of *n*-C₁₇H₃₆ at 175°C, using N₂, He, or H₂, mobile phase. [From R. R. Freeman, ed., *High Resolution Gas Chromatography* (Palo Alto, CA: Hewlett Packard Co., 1981).]

injected into the column. In chromatography, the farther a band has traveled, the more time it has had to diffuse and the broader it becomes (Figure 21-5b and c).

The faster the flow rate, the less time a band spends in the column and the less time there is for diffusion to occur. The faster the flow, the sharper the peaks. Broadening by longitudinal diffusion is inversely proportional to flow rate:

$$\text{Broadening by longitudinal diffusion:} \quad \text{broadening} \propto \frac{1}{u} \quad (21-5)$$

where u is the flow rate, usually measured in milliliters per minute.

Solute Requires Time to Equilibrate Between Phases

Imagine a solute that is distributed between the mobile and stationary phases at some moment in time at some position in a column with zero flow rate. Now set the flow into motion. If the solute cannot equilibrate rapidly enough between the phases, then solute in the stationary phase tends to lag behind solute in the mobile phase (Figure 21-6). This broadening due to the *finite rate of mass transfer* between phases becomes worse as the flow rate increases:

$$\text{Broadening by finite rate of mass transfer:} \quad \text{broadening} \propto u \quad (21-6)$$

A Separation Has an Optimum Flow Rate

When trying to separate closely spaced bands, we want to minimize band broadening. If the bands broaden too much, they will not be resolved. Because broadening by longitudinal diffusion *decreases* with increasing flow rate (Equation 21-5) but broadening by the finite rate of mass transfer *increases* with increasing flow rate (Equation 21-6), there is an intermediate flow rate that gives minimum broadening and optimum resolution (Figure 21-7). Part of the science and art of chromatography is to find conditions such as flow rate and solvent composition to obtain adequate separation between components of a mixture.

The rate of mass transfer between phases increases with temperature. Raising the column temperature might improve the resolution or allow faster separations without reducing resolution.

Some Band Broadening Is Independent of Flow Rate

Some mechanisms of band broadening are independent of flow rate. Figure 21-8 shows a mechanism that is called *multiple paths* and occurs in any column packed with solid particles. Because some of the random flow paths are longer than others, solute molecules entering the column at the same time on the left are eluted at different times on the right.

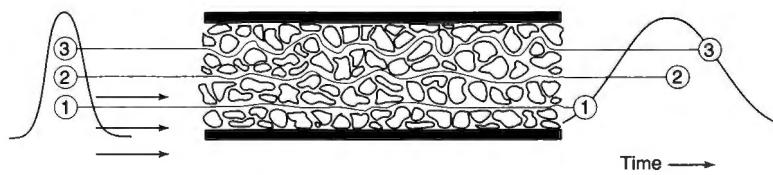


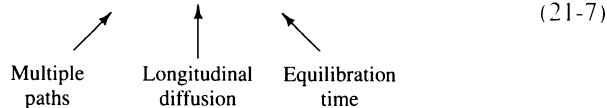
Figure 21-8 Band spreading from multiple flow paths. The smaller the stationary phase particles, the less serious is this problem. This process is absent in an open tubular column. [From H. M. McNair and E. J. Bonelli, *Basic Gas Chromatography* (Palo Alto, CA: Varian Instruments Division, 1968).]

Plate Height Equation

The van Deemter equation for plate height (H) as a function of flow rate (u) is the net result of the three band-broadening mechanisms just discussed:

van Deemter equation
for plate height:

$$H \approx A + \frac{B}{u} + Cu \quad (21-7)$$



where A , B , and C are constants determined by the column, stationary phase, mobile phase, and temperature. Each of the curves in Figure 21-7 is described by Equation 21-7 with different values of A , B , and C .

Equation 21-7 describes the broadening of a band of solute as it passes through a chromatography column. If the band has some finite width when it is applied to the column, the eluted band emerging from the other end will be broader than we predict with the van Deemter equation. Bands can broaden outside the column if there is too much tubing to flow through or if the detector has too large a volume. Best results are obtained if the lengths and diameters of all tubing outside the column are kept to a minimum. Also, there should be no void spaces inside the column where mixing can occur.

Open Tubular Columns

In contrast with a **packed column** that is filled with solid particles coated with stationary phase, an **open tubular column** is a hollow capillary whose inner wall is coated with a thin layer of stationary phase (Figure 21-9). In an open tubular column, there is no broadening from multiple paths, because there are no particles of stationary phase in the flow path. Therefore a given length of open tubular column generally gives better resolution than the same length of packed column. We say that the open tubular column has more theoretical plates (or a smaller plate height) than the packed column.

A packed gas chromatography column has greater resistance to gas flow than does an open tubular column. Therefore an open tubular column can be made much longer than a packed column with the same operating pressure. Because of its resistance to gas flow, a packed gas chromatography column is usually just 2–3 m in

Open tubular columns give better separations than packed columns because

- there is no multiple path band broadening ($A = 0$ in van Deemter equation)
- the open tubular column can be much longer

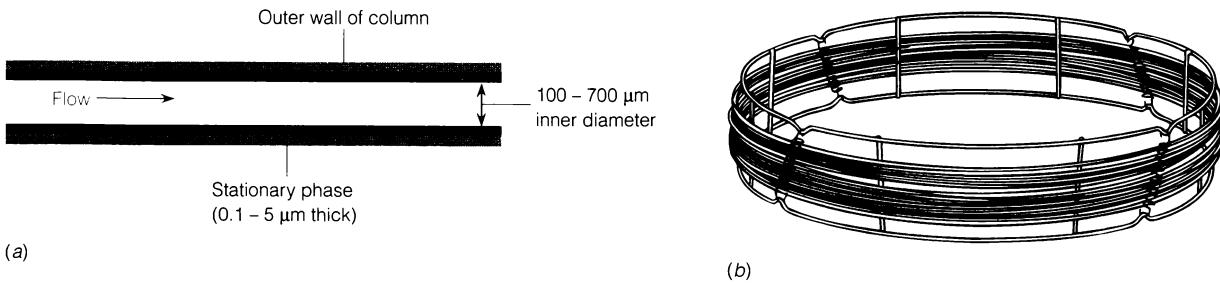


Figure 21-9 (a) Typical dimensions of open tubular column for gas chromatography.
(b) A fused silica column with a length of 15–100 m is wound in a small coil to fit inside the chromatograph.

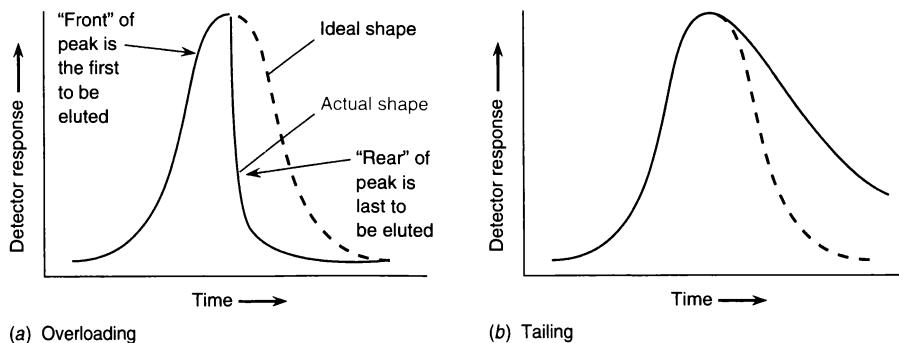


Figure 21-10 (a) Overloading gives rise to a chromatographic peak with an ordinary front and an abruptly cut off rear. (b) Tailing is a peak shape with a normal front and an elongated rear.

length, whereas an open tubular column can be 100 m in length. The greater length and smaller plate height of the open tubular column provide much better resolution than a packed column does.

An open tubular column cannot handle as much solute as a packed column because there is less stationary phase in the open tubular column. Therefore open tubular columns are useful for analytical separations but not for preparative separations.

Sometimes Peaks Have Funny Shapes

When a column is overloaded by too much solute in one band, the observed chromatographic peak gradually rises at its front and then abruptly falls off at its back (Figure 21-10a). The reason for this behavior is that a compound is most soluble in itself. As the concentration rises from zero at the front of the band to some high value inside the band, *overloading* can occur. When overloaded, the solute is so soluble in the concentrated part of the band that little trails behind the concentrated region.

Tailing is an asymmetric peak shape in which the trailing part of the band is elongated (Figure 21-10b). Such a peak occurs when there are strongly polar, highly adsorptive sites (such as exposed —OH groups) in the stationary phase that retain solute more strongly than other sites. To reduce tailing, column manufacturers use a chemical treatment called *silanization* to convert polar —OH groups into nonpolar —OSi(CH₃)₃ groups. Increased tailing during the life of a column is a signal that the column needs to be replaced.

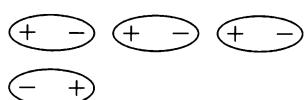
Box 21-1 discusses *polarity*.

Ask Yourself

- 21-C. (a) Why is longitudinal diffusion a more serious problem in gas chromatography than in liquid chromatography? (Think—the answer is not in the text.)
 (b) (i) In Figure 21-7, what is the optimum flow rate for best separation of solutes with He mobile phase? (ii) Why does plate height increase at high flow rate? (iii) Why does plate height increase at low flow rate?
 (c) Why does an open tubular gas chromatography column give better resolution than that of the same length of packed column?
 (d) Why is it desirable to use a very long open tubular column? What difference between packed and open tubular columns allows much longer open tubular columns to be used?

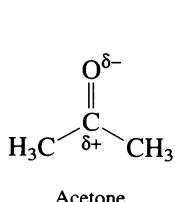
Box 21-1 Polarity

Polar compounds have positive and negative regions that attract neighboring molecules by electrostatic forces.

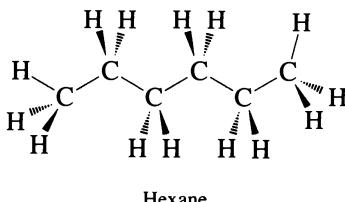


Polar molecules attract each other by electrostatic forces. Positive attracts negative.

Polarity arises because different atoms have different electronegativity—different abilities to attract electrons from the chemical bonds. For example, oxygen is more electronegative than carbon. In acetone, the oxygen attracts electrons from the C=O double bond and takes on a partial negative charge, designated δ^- . This shift leaves a partial positive charge (δ^+) on the neighboring carbon.



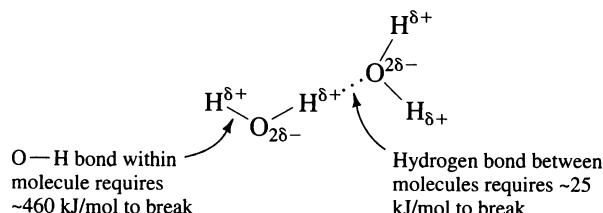
Acetone



Hexane

In contrast with acetone, hexane is considered to be a **nonpolar compound** because there is little charge separation within a hexane molecule.

Water is a very polar molecule that forms hydrogen bonds between the electronegative oxygen atom in one molecule and the electropositive hydrogen atom of another molecule:



Ionic compounds usually dissolve in water. In general, polar organic compounds tend to be most soluble in polar solvents and least soluble in nonpolar solvents. Nonpolar compounds tend to be most soluble in nonpolar solvents. “Like dissolves like.”

Typical nonpolar and weakly polar compounds		Typical polar compounds	
	octane (C_8H_{18})	CH_3OH methanol	
	benzene	CH_3CH_2OH ethanol	
	toluene	$CHCl_3$ chloroform	
	carbon tetrachloride		acetic acid
	diethyl ether ($C_4H_{10}O$)	$CH_3C\equiv N$ acetonitrile	

21-4 Mass Spectrometry

Mass spectrometry measures the masses and abundances of ions in the gas phase. A mass spectrometer is the most powerful detector for chromatography because the spectrometer is sensitive to low concentrations of analyte, provides both qualitative

Francis W. Aston (1877–1945) developed a “mass spectrograph” in 1919 that could separate ions and focus them onto a photographic plate. Aston immediately found that neon consists of two isotopes (^{20}Ne and ^{22}Ne) and went on to discover 212 of the 281 naturally occurring isotopes. He received the Nobel Prize in 1922.

Atomic mass units are called *daltons*, Da. The mass of $^{37}\text{Cl}^-$ is 36.966 Da.

and quantitative information, and can distinguish different substances with the same retention time. Selected reaction monitoring described on page 487 can separately observe two substances that lie under the same chromatographic peak. Mass spectrometry has vastly more use than just chromatography detection. For example, it is used to identify biological macromolecules desorbed from a surface by a laser pulse, and it is used to decipher the sequence of amino acids in proteins.

Prior to separation in the mass spectrometer, molecules must be converted into ions. These ions are then separated according to their **mass-to-charge ratio**, m/z . For an ion with a charge of $z = \pm 1$, such as $^{37}\text{Cl}^-$, m/z is numerically equal to m , which is close to 37. For the protein cytochrome *c* with 16 excess protons, $m = 12\,230$, $z = +16$, and $m/z = 12\,230/16 = 764.4$.

A Mass Spectrometer

Figure 21-11 shows a **transmission quadrupole mass spectrometer**, which is the most common mass separator in use today. It is connected to a gas chromatography column at the left to record the spectrum of each component as it is eluted. Compounds exiting the column pass through a heated connector into the ionization chamber where they are converted into ions and accelerated by 15 V before entering the quadrupole mass separator.

The mass separator consists of four parallel metal rods to which a constant voltage and a radio-frequency oscillating voltage are applied. The electric field deflects ions in complex trajectories as they migrate from the ionization chamber toward the detector, allowing only ions with one particular mass-to-charge ratio to reach the detector. Other (nonresonant) ions collide with the rods and are lost before they reach the detector. The mass spectrometer is evacuated to $\sim 10^{-9}$ bar to minimize collisions of ions with background gas as they pass through the quadrupole. Ions of different

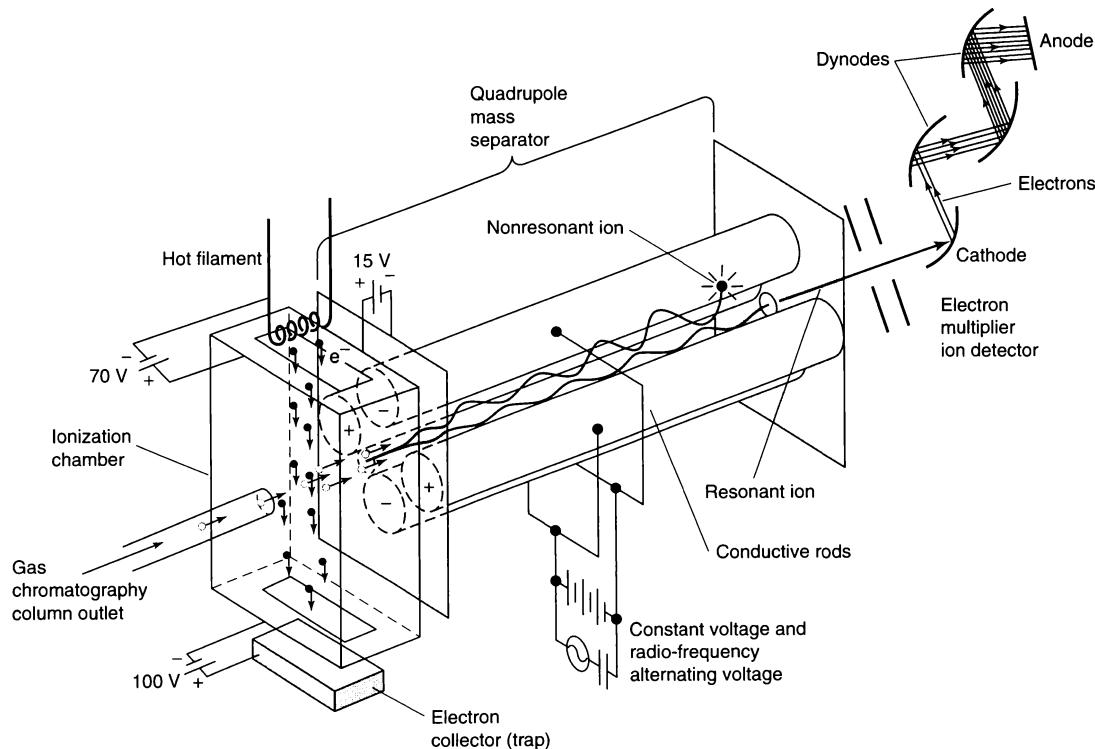


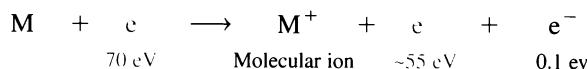
Figure 21-11 Transmission quadrupole mass spectrometer.

masses are selected to reach the detector by changing the voltages applied to the rods. Transmission quadrupoles can record from two to eight complete spectra per second, reaching as high as 4 000 m/z units. They can resolve peaks separated by m/z 0.3.

The *electron multiplier* ion detector at the right of Figure 21-11 is similar to the photomultiplier tube in Figure 19-10. Ions strike a cathode, dislodging electrons that are then accelerated into a more positive dynode. Electrons knocked off the first dynode are accelerated into a second dynode, where even more electrons are dislodged. Approximately 10^5 to 10^6 electrons reach the anode for each ion striking the cathode. Some types of detectors count the number of ions per second that reach the detector (as in Figure 22-27). The detector cannot discriminate between ions that arrive too close together, so signal is lost if the peak is too intense.

Ionization

Two common methods to convert molecules into ions are *electron ionization* and *chemical ionization*. Molecules entering the ionization chamber in Figure 21-11 are converted into ions by **electron ionization**. Electrons emitted from a hot filament (like the one in a light bulb) are accelerated by 70 eV before encountering incoming molecules. Some ($\sim 0.01\%$) analyte molecules (M) absorb enough energy (9–15 electron volts, eV) to ionize:



The resulting cation, M^+ , is called the **molecular ion**. After ionization, M^+ usually has enough residual internal energy (~ 1 eV) to break into fragments.

There might be so little M^+ that its peak is small or absent in the mass spectrum. The electron ionization mass spectrum at the left side of Figure 21-12 does not exhibit an M^+ peak, which would be at m/z 226. Instead, fragments appear at m/z 197, 156, 141, 112, 98, 69, and 55. These peaks provide clues about the structure of the molecule. A computer search is commonly used to match the spectrum of an unknown to similar spectra in a library.

Although we give examples of mass spectrometry of cations in this section, anions also can be produced and separated by mass spectrometry.

You can find electron ionization mass spectra of many compounds at <http://webbook.nist.gov/chemistry>.

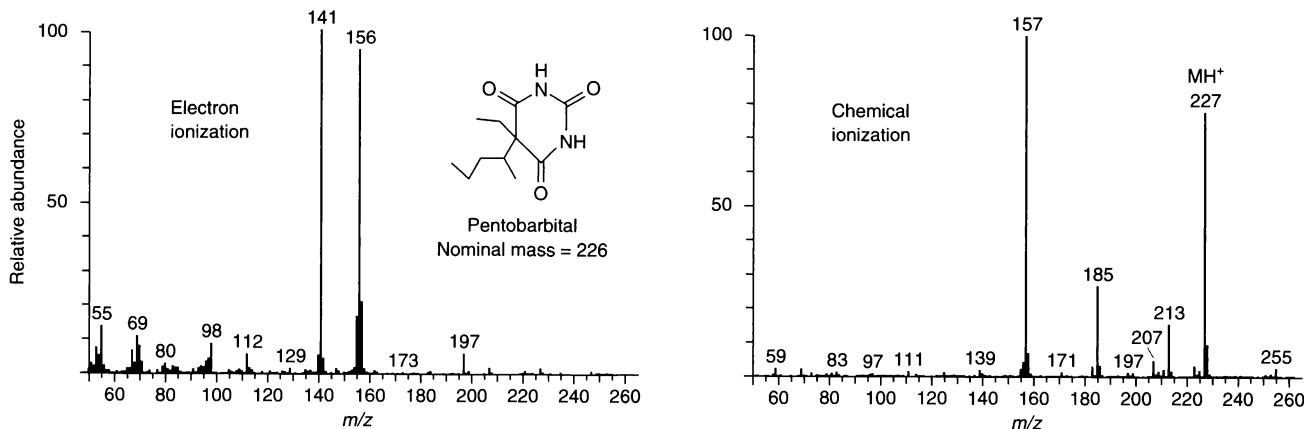


Figure 21-12 Mass spectra of the sedative pentobarbital with electron ionization (left) or chemical ionization (right). The molecular ion (m/z 226) is not evident with electron ionization. The dominant ion from chemical ionization is MH^+ . The peak at m/z 255 in the chemical ionization spectrum is $\text{M}(\text{C}_2\text{H}_5)^+$. C_2H_5^+ can be formed through chemical ionization by the reactions (1) $\text{CH}_4 \rightarrow \text{CH}_3^+ + \text{H}$ and (2) $\text{CH}_3^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2$. [Courtesy Varian Associates, Sunnyvale, CA.]

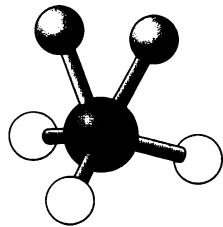
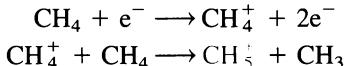


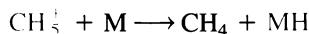
Figure 21-13 CH_5^+ is described as a CH_3 tripod with an added H_2 unit. $[\text{H}-\text{C}-\text{H}]$ is held together by two electrons distributed over three atoms. Atoms of the H_2 unit rapidly exchange with atoms of the CH_3 unit. [O. Asvany, P. Kumar P, B. Redlich, I. Hegemann, S. Schlemmer, and D. Marx, *Science* **2005**, 309, 1219.]

The most intense peak in a mass spectrum is called the **base peak**. Intensities of other peaks are expressed as a percentage of the base peak intensity. In the electron ionization spectrum in Figure 21-12, the base peak is at m/z 141.

Chemical ionization usually produces less fragmentation than electron ionization. For chemical ionization, the ionization chamber contains a *reagent gas* such as methane at a pressure of ~ 1 mbar. Energetic electrons (100–200 eV) convert CH_4 into a variety of products:



CH_5^+ (Figure 21-13) is a potent proton donor that reacts with analyte to give the *protonated molecule*, MH^+ , which is usually the most abundant ion.



In the chemical ionization mass spectrum in Figure 21-12, MH^+ at m/z 227 is a strong peak and there are fewer fragments than in the electron ionization spectrum.

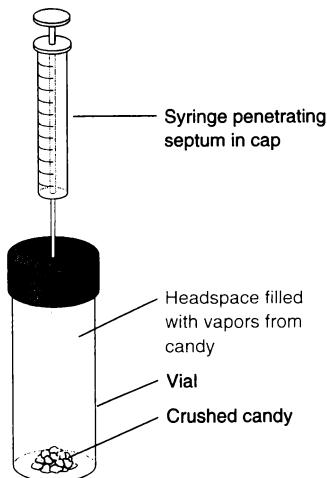
Total Ion and Selected Ion Chromatograms

One way to use a mass spectrometer as a detector for chromatography is to record the total current from all ions produced by eluate. Box 21-2 shows a

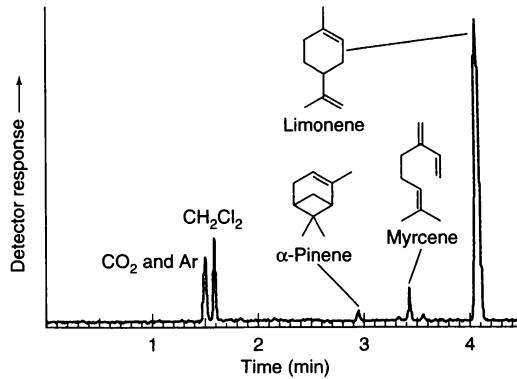
Box 21-2 Volatile Flavor Components of Candy

Students at Indiana State University identify components of candy and gum by using gas chromatography with a mass spectrometer as a detector. The extremely simple sampling technique for this qualitative analysis is called *headspace analysis*. A crushed piece of hard candy or a piece of gum is placed in a vial and allowed to stand for a few minutes so that *volatile* compounds (compounds

with a high vapor pressure) can evaporate and fill the gas phase (the *headspace*) with vapor. A 5- μL sample of the vapor phase is then drawn into a syringe and injected into a gas chromatograph. Peaks are identified by comparison of their retention times and mass spectra with those of known compounds.



Headspace sampling. A syringe is inserted through a rubber *septum* (a rubber disk) in the cap of the vial to withdraw gas for analysis.



Reconstructed total ion gas chromatogram of headspace vapors from Orange Life Savers.[®] The mass spectral detector measures ions above 34 atomic mass units. CO_2 and Ar are from air, and CH_2Cl_2 is the solvent used to clean the syringe. [From R. A. Kjonaas, J. L. Soller, and L. A. McCoy, *J. Chem. Ed.* **1997**, 74, 1104.]

reconstructed total ion chromatogram of vapors from candy. This chromatogram is “reconstructed” by a computer from individual mass spectra recorded during chromatography. In this case, the spectrometer measures all ions above m/z 34. Therefore it responds to all compounds shown in the figure, but not to carrier gas, H_2O , N_2 , or O_2 .

In Figure 21-14, trace *a* is a reconstructed total ion chromatogram showing all ions from seven opium alkaloids found in street heroin. Traces *b–h* are **selected ion chromatograms** in which the mass spectrometer is set to respond to just one mass in each trace. In trace *f*, the spectrometer responds only to m/z 370, corresponding to the protonated ion (MH^+) of heroin. The peak in trace *f* arises only from heroin because other components of this mixture do not have significant intensity at m/z 370. Even if heroin were eluted at the same time as another component, only heroin would be observed in the selected ion chromatogram.

The selected ion chromatogram simplifies chromatographic analysis and improves the signal-to-noise ratio for the desired analyte. Signal-to-noise is increased because more time is spent collecting data at the selected value of m/z . Box 21-3 shows a selected ion chromatogram from the analysis of bisphenol A found in canned food.



Ask Yourself

- 21-D. (a) What is the difference between a reconstructed total ion chromatogram and a selected ion chromatogram? Why does the selected ion chromatogram have a higher signal-to-noise ratio than that of the total ion chromatogram?
 (b) Why does trace *h* in Figure 21-14 have just one peak, even though a mixture of seven compounds was injected into the chromatograph?

21-5 Information in a Mass Spectrum

The mass spectrum of a molecule provides information about its structure. At the frontier of mass spectrometry today, scientists are elucidating the sequences of amino acids in proteins and the structures of complex carbohydrates by their fragmentation patterns. In this section, we touch on some of the simplest information available from mass spectrometry.

Nominal Mass

The unit of atomic mass is the dalton, Da, defined as 1/12 of the mass of ^{12}C . **Atomic mass** is the weighted average of the masses of the isotopes of an element. Table 21-1 tells us that bromine consists of 50.69% ^{79}Br with a mass of 78.918 34 Da and 49.31% ^{81}Br with a mass of 80.916 29 Da. In the weighted average, each mass is multiplied by its abundance. Therefore the atomic mass of Br is $(0.5069)(78.918\ 34) + (0.4931)(80.916\ 29) = 79.904$ Da.

The **molecular mass** of a molecule or ion is the sum of atomic masses listed in the periodic table. For 1-bromobutane, $\text{C}_4\text{H}_9\text{Br}$, the molecular mass is $(4 \times 12.010\ 7) + (9 \times 1.007\ 94) + (1 \times 79.904) = 137.018$.

The **nominal mass** of a molecule or ion is the *integer* mass of the species with the most abundant isotope of each of the constituent atoms. For carbon, hydrogen, and bromine, the most abundant isotopes are ^{12}C , ^1H , and ^{79}Br . Therefore the nominal mass of $\text{C}_4\text{H}_9\text{Br}$ is $(4 \times 12) + (9 \times 1) + (1 \times 79) = 136$.

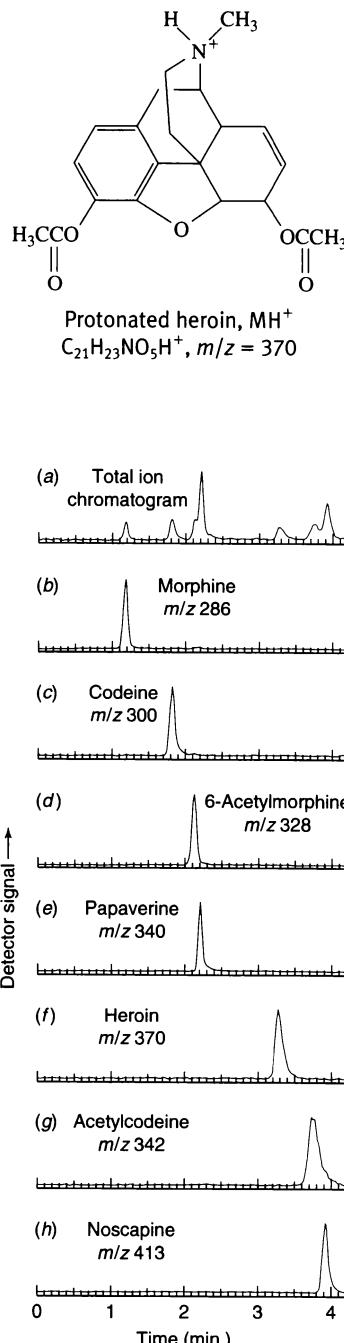
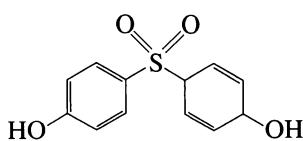


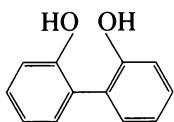
Figure 21-14 Liquid chromatography of opium alkaloids found in street heroin. Trace *a* is the reconstructed total ion chromatogram showing all masses in the range m/z 100–450. Traces *b–h* are selected ion chromatograms monitoring just a single value of m/z in each case. [From R. Dams, T. Benjits, W. Günther, W. Lambert, and A. De Leenheer, *Anal. Chem.* **2002**, 74, 3206.]

Box 21-3 Bisphenol A in Canned Foods

Bisphenol A (BPA), described at the opening of this chapter, is leached from the plastic liner of food cans into food. Two related compounds, bisphenol S (BPS) and 2,2'-biphenol (BP), which are components of epoxy resins and glues, rubber, and plastics, are also leached into food from can liners.

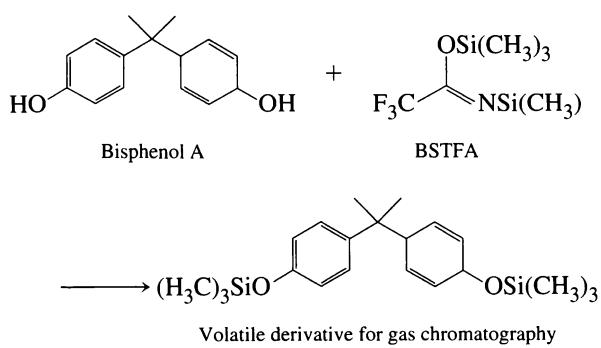


Bisphenol S (BPS), $C_{12}H_{10}SO_4$



2,2'-biphenol (BP), $C_{12}H_{10}O_2$

For chemical analysis, canned vegetables were ground and then extracted with water, using ultrasonic agitation. Polar substances were collected by dipping a *solid-phase microextraction* fiber (described in Section 22-4) made of polyacrylic acid into the stirring solution held at 70°C for 40 min. Extraction of BPA, BPS, and BP was most efficient near pH 6, using 0.01 M phosphate buffer in the food suspension. The fiber was dried in a stream of N₂ and then *derivatized* with vapors of bis(trimethylsilyl)trifluoroacetamide (BSTFA). This reagent replaces acidic hydrogens with trimethylsilyl groups to eliminate hydrogen bonding and increase vapor pressure for gas chromatography.



Gas chromatogram with electron-impact ionization selected ion monitoring for BPA, BPS, and BP extracted from liquid in a can of peas and carrots. [From P. Viñas, N. Campillo, N. Martínez-Castillo, and M. Hernández-Córdoba, *Anal. Bioanal. Chem.* 2010, 397, 115.]

The solid-phase microextraction fiber was introduced into the port of a gas chromatograph held at 280°C for 4 min to desorb derivatized analytes from the fiber onto a chromatography column held at 80°C. Analytes have negligible vapor pressure at 80°C, so they remain at the start of the column until the column temperature is raised.

The *selected ion chromatogram* shows BP, BPS, BPA, and a host of other peaks. BP, BPS, and BPA were identified by their retention times and mass spectra. In the selected ion chromatogram, the mass spectrometer is set to respond to just one mass during a certain time. BP has its strongest signal at m/z 186, so the detector was set to respond only to m/z 186 between 6.0 and 8.0 min. The strong signal observed near 7.7 min is m/z 186 from BP. Other signals seen prior to 8.0 min are from other components of the food extract giving ions at m/z 186. Between 8.0 and 9.6 min, the detector monitored m/z 165, which is the strongest signal for BPS. After 9.6 min, the detector was set to m/z 213, which is the strongest signal for BPA.

The chromatogram shows BPA, BPS, and BP isolated from the liquid in a can of peas and carrots from a grocery store. Observed levels of these substances in the liquid are 254, 175, and 176 ppb (ng/g), respectively. Observed concentrations in the solid peas and carrots from the same can are 78, 36, and 21 ppb. Health effects, if any, of traces of these substances in our food are unknown.

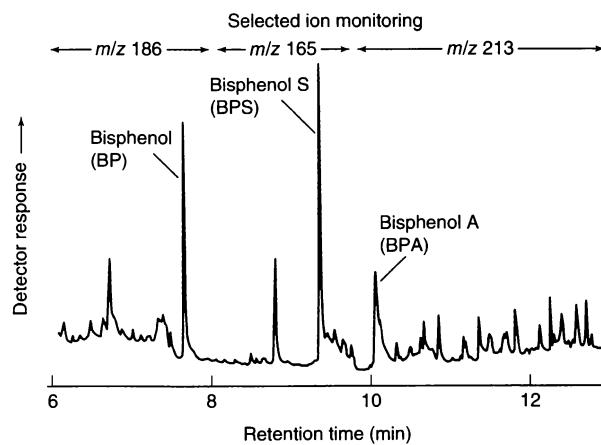


Table 21-1 Isotopes of selected elements

Element	Mass number	Mass (Da) ^a	Abundance (atom %) ^b	Element	Mass number	Mass (Da) ^a	Abundance (atom %) ^b
Electron	—	0.000 548 580	—	F	19	18.998 40	100
H	1	1.007 825	99.988	P	31	30.973 76	100
	2	2.014 10	0.012	S	32	31.972 07	94.93
C	12	12 (exact)	98.93		33	32.971 46	0.76
	13	13.003 35	1.07		34	33.967 87	4.29
N	14	14.003 07	99.632		36	35.967 08	0.02
	15	15.000 11	0.368	Cl	35	34.968 85	75.78
O	16	15.994 91	99.757		37	36.965 90	24.22
	17	16.999 13	0.038	Br	79	78.918 34	50.69
	18	17.999 16	0.205		81	80.916 29	49.31

a. 1 dalton (Da) $\equiv 1/12$ of the mass of $^{12}\text{C} = 1.660\ 538\ 783\ (83) \times 10^{-27}$ kg (<http://physics.nist.gov/constants>). Nuclide masses from G. Audi, A. H. Wapstra, and C. Thibault, *Nucl. Phys.* **2003**, A729, 337 (found at www.nndc.bnl.gov/masses/).

b. Abundance is representative of what is found in nature. See J. K. Böhlke et al., *J. Phys. Chem. Ref. Data* **2005**, 34, 57.

Fragmentation Patterns

The electron ionization mass spectrum of 1-bromobutane in Figure 21-15 has two peaks of almost equal intensity at m/z 136 and 138. The peak at m/z 136 is the molecular ion $\text{C}_4\text{H}_9\text{Br}^+$. Because bromine has almost equal abundance of the isotopes ^{79}Br and ^{81}Br , the second peak of almost equal intensity is $\text{C}_4\text{H}_9\text{Br}^+$. Any molecule or fragment containing just one Br will have pairs of peaks of nearly equal intensity in its mass spectrum. Other major peaks at m/z 107, 57, and 41 are explained by rupture of the bonds of 1-bromobutane in Figure 21-16. The peak at 107 has an equal-intensity partner at 109, so it must contain Br. Peaks at 57 and 41 do not have equal-intensity partners, so they cannot contain Br.

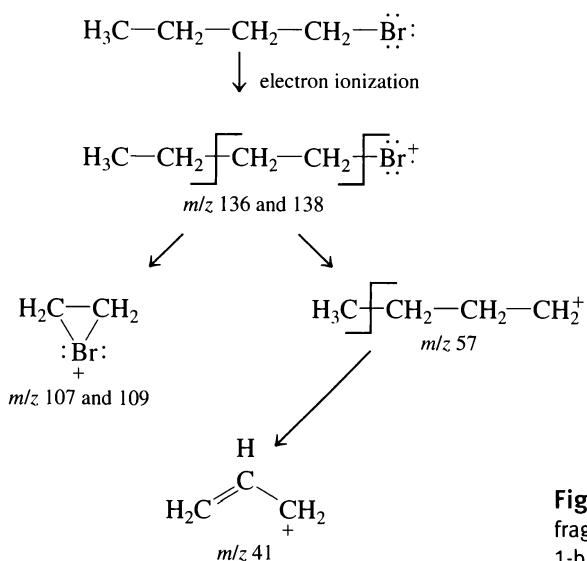
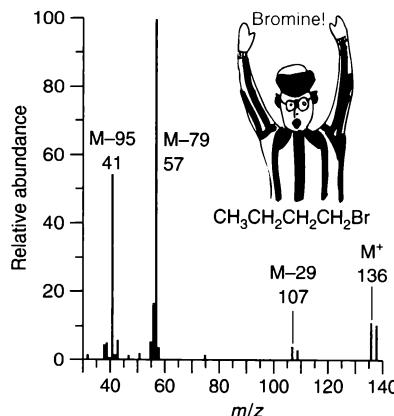


Figure 21-16 Major fragmentation pathways of 1-bromobutane cation.

Figure 21-15 Electron ionization mass spectrum of 1-bromobutane. [From A. Illies, P. B. Shevlin, G. Childers, M. Peschke, and J. Tsai, *J. Chem. Ed.* **1995**, 72, 717. Referee from Maddy Harris.]

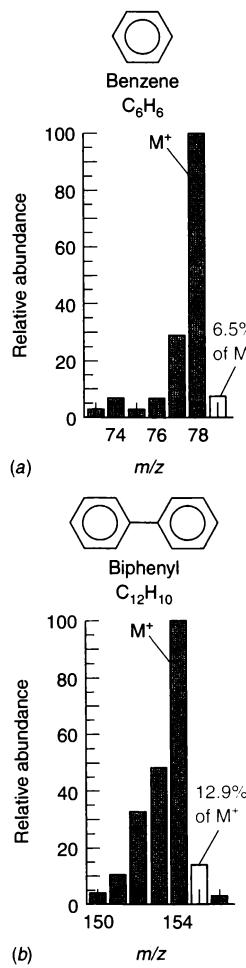


Figure 21-17 Electron ionization mass spectra of molecular ion region of benzene (C_6H_6) and biphenyl ($C_{12}H_{10}$). [From NIST/EPA/NIH Mass Spectral Database, SRData@enh.nist.gov.]

Isotope Patterns and the Nitrogen Rule

Peaks for ^{79}Br and ^{81}Br in Figure 21-15 are a characteristic *isotope pattern*. Information on the composition of organic compounds is obtained from the relative intensities at $M + 1$ and M^+ , where $M + 1$ is one mass unit above the molecular ion. Table 21-1 tells us that ^{12}C is the common isotope of carbon and ^{13}C has a natural abundance of 1.1%. Other common elements in organic compounds, H, O, and N, each have just one major isotope with little of the next-higher-mass isotope. Therefore the compound $C_nH_xO_yN_z$ has a ratio of intensities of the molecular ion given by

$$\frac{\text{Intensity of } M + 1 \text{ relative}}{\text{to } M^+ \text{ for } C_nH_xO_yN_z} = n \times 1.1\% \quad (21-8)$$

Figure 21-17 shows the molecular ion region of the mass spectrum of benzene. For C_6H_6 , Equation 21-8 predicts relative intensities $(M + 1)/M^+ = 6 \times 1.1\% = 6.6\%$. The observed ratio is 6.5%. Ordinary mass spectral intensity ratios are not more accurate than $\pm 10\%$, so a value in the range 5.9–7.3% is within expected uncertainty from 6.6%.

The **nitrogen rule** helps us propose compositions for molecular ions: If a compound has an odd number of nitrogen atoms—in addition to any number of C, H, halogens, O, S, Si, and P—then M^+ has an odd nominal mass. For a compound with an even number of nitrogen atoms (0, 2, 4, and so on), M^+ has an even nominal mass. A molecular ion at m/z 128 can have 0 or 2 N atoms, but it cannot have 1 N atom.

Example Elemental Information from the Mass Spectrum

Figure 21-17 shows the molecular ion region of the spectrum of biphenyl. M^+ is observed at m/z 154 and the intensity of $M + 1$ is 12.9% of M^+ . What formulas of the type $C_nH_xO_yN_z$ are consistent with the spectrum?

SOLUTION From the even nominal mass of the molecular ion (154), there must be an even number of N atoms (0, 2, 4, and so on). From the intensity ratio $(M + 1)/M^+ = 12.9\%$, we use Equation 21-8 to estimate that the number of carbon atoms is $12.9\% / 1.1\% = 11.7 \approx 12$. A possible formula is $C_{12}H_{10}$ because $(12 \times 12) + (10 \times 1) = 154$. Another plausible formula is $C_{11}H_6O$, whose nominal mass also is 154. The predicted intensity ratio for $C_{11}H_6O$ is $(M + 1)/M^+ = 11 \times 1.1\% = 12.1\%$, which is consistent with the observed value of 12.9%. The formula $C_{10}H_6N_2$ would have $(M + 1)/M^+ = 10 \times 1.1\% = 10.8\%$, which is somewhat low to match the observed ratio.

 **Test Yourself** What formula $C_nH_xO_yN_z$ is consistent with a molecular ion at m/z 94 and the intensity ratio $(M + 1)/M^+ = 6.8\%$? (Answer: $n = 6$ and z is even. Possible formulas are C_6H_6O and C_6H_{22} , but the most H atoms that can bond to 6 C atoms is 14. $C_5H_6N_2$ would have $(M + 1)/M = 5.5\%$, which is too low to match the observed value of 6.8%. C_7H_{10} would have $(M + 1)/M = 7.7\%$, which is too high. The best answer is C_6H_6O .)

Chromatographic and isotopic analysis are used in testing athletes for illegal use of synthetic testosterone to build muscle mass. In men, the natural ratio of testosterone to its stereoisomer, epitestosterone, is typically near 1:1 and rarely exceeds 4:1. A ratio exceeding 4:1 in urine measured by chromatography suggests that the athlete is taking testosterone. If a second sample obtained at the same time as the first sample replicates the result, there is reason to suspect that the athlete is taking synthetic testosterone. Isotope measurements distinguish natural from synthetic testosterone. Testosterone from urine is isolated by gas chromatography and combusted to CO_2 . The $^{13}\text{C}/^{12}\text{C}$ ratio in the CO_2 is measured accurately by *isotope ratio mass spectrometry*, which uses two

calibrated detectors to collect m/z 44 ($^{12}\text{CO}_2^+$) and m/z 45 ($^{13}\text{CO}_2^+$) ions. Synthetic testosterone is made from plant oils whose $^{13}\text{C}/^{12}\text{C}$ ratio is $\sim 0.5\%$ lower than the ratio in the human body. A high testosterone:epitestosterone ratio coupled with a low $^{13}\text{C}/^{12}\text{C}$ ratio strongly suggests illegal use of testosterone. Athletes have been stripped of hard-won titles on the basis of gas chromatography–isotope ratio mass spectrometry tests.⁶

High-Resolution Mass Spectrometry

Thus far, we have considered only mass spectra showing ions differing by one or more mass units, which is the limit of resolution of a transmission quadrupole mass spectrometer. Instruments such as the *time-of-flight* and *orbitrap* mass spectrometers can, for example, distinguish $\text{C}_8\text{H}_{15}\text{N}_5\text{Cl}^+$ from $\text{C}_7\text{H}_{14}\text{N}_5\text{O}_3^+$, both of which have a nominal mass of 216. Adding the exact masses of ^{12}C , ^1H , ^{14}N , ^{16}O , and ^{35}Cl from Table 21-1, and subtracting the mass of one lost electron, we find that these two ions differ by 0.008 1 Da:

$\text{C}_8\text{H}_{15}\text{N}_5\text{Cl}^+$ (atrazine H^+)	$\text{C}_7\text{H}_{14}\text{N}_5\text{O}_3^+$ (cymoxanil NH_4^+)
8^{12}C	$8 \times 12.000\ 00$
15^1H	$+15 \times 1.007\ 825$
5^{14}N	$+5 \times 14.003\ 07$
1^{35}Cl	$+1 \times 34.968\ 85$
$-\text{e}^-$	$-1 \times 0.000\ 55$
	216.101 0
	216.109 1

Exact mass:

$$\text{Difference} = 216.109\ 1 - 216.101\ 0 = 0.008\ 1\ \text{Da}$$

Figure 21-18 shows the resolution of these two ions by an orbitrap mass spectrometer. The spectrum is from a study that resolved, identified, and measured 510 pesticides at parts-per-billion levels in vegetables with a 12-min liquid chromatographic run. The ion $\text{C}_8\text{H}_{15}\text{N}_5\text{Cl}^+$ from the pesticide atrazine is resolved from the ion $\text{C}_7\text{H}_{14}\text{N}_5\text{O}_3^+$ from cymoxanil. Errors in the exact mass are 0.000 3 for atrazine and 0.001 0 for cymoxanil.

The mass *accuracy* of time-of-flight and orbitrap spectrometers interfaced to a liquid chromatograph is typically around 3 to 5 parts per million. For $m/z \approx 200$, a 3-ppm error is $(200)(3 \times 10^{-6}) = m/z\ 0.000\ 6$ and a 5-ppm error is $m/z\ 0.001\ 0$. If the spectrometer gives a value of $m/z\ 184.126$ for a particular peak, you could tentatively begin to rule out a proposed composition that differs by more than $m/z\ 0.001$ from this value.

If peaks are too close together, the spectrometer cannot resolve them as separate peaks. At $m/z \approx 200$, a time-of-flight spectrometer can resolve ions with differences of $m/z \approx 0.02$ and the orbitrap spectrometer can resolve differences of $m/z \approx 0.01$, as in Figure 21-18. Even though the location of a peak is accurate to $m/z\ 0.001$, two peaks closer together than $m/z \approx 0.01$ are not well resolved.



Ask Yourself

21-E. (a) $\text{C}_n\text{H}_x\text{O}_y\text{N}_z$ has a nominal mass of 194 with $(M + 1)/M^+ = 8.8\%$. How many C atoms are in the formula and what number of N atoms are allowed? Write possible formulas for the compound.

(b) Calculate the exact masses of the ions $\text{C}_5\text{H}_8\text{O}^+$ and $\text{C}_6\text{H}_{12}^+$, both of which have a nominal mass of 84. Would the instrument used for Figure 21-18 be expected to resolve these two ions?

Possible structures:

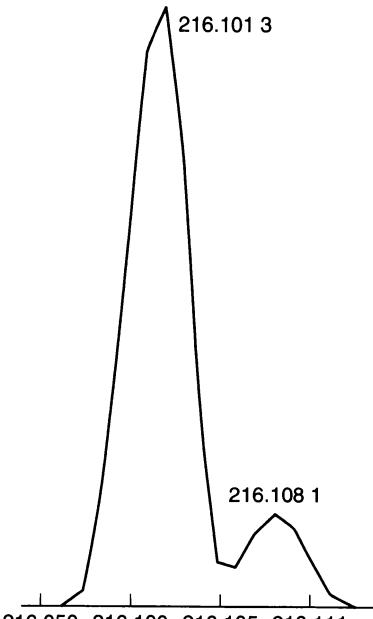
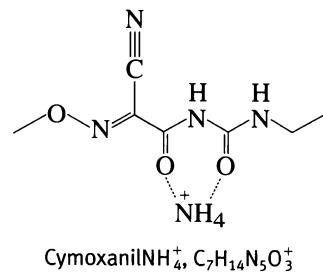
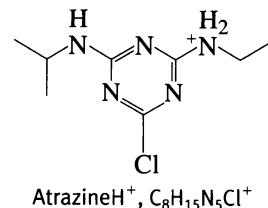


Figure 21-18 Orbitrap spectral resolution of two pesticides differing in m/z by 0.008 1. [From A. Zhang, J. S. Chang, C. Gu, and M. Sanders, *Current Trends in Mass Spectrometry*, July 2010, p. 40.]

Key Equations

Number of theoretical plates

$$N = \frac{5.55 t_r^2}{w_{1/2}^2}$$

t_r = retention time of analyte

$w_{1/2}$ = peak width at half-height

(t_r and $w_{1/2}$ must be measured in the same units.)

Plate height

$$H = L/N$$

L = length of column

Resolution

$$\text{resolution} = \frac{\Delta t_r}{w_{av}} = \frac{0.589 \Delta t_r}{w_{1/2av}}$$

Δt_r = difference in retention times between two peaks

w_{av} = average width of two peaks at baseline

$w_{1/2av}$ = average width of two peaks at half-height

(Δt_r and w must be measured in the same units.)

Scaling equation

$$\frac{\text{large load}}{\text{small load}} = \left(\frac{\text{large column radius}}{\text{small column radius}} \right)^2$$

van Deemter equation

$$H \approx A + \frac{B}{u} + Cu$$

H = plate height; u = flow rate

A = constant due to multiple flow paths

B = constant due to longitudinal diffusion of solute

C = constant due to equilibration time of solute between phases

Important Term

adsorption
chromatography
affinity chromatography
atomic mass
base peak
chemical ionization
chromatogram
chromatography
electron ionization
eluate
eluent

elution
gas chromatography
ion-exchange
chromatography
liquid chromatography
mass spectrometry
mass-to-charge ratio, m/z
mobile phase
molecular exclusion
chromatography
molecular ion

molecular mass
nitrogen rule
nominal mass
nonpolar compound
open tubular column
packed column
partition chromatography
plate height
polar compound
reconstructed total ion
chromatogram

resolution
retention time
selected ion chromatogram
spiking
stationary phase
theoretical plate
transmission quadrupole
mass spectrometer
van Deemter equation

Problems

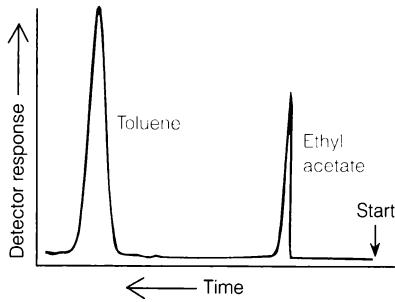
- 21-1. What is the difference between eluent and eluate?
- 21-2. Which column gives narrower peaks in a chromatographic separation: plate height = 0.1 mm or 1 mm?
- 21-3. (a) Figure 21-5b shows the concentration profile of a band of material after it has traveled through a column for 2 min and for 26 min. Why is the band broader after 26 min?
(b) Explain why a chromatographic separation normally has an optimum flow rate that gives the best separation.
- 21-4. Suggest a reason why the optimum linear flow rate is much higher in gas chromatography than in liquid chromatography.
- 21-5. Why does silanization reduce tailing of chromatographic peaks?
- 21-6. What kind of information does a mass spectrometer detector give in gas chromatography that is useful for qualitative analysis? For quantitative analysis?

21-7. How is spiking used in qualitative analysis? Why are several different types of columns necessary to make a convincing case for the identity of a compound?

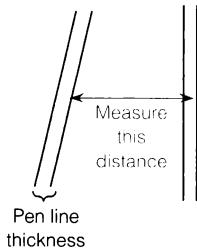
21-8. (a) How many theoretical plates produce a chromatography peak eluting at 12.83 min with a width at half-height of 8.7 s?

(b) The length of the column is 15.8 cm. Find the plate height.

21-9. A gas chromatogram of a mixture of toluene and ethyl acetate is shown here.



(a) Measure $w_{1/2}$ for each peak to the nearest 0.1 mm. When the thickness of the pen trace is significant relative to the length being measured, it is important to take the pen width into account. It is best to measure from the edge of one trace to the corresponding edge of the other trace, as shown here.



(b) Find the number of theoretical plates for each peak.

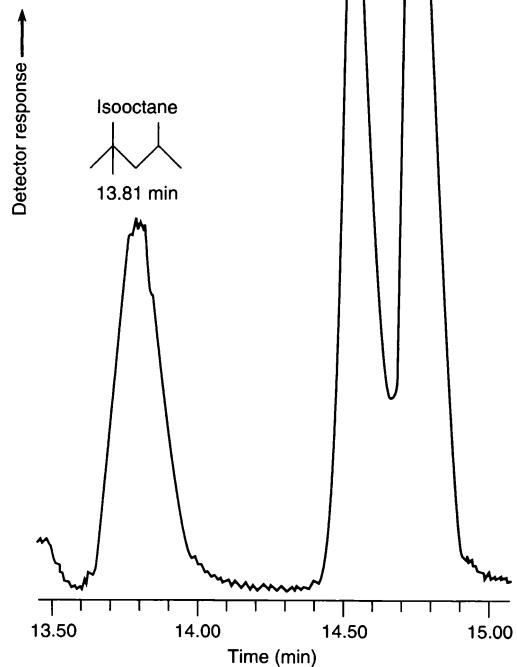
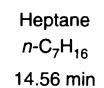
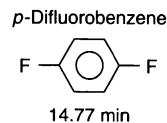
21-10. Two components of a 12-mg sample are adequately separated by chromatography through a column 1.5 cm in diameter and 25 cm long at a flow rate of 0.8 mL/min. What size column and what flow rate should be used to obtain similar separation with a 250-mg sample?

21-11. The chromatogram in the figure at the top of the right-hand column has a peak for isoctane at 13.81 min. The column is 30.0 m long.

(a) Measure $w_{1/2}$ and find the number of theoretical plates for this peak.

(b) Find the plate height.

(c) Figure 21-3 tells us that the ratio $w/w_{1/2}$ for a Gaussian peak is $4\sigma/2.35\sigma = 1.70$. Measure the width at the base for isoctane in the chromatogram for this problem. Compare the measured ratio $w/w_{1/2}$ to the theoretical ratio.



21-12. Consider the peaks for heptane (14.56 min) and *p*-difluorobenzene (14.77 min) in the chromatogram for Problem 21-11. The column is 30.0 m long.

(a) Measure $w_{1/2}$ for each peak and calculate the number of plates and the plate height for each.

(b) From $w_{1/2}$, compute w for each peak and find the resolution between the two peaks.

21-13. A column 3.00 cm in diameter and 32.6 cm long gives adequate resolution of a 72.4-mg mixture of unknowns, initially dissolved in 0.500 mL.

(a) If you wish to scale down to 10.0 mg of the same mixture with minimum use of chromatographic stationary phase and solvent, what length and diameter column would you use?

(b) In what volume would you dissolve the sample?

(c) If the flow rate in the large column is 1.85 mL/min, what should be the flow rate in the small column?

21-14. In Figure 21-7, flow rate is expressed as gas velocity in cm/s. The gas is flowing through an open tubular column with an inner diameter of 0.25 mm. What volume flow rate (mL/min) corresponds to a gas velocity of 50 cm/s? (The volume of a cylinder is $\pi r^2 \times$ length, where r is the radius.)

21-15. *Internal standard.* A solution containing 3.47 mM X (analyte) and 1.72 mM S (standard) gave peak areas of 3 473 and 10 222, respectively, in a chromatographic analysis. Then 1.00 mL of 8.47 mM S was added to 5.00 mL of unknown X, and the mixed solution was diluted to 10.0 mL. This solution gave peak areas of 5 428 and 4 431 for X and S, respectively.

- (a) Find the response factor for X relative to S in Equation 5-9.
- (b) Find [S] (mM) in the 10.0 mL of mixed solution.
- (c) Find [X] (mM) in the 10.0 mL of mixed solution.
- (d) Find [X] in the original unknown.

21-16. *Internal standard.* A known mixture of compounds C and D gave the following chromatography results:

Compound	Concentration ($\mu\text{g/mL}$) in mixture	Peak area (cm^2)
C	236	4.42
D	337	5.52

A solution was prepared by mixing 1.23 mg of D in 5.00 mL with 10.00 mL of unknown containing just C and diluting to 25.00 mL. Peak areas of 3.33 and 2.22 cm^2 were observed for C and D, respectively. Find the concentration of C ($\mu\text{g/mL}$) in the unknown.

21-17. *Internal standard graph.* When we develop a method using an internal standard, it is important to verify that the response factor F in Equation 5-9 is constant over the intended calibration range. The table gives data for gas chromatography of naphthalene (C_{10}H_8), using deuterated naphthalene (C_{10}D_8 in which D is the isotope ^2H) as an internal standard. The two compounds emerge from the column at almost identical times and are measured by a mass spectrometer. It is convenient to plot Equation 5-9 in the rearranged form

$$\frac{\text{area of analyte signal}}{\text{area of standard signal}} = F \left(\frac{\text{concentration of analyte}}{\text{concentration of standard}} \right) \quad (5-9)$$

Sample	C_{10}H_8 (ppm)	C_{10}D_8 (ppm)	C_{10}H_8 peak area	C_{10}D_8 peak area
1	1.0	10.0	303	2 992
2	5.0	10.0	3 519	6 141
3	10.0	10.0	3 023	2 819

The volume of solution injected into the column was different in all three runs.

If Equation 5-9 is obeyed, a graph of peak area ratio ($\text{C}_{10}\text{H}_8/\text{C}_{10}\text{D}_8$) versus concentration ratio ($[\text{C}_{10}\text{H}_8]/[\text{C}_{10}\text{D}_8]$) should be

a straight line through the origin with a slope of F . Prepare such a graph and find the slope and intercept and their standard deviations by the method of least squares. Find the response factor, F , and its standard deviation. Does the graph go through the origin? That is, is the intercept significantly different from zero?

21-18. (a) The plate height in a particular packed gas chromatography column is characterized by the van Deemter equation H (mm) = $A + B/u + Cu$, where $A = 1.50$ mm, $B = 25.0$ mm · mL/min, $C = 0.025$ 0 mm · min/mL, and u is flow rate in mL/min. Construct a graph of plate height versus flow rate and find the optimum flow rate for minimum plate height.

(b) In the van Deemter equation, B is proportional to the rate of longitudinal diffusion. Predict whether the optimum flow rate would increase or decrease if the rate of longitudinal diffusion were doubled. To confirm your prediction, increase B to 50.0 mm · mL/min, construct a new graph, and find the optimum flow rate. Does the optimum plate height increase or decrease?

(c) The parameter C is inversely proportional to the rate of equilibration of solute between the mobile and stationary phase ($C \propto 1/\text{rate of equilibration}$). Predict whether the optimum flow rate would increase or decrease if the rate of equilibration between phases were doubled (that is, $C = 0.012$ 5 mm · min/mL). Does the optimum plate height increase or decrease?

21-19. (a) Using van Deemter parameters in part (a) of the previous problem, find the plate height for a flow rate of 20.0 mL/min.

(b) How many plates are on the column if the length is 2.00 m?

(c) What will be the width at half-height of a peak eluted in a time of 8.00 min?

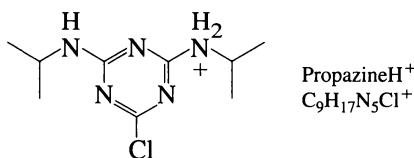
21-20. Explain the difference between molecular mass and nominal mass. Give the value of the molecular mass and nominal mass of benzene, C_6H_6 .

21-21. From information in Table 21-1, compute the atomic mass of Cl and compare your answer with the value in the periodic table on the inside cover of this book.

21-22. *Elemental analysis by mass spectrometry.* A particular high-resolution mass spectrometer measures m/z to an accuracy of 10 parts per million. This means that m/z 100 can be measured to an accuracy of $(100)(10 \times 10^{-6}) = 0.001$ (and precision into the next decimal place). A molecular ion thought to be $\text{C}_4\text{H}_{11}\text{N}_3\text{S}^+$ or $\text{C}_4\text{H}_{11}\text{N}_3\text{O}_2^+$ was observed at m/z 133.068 6. Compute the expected mass of each ion by adding the masses of the correct isotope of each atom and subtracting the mass of an electron. Which formula is correct?

21-23. (a) The herbicide propazine forms propazine H^+ with a nominal mass of 230 Da in liquid chromatography–mass spectrometry. Calculate the exact mass of propazine H^+ containing

the most common isotope of each of its elements in Table 21-1. The ion is observed at m/z 230.116 4.⁷



(b) The predominant isotopic composition of the ion at m/z 231 is $^{12}\text{C}_8^{13}\text{CH}_7\text{N}_5\text{Cl}^+$ with one ^{13}C . Calculate the exact mass of this ion. Relative to propazineH⁺ near m/z 230, what is the expected intensity of the peak at m/z 231? The ion is observed at m/z 231.118 8 with an intensity of 11.7%.

(c) The ion at m/z 232 has ^{37}Cl in place of ^{35}Cl . The natural abundance of ^{37}Cl is 24.22% and the natural abundance of ^{35}Cl is 75.78%. The expected relative intensities of the peaks at m/z 232 and m/z 230 are $24.22/75.78 = 0.319$ 6 : 1. Compute the exact mass for the peak near m/z 232. The ion is observed at m/z 232.113 4 with an intensity of 32.4%.

21-24. Suggest molecular formulas for the major peaks at m/z 31, 41, 43, and 56 in the mass spectrum of 1-butanol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$.

21-25. For each case below, suggest a plausible formula of the type $\text{C}_n\text{H}_x\text{O}_y\text{N}_z$:

- (a) Nominal mass of $\text{M}^+ = 79$; $(\text{M} + 1)/\text{M}^+ = 5.9\%$
- (b) Nominal mass of $\text{M}^+ = 123$; $(\text{M} + 1)/\text{M}^+ = 6.1\%$
- (c) Nominal mass of $\text{M}^+ = 148$; $(\text{M} + 1)/\text{M}^+ = 7.4\%$
- (d) Nominal mass of $\text{M}^+ = 168$; $(\text{M} + 1)/\text{M}^+ = 12.5\%$

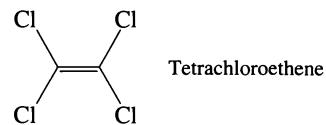
21-26. Use Table 21-1 to predict relative intensities at m/z 36, 37, and 38 for HCl. Let the intensity of the molecular ion be 100 and disregard contributions <0.1%.

21-27. Use Table 21-1 to predict relative intensities at m/z 34, 35, and 36 for H_2S . Let the intensity of the molecular ion be 100 and disregard contributions <0.1%.

Notes and References

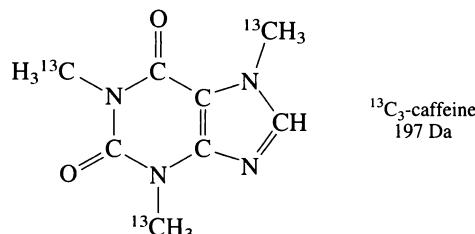
1. A. V. Krishnan, P. Stathis, S. F. Permuth, L. Tokes, and D. Feldman, *Endocrinology* **1993**, *132*, 2279; P. Viñas, N. Campillo, N. Martínez-Castillo, and M. Hernández-Córdoba, *Anal. Bioanal. Chem.* **2010**, *397*, 115.
2. National Toxicology Program Monograph, *Potential Human Reproductive and Developmental Effects of Bisphenol A*, NIH Publication No. 08-5994, 2008; <http://cerhr.niehs.nih.gov/evals/bisphenol/bisphenol.pdf>.
3. S. K. Ritter, *Chem. Eng. News*, 6 June 2011, p. 13; <http://pubs.acs.org/cen/coverstory/89/8923cover.html>.
4. T. Mendum, E. Stoler, H. VanBenschoten, and J. C. Warner, *Green Chem. Lett. & Rev.* **2010**, *4*, 81; <http://www.tandfonline.com/doi/abs/10.1080/17518253.2010.502908>.
5. S. Biedermann, P. Tschudin, and K. Grob, *Anal. Bioanal. Chem.* **2010**, *398*, 571.
6. T. C. Werner and C. K. Hatton, *J. Chem. Ed.* **2011**, *88*, 34.
7. E. M. Thurman and I. Ferrer, *Anal. Bioanal. Chem.* **2010**, *397*, 2807.
8. J. Richer, J. Spencer, and M. Baird, *J. Chem. Ed.* **2006**, *83*, 1196.
9. M. J. Yang, M. L. Orton, and J. Pawliszyn, *J. Chem. Ed.* **1997**, *74*, 1130.

21-28. A student experiment in headspace gas chromatography–mass spectrometry of glue vapors identified tetrachloroethene in some household adhesives.⁸ In the region of the molecular ion M^+ , the following peaks were found (with relative intensities in parentheses: m/z 164 (779), 166 (999), 168 (479), 170 (101), 172 (10). Draw a stick diagram showing the peaks and their intensities. Label which species accounts for each peak and give a qualitative explanation for the pattern of intensities. Refer to isotopic abundance in Table 21-1.



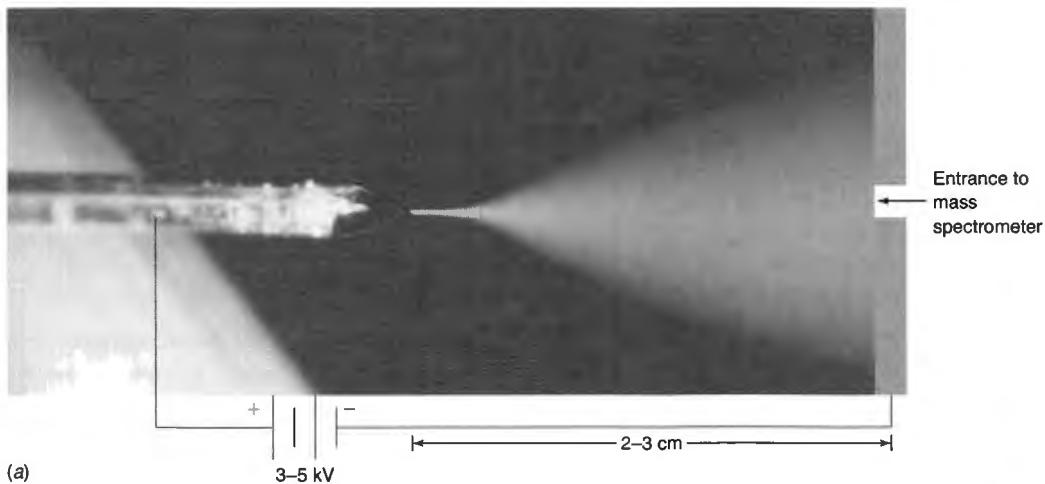
How Would You Do It?

21-29. Caffeine can be measured by chromatography with $^{13}\text{C}_3$ -caffeine as an internal standard.⁹ $^{13}\text{C}_3$ -caffeine has the same retention time as ordinary ^{12}C -caffeine.



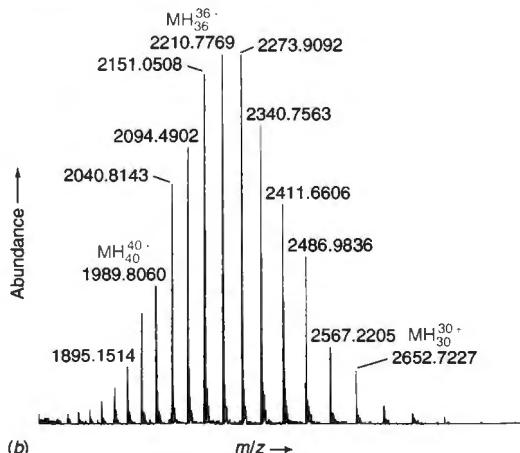
Caffeine can be extracted from aqueous solution by *solid-phase microextraction* (Section 22-4). In this procedure, a fused-silica (SiO_2) fiber coated with a polymer is dipped into the liquid and solutes from the liquid distribute themselves between the polymer phase and the liquid. Then the fiber is withdrawn from the liquid and heated in the port of a gas chromatograph. Solutes evaporate from the polymer and are carried into the column. Suggest a procedure for the quantitative analysis of caffeine in coffee by using solid-phase microextraction with a $^{13}\text{C}_3$ -caffeine internal standard.

Protein Electrospray



(a)

(a) Electrospray of liquid from a capillary held at a potential of 5 kV with respect to the entrance of a nearby mass spectrometer. [Courtesy R. D. Smith, Pacific Northwest Laboratory, Richland, WA.]



(b)

(b) Electrospray time-of-flight mass spectrum of the protein transferrin eluted from a liquid chromatography column. Peaks arise from protonated species MH_n^{n+} with $n = 27$ to 47. [From M. E. Del Castillo Busto, M. Montes-Bayón, E. Blanco-González, J. Meija, and A. Sanz-Medel, *Anal. Chem.* 2005, 77, 5615.]

Liquid exiting a chromatography column can be converted into the fine mist in panel *a* by **electrospray**, in which a high voltage is applied between the column and the entrance to a mass spectrometer. Micrometer-size droplets rapidly evaporate, leaving their solutes—including ions—free in the gas phase.

Electrospray has major impact in biological chemistry because it is one of the few means to introduce macromolecules into a mass spectrometer. A typical protein has amine and carboxylic acid side chains (Table 11-1) that give it a net positive or negative charge, depending on pH. Electrospray ejects preexisting ions from solution into the gas phase. Panel *b* shows the mass spectrum of the 80-kDa protein transferrin (Figure 19-15) eluted from a liquid chromatography column. In the $\text{pH} \approx 2.6$ eluate, the charge of transferrin ranges from +27 to +47, depending on protonation of arginine, lysine, and histidine amine side chains.

Electrospray was developed in the 1980s by John B. Fenn, who shared the Nobel Prize in 2002. More than 50 years earlier, Fenn's poor showing on an algebra exam came back with his teacher's comment, “Don't ever try to be a scientist or engineer.”¹

Gas and Liquid Chromatography

Gas and liquid chromatography are workhorses of the analytical and environmental chemistry laboratories. This chapter describes equipment and basic techniques.²

22-1 Gas Chromatography

In **gas chromatography**, a gaseous mobile phase transports gaseous solutes through a long, thin column containing stationary phase. We begin the process in Figure 22-1 by injecting a volatile liquid through a rubber *septum* (a thin disk) into a heated port, which vaporizes the sample. The sample is swept through the column by He, N₂, or H₂ *carrier gas*, and the separated solutes flow through a detector, whose response is displayed on a computer. The column must be hot enough to produce sufficient vapor pressure for each solute to be eluted in a reasonable time. The detector is maintained at a higher temperature than that of the column so that all solutes are gaseous. The injected sample size for a liquid is typically 0.1–2 μL for analytical chromatography, whereas preparative columns can handle 20–1 000 μL. Gases can be introduced in volumes of 0.5–10 mL by a gas-tight syringe or a gas-sampling valve.

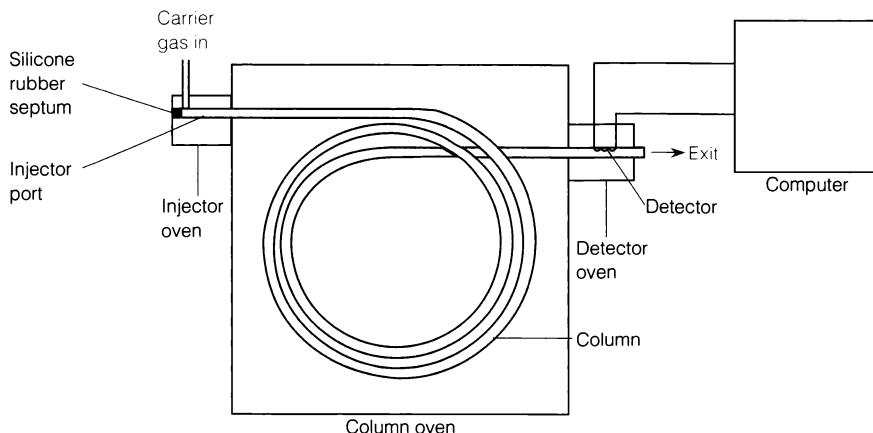


Figure 22-1 Schematic representation of a gas chromatograph.

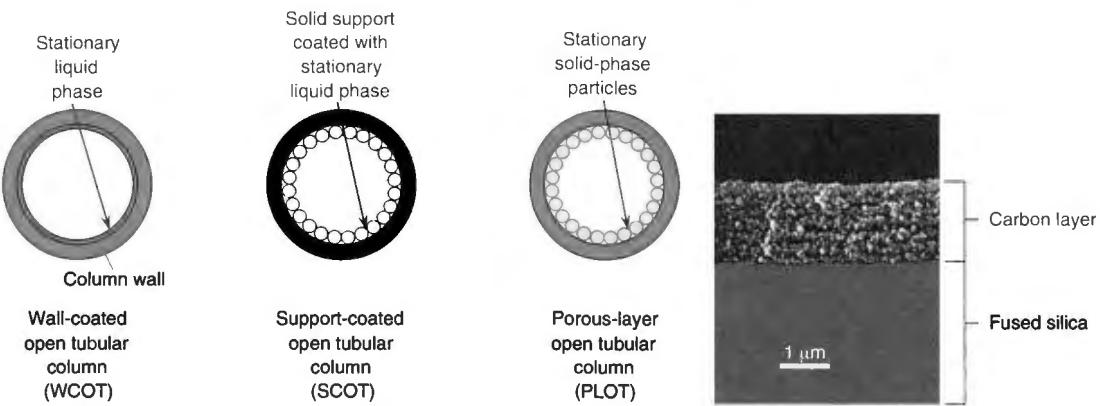


Figure 22-2 Cross-sectional view of wall-coated, support-coated, and porous-layer columns. Micrograph shows porous carbon stationary phase on inside wall of a fused-silica open tubular column.

Columns

Open tubular columns (Figure 21-9) have a liquid or solid stationary phase coated on the inside wall (Figure 22-2). Open tubular columns are usually made of fused silica (SiO_2). As the column ages, stationary phase bakes off and exposes silanol groups ($\text{Si}—\text{O}—\text{H}$) on the silica surface. The silanol groups strongly retain some polar compounds by hydrogen bonding, thereby causing *tailing* (Figure 21-10b) of chromatographic peaks. To reduce the tendency of a stationary phase to *bleed* from the column at high temperature, the stationary phase is normally *bonded* (chemically attached) to the silica surface and *cross-linked* to itself by covalent chemical bridges.

Polarity was discussed in Box 21-1.

Liquid stationary phases in Table 22-1 have a range of polarities. The choice of liquid phase for a given problem is based on the rule “like dissolves like.” Nonpolar columns are usually best for nonpolar solutes, and polar columns are usually best for polar solutes.

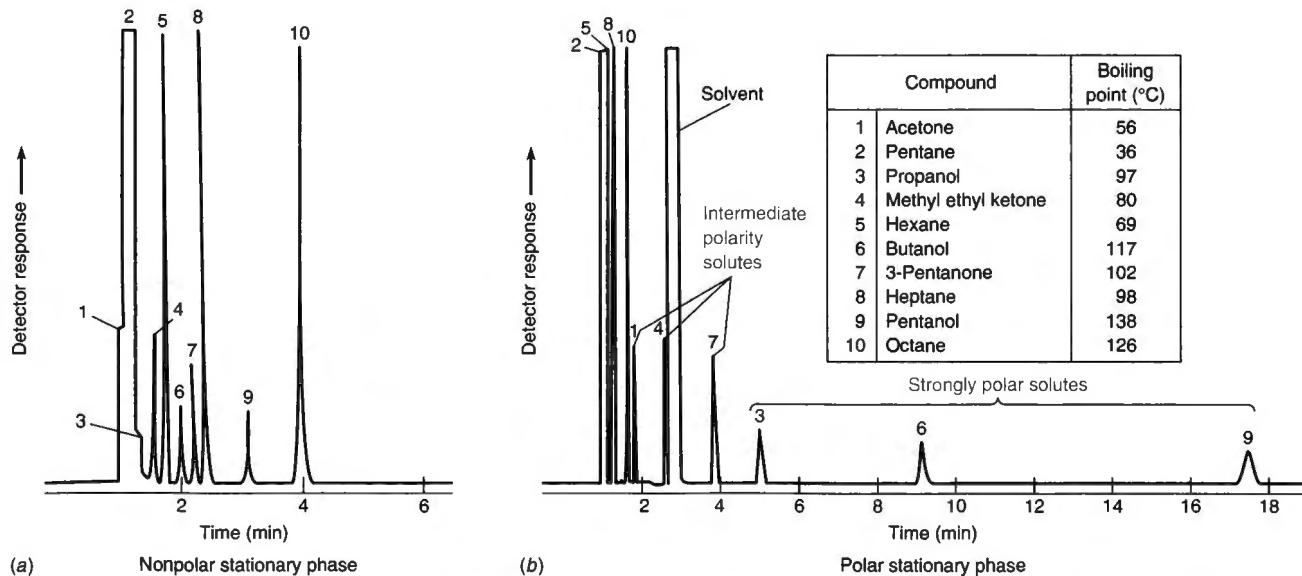


Figure 22-3 Separation of compounds on (a) nonpolar poly(dimethylsiloxane) and (b) strongly polar polyethylene glycol stationary phases (1 μm thick) in open tubular columns (30 m long \times 0.32 mm diameter) at 70°C. [Courtesy Restek Co., Bellefonte, PA.]

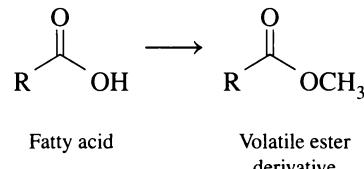
Table 22-1 Common stationary phases in capillary gas chromatography

Structure	Polarity	Temperature range
 (Diphenyl) _x (dimethyl) _{1-x} polysiloxane	$x = 0$ Nonpolar $x = 0.05$ Nonpolar $x = 0.35$ Intermediate polarity $x = 0.65$ Intermediate polarity	-60° to 360°C -60° to 360°C 0° to 300°C 50° to 370°C
 Arylene polysiloxane	Arylene stationary phases with low “bleed” (less thermal decomposition) at high temperature are available in compositions similar to other polysiloxanes in this table.	
 (Cyanopropylphenyl) _{0.14} (dimethyl) _{0.86} polysiloxane	Intermediate polarity	-20° to 280°C
 Carbowax (polyethylene glycol)	Strongly polar	40° to 250°C
 (Biscyanopropyl) _{0.9} (cyanopropylphenyl) _{0.1} polysiloxane	Strongly polar	0° to 275°C

We see the effects of column polarity on a separation in Figure 22-3. In Figure 22-3a, 10 compounds are eluted nearly in order of increasing boiling point from a poly(dimethylsiloxane) stationary phase: The higher the vapor pressure, the faster the compound is eluted. In Figure 22-3b, the strongly polar polyethylene glycol stationary phase strongly retains the polar solutes. The three alcohols (with $-\text{OH}$ groups) are the last to be eluted, following the three ketones (with $\text{C}=\text{O}$ groups), which follow four alkanes (having only C—H bonds). Hydrogen bonding between solute and the stationary phase causes strong retention.

Figure 22-4 illustrates excellent separation of 22 fatty acids for the measurement of *trans* fat for food labels (Figure 5-3). Peaks 1, 2, 3, 4, 11, 12, 13, 15, 16, 17, 18, 19, 20, and 21 are all *trans* fats. Fatty acids were converted (*derivatized*) to methyl esters to make them volatile enough for gas chromatography. The stationary phase is an *ionic liquid* that melts below room temperature and has a wide liquid

Derivatization:



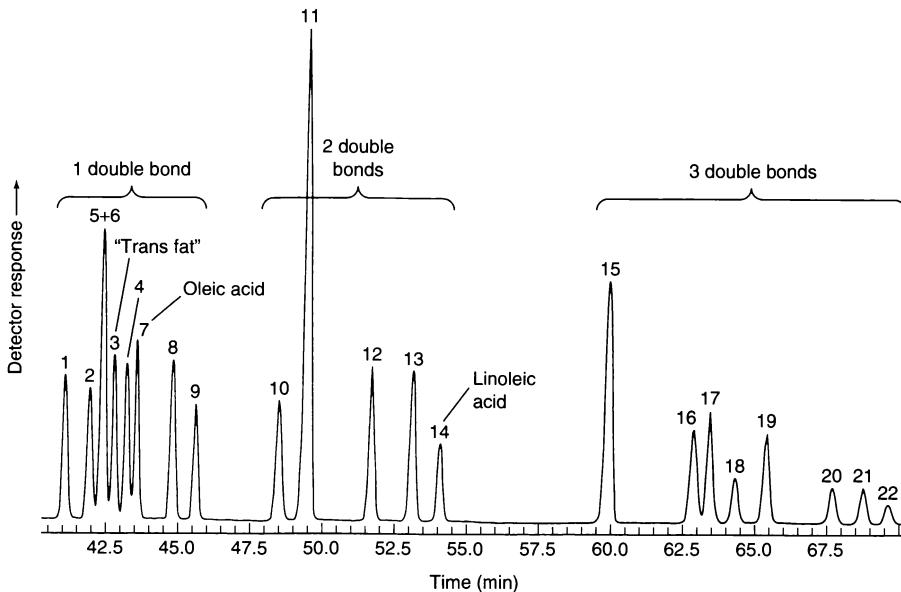
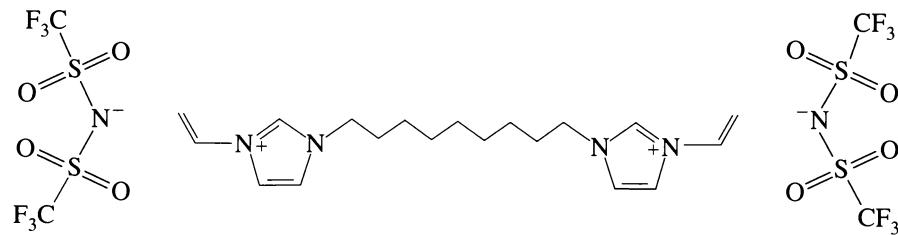


Figure 22-4 Resolution of 22 18-carbon fatty acid methyl esters with 1, 2, or 3 double bonds. Fatty acids shown in Figure 5-3 are labeled (peaks 3, 7, and 14). A Supelco IL100 ionic liquid wall-coated open tubular gas chromatography column ($100\text{ m} \times 0.25\text{ mm}$ inner diameter with $0.20\text{ }\mu\text{m}$ film thickness) was operated at 150°C with H_2 carrier gas at a linear flow rate of 25 cm/s and flame ionization detection. [From C. Ragonese, P. Q. Tranchida, P. Dugo, G. Dugo, L. M. Sidisky, M. V. Robillard, and L. Mondello, *Anal. Chem.* **2009**, *81*, 5561.]

range with low volatility at elevated temperature. Ionic liquids offer novel selectivities for polar analytes and increased operating temperature with low bleed (low loss of stationary phase). Analysis of the same mixture with a polar biscyanopropyl stationary phase did not resolve all of the fatty acids.



Ionic liquid 1,9-di(3-vinylimidazolium)nonane bis(trifluoromethyl)sulfonylimide (Supelco IL 100 stationary phase)

Molecular sieves are also used to dry gases because sieves strongly retain water. Sieves are regenerated (freed of water) by heating to 300°C in vacuum.

Common solid stationary phases include porous carbon (Figure 22-2 micrograph) and *molecular sieves*, which are inorganic materials with nanometer-size cavities that retain and separate small molecules such as H_2 , O_2 , N_2 , CO_2 , and CH_4 . Figure 22-5 compares the separation of gases by molecular sieves in a wall-coated open tubular column and in a *packed column* filled with particles of the solid stationary phase. Open tubular columns typically give better separations (narrower peaks), but packed columns can handle larger samples. In Figure 22-5, the sample injected into the packed column was 250 times larger than the sample injected into the open tubular column.

Chromatographers often use a 5- to 10-m-long **guard column** attached to the front of a chromatography column. The guard column for gas chromatography contains no stationary phase and the inner walls are *silanized* (page 464) to minimize retention of solutes. The purpose of the guard column is to collect nonvolatile components that would otherwise be injected into the chromatography column and never be eluted. Nonvolatile “junk” eventually ruins a chromatography column. A buildup of nonvolatiles in the guard column is manifested by distortion of chromatographic peaks. When this happens, we cut off and discard the beginning of the guard column.

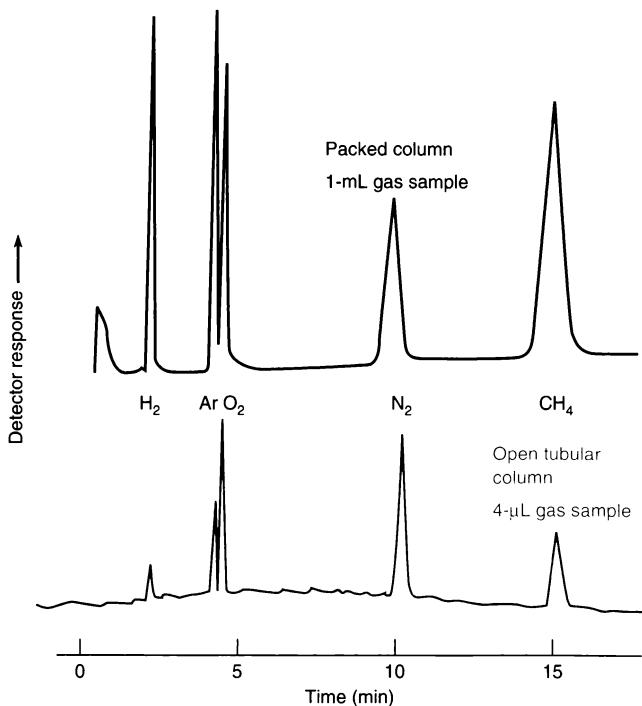


Figure 22-5 Gas chromatography with 5A molecular sieves. Upper chromatogram was obtained with a packed column (4.6 m long \times 3.2 mm diameter) at 40°C by using 1 mL of sample containing 2 ppm (by volume) of each analyte in He. Lower chromatogram was obtained with an open tubular column (30 m long \times 0.32 mm diameter) at 30°C by using 4 μL of the same sample. [From J. Madabushi, H. Cai, S. Steams, and W. Wentworth, *Am. Lab.*, October 1995, p. 21.]

Temperature Programming

If we increase the column temperature in Figure 22-1, solute vapor pressure increases and retention times decrease. To separate compounds with a wide range of boiling points or polarities, we raise the column temperature *during* the separation, a technique called **temperature programming**. Figure 22-6 shows the effect of temperature programming on the separation of nonpolar compounds with a range of boiling points from 69°C for C_6H_{14} to 356°C for $C_{21}H_{44}$. At a constant column temperature of 150°C, low-boiling compounds emerge close together and the high-boiling compounds may not be eluted. If temperature is programmed to increase from 50° to 250°C, all compounds are eluted and the separation of peaks is fairly uniform. Even though 250°C is below the boiling point of some compounds in the mixture, these compounds have sufficient vapor pressure to be eluted.

Carrier Gas

Figure 21-7 showed that H_2 and He give better resolution (smaller plate height) than N_2 at high flow rate. The reason is that solutes diffuse more rapidly through H_2 and He and therefore equilibrate between the mobile and stationary phases more rapidly than they can in N_2 . To help protect the stationary phase, carrier gas is passed through traps to remove traces of O_2 , H_2O , and hydrocarbons prior to entering the chromatograph. Metal tubing is strongly recommended over any kind of plastic tubing to maintain gas purity.

Sample Injection

Liquid volumes of $\sim 1 \mu L$ are injected through a rubber septum into a heated glass port. Gas volumes of $\sim 10 \mu L$ up to 5 mL can be injected with a gas-tight syringe or introduced via a sample injection valve similar to that shown later for liquid chromatography

Raising column temperature

- decreases retention time
- sharpens peaks

We refer to constant-temperature conditions as *isothermal* conditions.

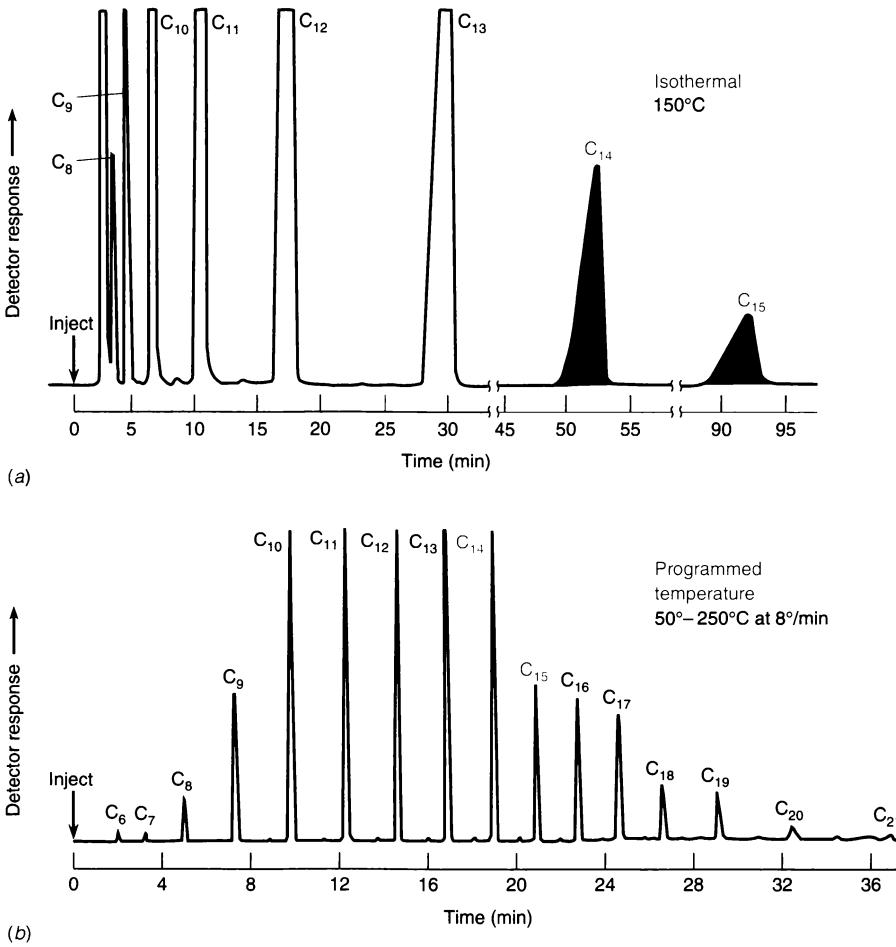


Figure 22-6 (a) Isothermal and (b) programmed temperature chromatography of linear alkanes through a packed column with a nonpolar stationary phase. Detector sensitivity is 16 times greater in (a) than in (b). [From H. M. McNair and E. J. Bonelli, *Basic Gas Chromatography* (Palo Alto, CA: Varian Instrument Division, 1968).]

in Figure 22-21. Carrier gas sweeps the vaporized sample into the chromatography column. A complete injection usually contains too much material for an open tubular capillary column. In **split injection** (Figure 22-7a), only 0.1–10% of the injected sample reaches the column. The remainder is blown out to waste. If the entire sample is not vaporized during injection, however, higher boiling components are not completely injected and there will be errors in quantitative analysis. The fatty acid analysis in Figure 22-4 used split injection with 99% of the sample diverted to waste.

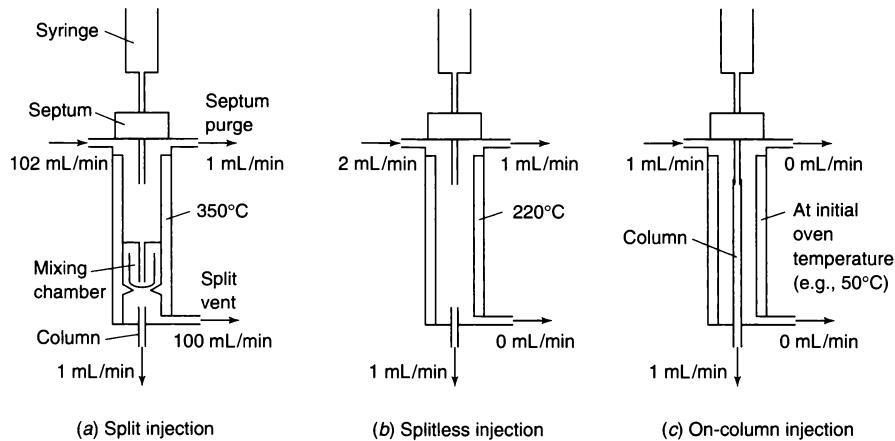


Figure 22-7 Injection port operation for (a) split, (b) splitless, and (c) on-column injection into an open tubular column. A slow flow of gas past the inside surface of the septum out to waste cools the rubber and prevents volatile emissions from the rubber from entering the chromatography column.

For quantitative analysis and for analysis of trace components of a mixture, **splitless injection** (Figure 22-7b) is appropriate. (*Trace components* are those present at extremely low concentrations.) For this purpose, a dilute sample in a low-boiling solvent is injected at a column temperature 40° below the boiling point of the solvent. Solvent condenses at the beginning of the column and traps a thin band of solute. (Hence, this technique is called **solvent trapping**.) After additional vapors have been purged from the injection port, the column temperature is raised and chromatography is begun. In splitless injection, ~80% of the sample is applied to the column, and little fractionation (selective evaporation of components) occurs during injection.

A technique called **cold trapping** is used to focus high-boiling solutes at the beginning of a column. In this case, the column is initially 150° lower than the boiling points of solutes of interest. Solvent and low-boiling solutes are eluted rapidly, but high-boiling solutes condense in a narrow band at the start of the column. The column is later warmed to initiate chromatography for the components of interest. The chromatogram of bisphenol A in Box 21-3 began with cold trapping the vapors from the solid-phase microextraction fiber.

For sensitive compounds that decompose above their boiling temperature, we use **on-column injection** of solution directly into the column (Figure 22-7c), without going through a hot port. Analytes are focused in a narrow band by solvent trapping or cold trapping. Warming the column initiates chromatography.

“Wide-bore” columns (diameter ≥ 0.53 mm) are wide enough to accept the common syringe needle for on-column injection. Narrower columns (typically 0.10–0.32 mm diameter) provide higher resolution (sharper peaks) but have less capacity and require higher operating pressure. Diameters ≥ 0.32 mm are too large to be used with mass spectral detection because the mass flow rate is too high for most vacuum pumps to handle.

Flame Ionization Detector

In the **flame ionization detector** in Figure 22-8, eluate is burned in a mixture of H₂ and air. Carbon atoms (except carbonyl and carboxyl carbon atoms) produce CH radicals, which go on to produce CHO⁺ ions in the flame:

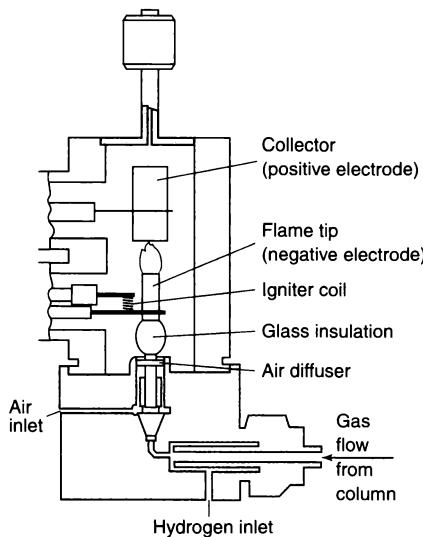
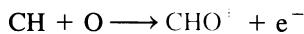


Figure 22-8 Flame ionization detector. [Courtesy Varian Associates, Palo Alto, CA.]

Injection into open tubular columns:

- *split*: routine method
- *splitless*: best for quantitative analysis
- *on-column*: best for thermally unstable solutes

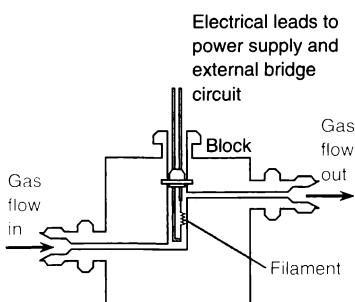


Figure 22-9 Thermal conductivity detector. [Courtesy Varian Associates, Palo Alto, CA.]

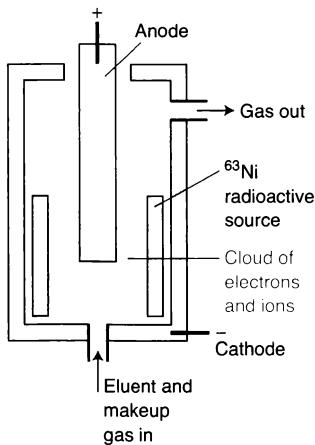
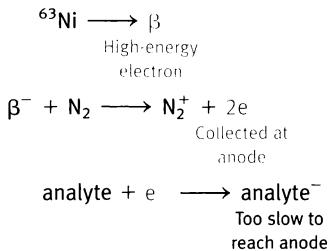


Figure 22-10 Electron capture detector.

Electron capture detector:



The flow of ions and electrons to the electrodes produces the detector signal. Only about 1 in 10^5 carbon atoms produces an ion, but ion production is proportional to the number of susceptible carbon atoms entering the flame. Response to organic compounds is proportional to solute mass over seven orders of magnitude. The detector is sensitive enough for narrow-bore columns. It responds to most hydrocarbons and is insensitive to nonhydrocarbons such as H₂, He, N₂, O₂, CO, CO₂, H₂O, NH₃, NO, H₂S, and SiF₄. The detection limit is 100 times smaller than that of the thermal conductivity detector and is best with N₂ carrier gas. For open tubular columns eluted with H₂ or He, N₂ *makeup gas* is added to the stream before the stream reaches the detector. Makeup gas provides the higher flow rate needed by the detector and improves sensitivity. Fatty acid analysis in Figure 22-4 uses flame ionization detection.

Thermal Conductivity Detector

Thermal conductivity is a measure of the ability of a substance to transport heat. In the **thermal conductivity detector** in Figure 22-9, gas emerging from the chromatography column flows over a hot tungsten-rhenium filament. When solute emerges from the column, the thermal conductivity of the gas stream decreases, the filament gets hotter, its electric resistance increases, and the voltage across the filament increases. The voltage change is the detector signal. Thermal conductivity detection is more sensitive at lower flow rates. To prevent overheating and oxidation of the filament, the detector should never be left on unless carrier gas is flowing.

The detector responds to *changes* in thermal conductivity, so the conductivities of solute and carrier gas should be as different as possible. Because H₂ and He have the highest thermal conductivity, these are the carriers of choice for thermal conductivity detection. A thermal conductivity detector responds to every substance except the carrier gas.

Thermal conductivity detectors are generally not sensitive enough to detect the small quantity of analyte eluted from open tubular columns smaller than 0.53 mm in diameter. For narrower columns, other detectors must be used.

Electron Capture Detector

The **electron capture detector** in Figure 22-10 is extremely sensitive to halogen-containing molecules, such as chlorinated pesticides and fluorocarbons in environmental samples, but relatively insensitive to hydrocarbons, alcohols, and ketones. Carrier gas entering the detector is ionized by high-energy electrons ("β-rays") emitted from a foil containing radioactive ⁶³Ni. Electrons liberated from the gas are attracted to an anode, producing a small, steady current. When analyte molecules with a high electron affinity enter the detector, they capture some of the electrons and reduce the current. The detector responds by varying the frequency of voltage pulses between anode and cathode to maintain constant current. Electron capture is extremely sensitive, with a detection limit of ~5 fg (femtogram, 10^{-15} g), comparable to that of selected ion monitoring by mass spectrometry. The carrier gas is usually N₂ or 5 vol% CH₄ in Ar. For open tubular columns, chromatography is conducted with H₂ or He at low flow rate and N₂ makeup gas is added to the stream prior to the detector.

Other Detectors

A **flame photometric detector** measures optical emission from phosphorus and sulfur compounds. When eluate passes through a H₂-air flame, excited sulfur- and phosphorus-containing species emit characteristic radiation, which is detected with a photomultiplier tube. Radiant emission is proportional to analyte concentration.

The *alkali flame detector*, also called a *nitrogen-phosphorus detector*, is a modified flame ionization detector that is selectively sensitive to phosphorus and nitrogen. It is especially important for drug analysis. Ions such as NO_2^- , CN^- , and PO_2^- , produced by these elements when they contact a Rb_2SO_4 -containing glass bead at the burner tip create the current that is measured. N_2 from air is inert to this detector and does not interfere. The bead must be replaced periodically because Rb_2SO_4 is consumed.

A *sulfur chemiluminescence detector* mixes the exhaust from a flame ionization detector with O_3 to form an excited state of SO_2 that emits light, which is detected.

Mass Spectrometric Detection and Selected Reaction Monitoring

A *mass spectrometer* (Section 21-4) is the single most versatile detector. The *reconstructed total ion chromatogram* (Figure 21-14a) shows all components in a mixture. The mass spectrum of each component recorded as it is eluted provides qualitative identification. Alternatively, *selected ion monitoring* at one value of m/z (Figures 21-14b–h) responds to one or a few components of a mixture. Because it does not respond to everything in the sample, selected ion monitoring reduces interference from overlapping chromatographic peaks.

Interference is further reduced and the *signal-to-noise ratio* is further increased by a powerful mass spectral technique called **selected reaction monitoring**. Figure 22-11 shows a *triple quadrupole mass spectrometer* in which a mixture of ions enters quadrupole Q1, which passes just one selected *precursor ion* to the second stage, Q2. The second stage passes all ions of all masses straight on to the third stage, Q3. However, while inside Q2, which is called a *collision cell*, the precursor ion collides with N_2 or Ar at a pressure of $\sim 10^{-8}$ to 10^{-6} bar and breaks into fragments called *product ions*. Quadrupole Q3 allows only specific product ions to reach the detector.

Selected reaction monitoring is extremely selective for the analyte of interest. For example, we can monitor traces of caffeine in natural waters to detect contamination by domestic wastewater. The global daily average consumption of caffeine in Europe and North America is ~ 200 – 400 mg per person, much of which ends up in sewage. If caffeine shows up in a municipal water supply, it probably migrated from wastewater. To measure parts per trillion levels of caffeine, 1 L of water was passed through a column containing 10 mL of adsorbent polystyrene beads that retain caffeine and a host of other

Gas chromatography detectors:

- *flame ionization*: responds to compounds with C—H
- *thermal conductivity*: responds to everything, but not sensitive enough for columns < 0.53 mm in diameter
- *electron capture*: halogens, conjugated $\text{C}=\text{O}$, $-\text{C}\equiv\text{N}$, $-\text{NO}_2$
- *flame photometer*: P and S
- *alkali flame*: P and N
- *sulfur chemiluminescence*: S
- *mass spectrometer*: responds to everything

Signal arises from what we seek to measure and *noise* is random variation in the instrument response. At the *signal detection limit* (Equation 5-2), signal is 3 times greater than noise. At the *lower limit of quantitation*, the signal-to-noise ratio is 10, which we can measure with moderate precision.

Because it uses two consecutive mass spectrometers, selected reaction monitoring is also called *tandem mass spectrometry* or *mass spectrometry-mass spectrometry*, or just *MS-MS*.

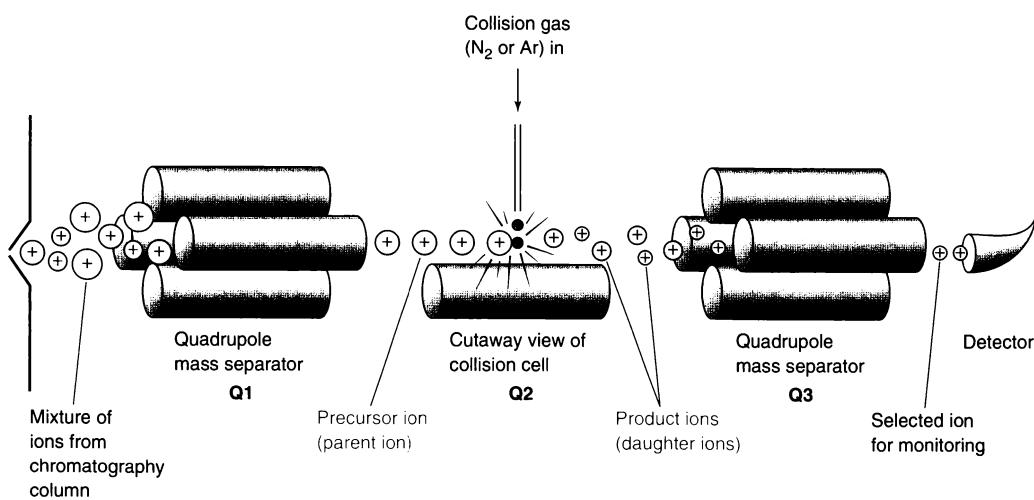


Figure 22-11 Principle of selected reaction monitoring.

organic compounds. Caffeine and many other substances were then eluted with organic solvent, dried, and evaporated to 0.1–1 mL. By this *solid-phase extraction* procedure, caffeine was *preconcentrated* by a factor of 10^3 – 10^4 prior to chromatography.

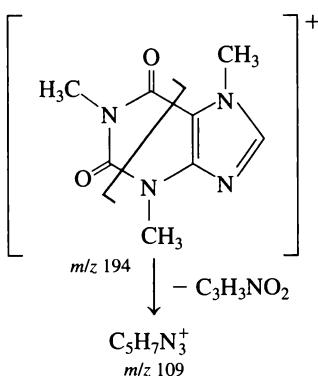


Figure 22-12a shows the electron ionization mass spectrum of caffeine. For selected reaction monitoring, the *m/z* 194 precursor ion was selected by quadrupole Q1 in Figure 22-11 and the *m/z* 109 product ion was selected by Q3 for detection. Few compounds other than caffeine produce the *m/z* 194 precursor ion, and it would be rare for any of them to give the *m/z* 109 product ion because they are unlikely to break into the same fragments as caffeine. The chromatogram in Figure 22-12b shows only one significant peak, even though the caffeine content was only 4 ng/L (parts per trillion) in the water sample and, undoubtedly, many other compounds were present at higher concentrations.

For quantitative analysis, ¹³C₃-caffeine was added to the sample as an *internal standard*. This isotopic molecule is eluted at the same time as ordinary caffeine, but the two are detected separately by selected reaction monitoring. The internal standard is detected by its precursor ion at *m/z* 197 and product ion at *m/z* 111. The paper cited in Figure 22-12 found that 1–4% of caffeine discharged by people in the catchment area of one Swiss lake entered the lake. Most caffeine apparently entered the lake

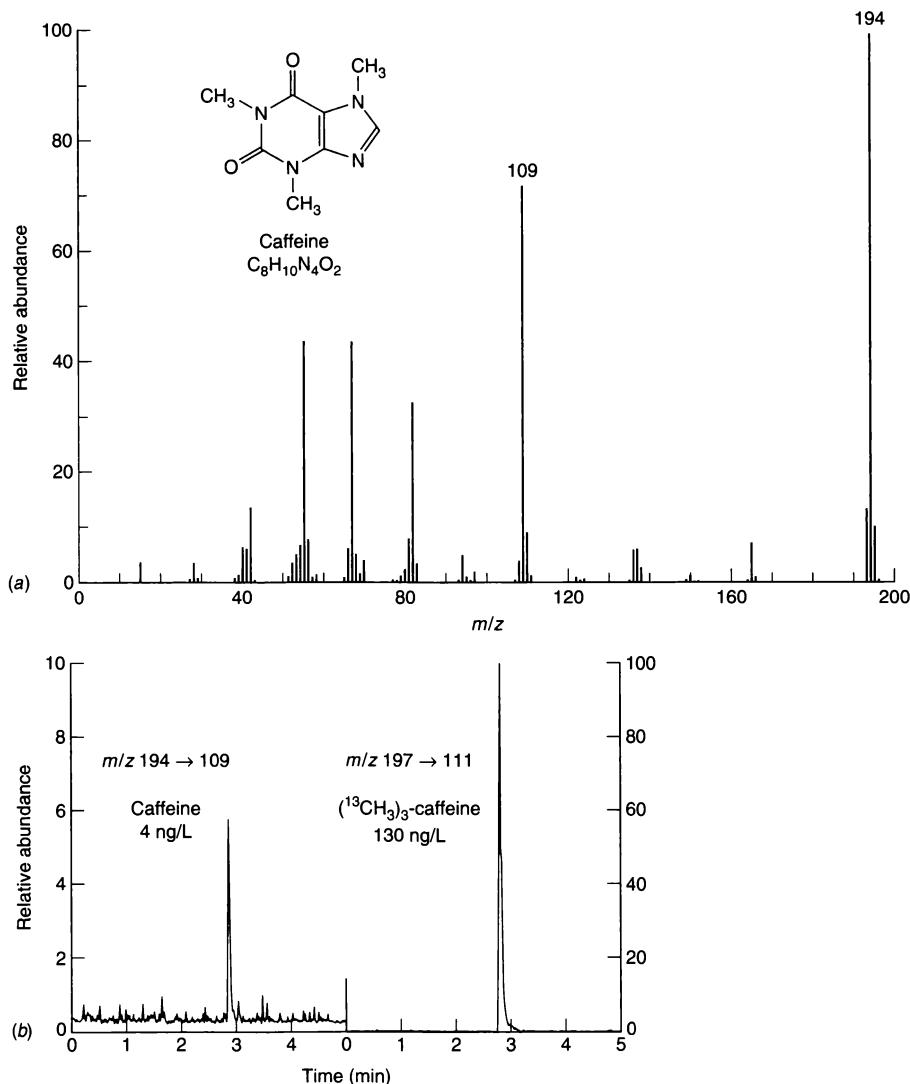


Figure 22-12 (a) Electron ionization mass spectrum of caffeine. [From NIST/EP/NIH Mass Spectral Database.] (b) Selected reaction monitoring gas chromatogram of water collected 5 m below the surface of the Mediterranean Sea and containing 4 ng of caffeine per liter. [From I. J. Buerge, T. Poiger, M. D. Müller, and H.-R. Buser, *Environ. Sci. Technol.* **2003**, 37, 691.]

during rainstorms when the capacity of wastewater treatment plants was exceeded and sewage flowed directly into the lake.

Ask Yourself

- 22-A. (a) What is the advantage of temperature programming in gas chromatography?
- (b) What is the advantage of an open tubular column over a packed column? Does a narrower or wider open tubular column provide higher resolution? What is the advantage of a packed column over an open tubular column?
- (c) Why do H₂ and He allow more rapid linear flow rates in gas chromatography than does N₂, without loss of column efficiency (see Figure 21-7)?
- (d) When would you use split, splitless, or on-column injection?
- (e) To which kinds of analytes do the following detectors respond: (i) flame ionization, (ii) thermal conductivity, (iii) electron capture, (iv) flame photometric, (v) alkali flame, (vi) sulfur chemiluminescence, and (vii) mass spectrometer?
- (f) What information is displayed in a reconstructed total ion chromatogram, selected ion monitoring, and selected reaction monitoring? Which technique is most selective and which is least selective and why?
- (g) Why does the chromatogram in Figure 22-12 show a single peak for caffeine when there are many compounds at higher concentration in the sample?

22-2 Classical Liquid Chromatography

Modern chromatography evolved from the experiment in Figure 21-1 in which sample is applied to the top of an open, gravity-fed column containing stationary phase. Section 22-3 describes high-performance liquid chromatography, which uses closed columns under high pressure and is the most common form of liquid chromatography today. Open columns are used for preparative separations in biochemistry and chemical synthesis.

There is an art to pouring uniform columns, applying samples evenly, and obtaining symmetric elution bands. Stationary solid phase is normally poured into a column by first making a *slurry* (a mixture of solid and liquid) and pouring the slurry gently down the wall of the column. Try to avoid creating distinct layers, which form when some of the slurry is allowed to settle before more is poured in. Do not drain solvent below the top of the stationary phase, because air spaces and irregular flow patterns will be created. Solvent should be directed gently down the wall of the column. In *no* case should the solvent be allowed to dig a channel into the stationary phase. Maximum resolution demands slow flow.

Stationary Phase

For adsorption chromatography, *silica* ($\text{SiO}_2 \cdot x\text{H}_2\text{O}$, also called silicic acid) is a common stationary phase. Its active adsorption sites are Si—O—H (silanol) groups, which are slowly deactivated by adsorption of water from the air. Silica is activated by heating to 200°C to drive off water. *Alumina* ($\text{Al}_2\text{O}_3 \cdot x\text{H}_2\text{O}$) is the other most common adsorbent. Preparative chromatography in the biochemistry lab is most often based on molecular exclusion and ion exchange, described in Chapter 23.

Solvents

In *adsorption chromatography*, solvent competes with solute for adsorption sites on the stationary phase. *The relative abilities of different solvents to elute a given solute*

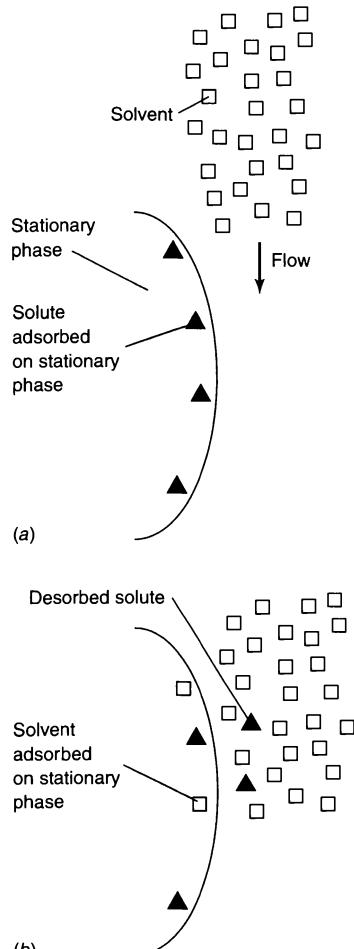


Figure 22-13 Solvent molecules compete with solute molecules for binding sites on the stationary phase. The more strongly the solvent binds to the stationary phase, the greater the *eluent strength* of the solvent.

Gradient elution in liquid chromatography is analogous to temperature programming in gas chromatography. Increased eluent strength is required to elute more strongly retained solutes.

Question According to the scaling rules in Section 21-2, if column diameter is increased from 4 mm to 40 mm, how much larger can the sample be to achieve the same resolution?

Table 22-2 Eluotropic series and ultraviolet cutoff wavelengths of solvents for adsorption chromatography on silica^a

Solvent	Eluent strength (ϵ°)	Ultraviolet cutoff (nm) ^b
Pentane	0.00	190
Hexane	0.01	195
Heptane	0.01	200
Trichlorotrifluoroethane	0.02	231
Toluene	0.22	284
Chloroform	0.26	245
Dichloromethane	0.30	233
Diethyl ether	0.43	215
Ethyl acetate	0.48	256
Methyl <i>t</i> -butyl ether	0.48	210
Dioxane	0.51	215
Acetonitrile	0.52	190
Acetone	0.53	330
Tetrahydrofuran	0.53	212
2-Propanol	0.60	205
Methanol	0.70	205

a. From L. R. Snyder in *High-Performance Liquid Chromatography* (C. Horváth, ed.), vol. 3 (New York: Academic Press, 1983); *Burdick & Jackson Solvent Guide*, 3rd ed. (Muskegon, MI: Burdick & Jackson Laboratories, 1990).

b. Ultraviolet cutoff is the approximate minimum wavelength at which solutes can be detected above the strong ultraviolet absorbance of solvent. The ultraviolet cutoff for water is 190 nm.

from the column are nearly independent of the nature of the solute. Elution can be described as a displacement of solute from the adsorbent by solvent (Figure 22-13).

An *eluotropic series* ranks solvents by their abilities to displace solutes from a given adsorbent. **Eluent strength** in Table 22-2 is a measure of solvent adsorption energy, with the value for pentane defined as 0. The more polar the solvent, the greater its eluent strength. The greater the eluent strength, the more rapidly solutes are eluted from the column.

A *gradient* (steady change) of eluent strength is used for many separations. First, weakly retained solutes are eluted with a solvent of low eluent strength. Then a second solvent is mixed with the first, either in discrete steps or continuously, to increase eluent strength and elute more strongly adsorbed solutes. A small amount of polar solvent markedly increases the eluent strength of a nonpolar solvent.



Ask Yourself

22-B. Why are the relative eluent strengths of solvents in adsorption chromatography fairly independent of solute?

22-3 High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) uses high pressure to force eluent through a closed column packed with micrometer-size particles that provide exquisite separations. The analytical HPLC equipment in Figure 22-14 uses columns

Autosampler

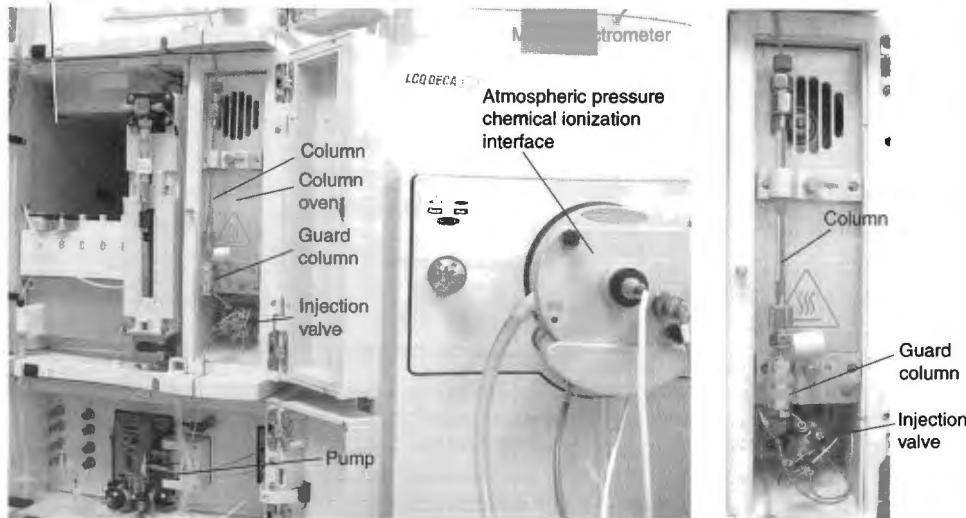


Figure 22-14 High-performance liquid chromatograph (HPLC) including a mass spectrometer for detection. The column is enlarged at the right. In operation, the door to the column oven would be closed to maintain constant temperature. [Courtesy E. Erickson, Michelson Laboratory, China Lake, CA..]



Figure 22-15 A 300-liter preparative chromatography column can purify a kilogram of material. [Courtesy Prochrom, Inc., Indianapolis, IN.]

with diameters of 1–5 mm and lengths of 5–30 cm, yielding 50 000 to 100 000 plates per meter. Essential components include a solvent delivery system, a sample injection valve, a detector, and a computer to control the system and display results. The industrial preparative column in Figure 22-15 can handle as much as 1 kg of sample.

Under optimum conditions, resolution increases as stationary phase particle size decreases. Notice how much sharper the peaks become in Figure 22-16 when particle size is changed from 4 μm to 1.7 μm . Sharper peaks allow us to decrease the time required for a separation by increasing the flow rate or increasing the solvent strength, while maintaining adequate resolution. In going from Figure 22-16b to Figure 22-16c, the time has been reduced by a factor of 4 by using a stronger solvent.

van Deemter curves in Figure 22-17 illustrates how decreasing particle size decreases optimum plate height and allows a higher flow rate with little increase in plate height. Smaller particles allow solute to diffuse shorter distances to equilibrate between the stationary and mobile phases, thereby decreasing the term C in the van Deemter equation 21-7. Smaller particles also decrease irregular flow paths (the term A).

The penalty for using fine particles is resistance to solvent flow. Until recently, HPLC operated at pressures of ~70–400 bar to attain flow rates of ~0.5–5 mL/min. In 2004, equipment became available to employ 1.5- to 2- μm -diameter particles at pressures up to 1 000 bar. These instruments provide increased resolution or decreased run time. Chromatography with 1.5- to 2- μm -diameter particles at high pressure is commonly called UPLC (Ultra-Performance Liquid Chromatography).

Decreased particle size increases resolution but requires high pressure to obtain a reasonable flow rate.

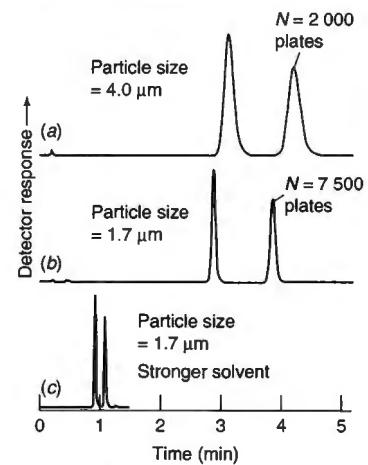


Figure 22-16 (a and b) Chromatograms of the same sample run at the same linear velocity on 5.0-cm-long columns packed with C₁₈-silica. (c) A stronger solvent was used to elute solutes more rapidly from the column in panel b. [From Y. Yang and C. C. Hodges, *LCGC Supplement*, May 2005, p. 31.]

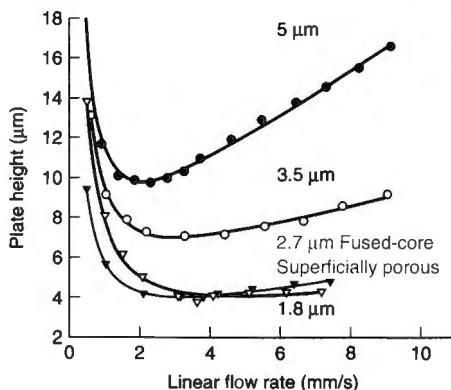


Figure 22-17 van Deemter curves: Plate height as a function of *linear flow rate* (mm/s) for microporous (Figure 22-18) stationary phase particle diameters of 5.0, 3.5, and 1.8 μm , as well as *superficially porous* (or *fused-core*) particles (Figure 22-19) with a diameter of 2.7 μm and a 0.5- μm porous layer thickness. Measurements for naphthalene eluted from C_{18} -silica (50 mm long \times 4.6 mm diameter) with 60 vol% acetonitrile/40 vol% H_2O at 24°C. [Courtesy MAC-MOD Analytical, Chadds Ford, PA.]

Stationary Phase

Normal-phase chromatography: polar stationary phase and less polar solvent

Reversed-phase chromatography: low-polarity stationary phase and polar solvent

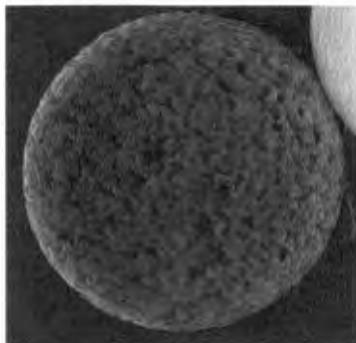


Figure 22-18 Scanning electron micrograph of 4.4- μm -diameter microporous silica chromatography particle from an experimental batch made by K. Wyndham at Waters Corporation. [Photo kindly provided by J. Jorgensen, University of North Carolina.]

Normal-phase chromatography uses a polar stationary phase and a less polar solvent. *Eluent strength is increased by adding a more polar solvent.* **Reversed-phase chromatography** is the more common scheme in which the stationary phase is nonpolar or weakly polar and the solvent is more polar. *Eluent strength is increased by adding a less polar solvent.* In general, eluent strength is increased by making the mobile phase more like the stationary phase. Reversed-phase chromatography eliminates tailing (Figure 21-10b) arising from adsorption of polar compounds by polar packings. Reversed-phase chromatography is also relatively insensitive to polar impurities (such as water) in the eluent.

Microporous particles of silica with diameters of 1.5–10 μm are the most common solid stationary phase support (Figure 22-18). These particles are permeable to solvent and have a surface area as large as 500 m^2 per gram of silica. Solute adsorption occurs directly on the silica surface.

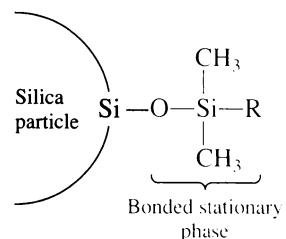
Most commonly, liquid-liquid partition chromatography is conducted with a **bonded stationary phase** covalently attached to silanol groups on the silica surface (Table 22-3). The octadecyl (C_{18}) stationary phase is, by far, the most common in HPLC. The Si—O—Si bond that attaches the stationary phase to the silica is stable only over the pH range 2–8. Strongly acidic or basic eluents generally cannot be used with silica.

Different stationary phases interact differently with the same set of solutes. For example, retention times and order of elution of different compounds are generally different when switching from a nonpolar octadecyl (C_{18}) column to a nonpolar pentafluorophenyl column. A separation that is not adequate on C_{18} might be adequate on pentafluorophenyl.

Figure 22-19 shows rapid separation of proteins on **superficially porous particles** (also called *fused-core* particles), which consist of a 0.25- μm -thick porous silica layer on a 5- μm -diameter nonporous silica core. A stationary phase such as C_{18} is bonded throughout the thin, porous outer layer. Diffusion of solute into a

Table 22-3 Some common bonded phases for liquid chromatography

Bonded polar phases		Bonded nonpolar phases
$R = (\text{CH}_2)_3\text{NH}_2$	Amino	$R = (\text{CH}_2)_{17}\text{CH}_3$ Octadecyl
$R = (\text{CH}_2)_3\text{C}\equiv\text{N}$	Cyano	$R = (\text{CH}_2)_7\text{CH}_3$ Octyl
$R = (\text{CH}_2)_3\text{OCH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$	Diol	$R = (\text{CH}_2)_3\text{C}_6\text{H}_5$ Phenyl
$R = (\text{spacer})\text{CH}_2\text{N}(\text{CH}_3)_2(\text{CH}_2)_3\text{SO}_3^-$	ZIC-HILIC®	$R = (\text{CH}_2)_3\text{C}_6\text{F}_5$ Pentafluorophenyl



0.25-μm-thick layer is faster than diffusion into fully porous particles with a radius of 2.5 μm, thus enabling high chromatographic efficiency at high flow rate. Superficially porous particles are especially suitable for separation of macromolecules such as proteins, which diffuse more slowly than small molecules. Figure 22-17 showed that the van Deemter curve for superficially porous particles with a total diameter of 2.7 μm and a porous layer thickness of 0.5 μm is similar to that of a totally porous particle with a diameter of 1.8 μm. The superficially porous particle enables separations similar to those achieved with 1.8-μm totally porous particles without requiring such high pressure.

Optical isomers (also called *enantiomers*) are mirror image compounds such as D- and L-amino acids that cannot be superimposed on each other. Most compounds with four different groups attached to one tetrahedral carbon atom exist in two mirror image isomers. Optical isomers can be separated from each other by chromatography on a stationary phase containing just one optical isomer of the bonded phase (called a *chiral column*). The pharmaceutical industry separates optical isomers of some drugs because one isomer could be biologically active while the other is inactive or toxic. The drug thalidomide, prescribed in the 1960s to prevent morning sickness in pregnant women, produced gross birth defects in more than 10 000 children before it was banned. It was later discovered that one enantiomer of thalidomide has a desirable

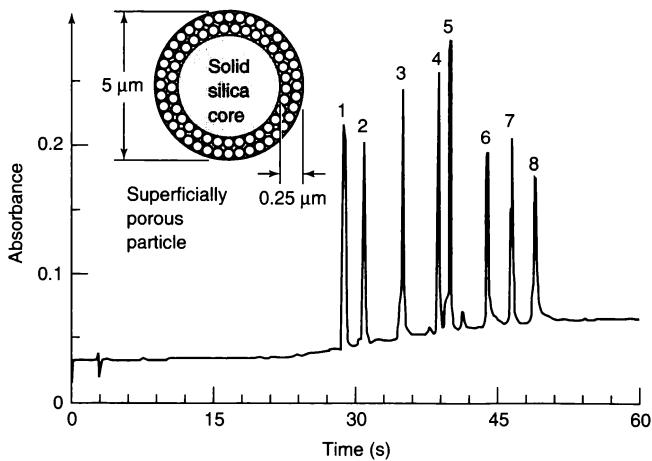
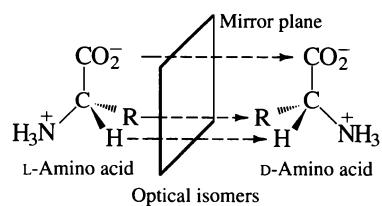


Figure 22-19 Rapid separation of eight proteins on superficially porous C₁₈-silica in 75 × 2.1-mm column containing Poroshell 300SB-C18 eluted at 3 mL/min at 70°C at 26 MPa (260 bar) with ultraviolet detection at 215 nm. [From R. E. Majors, *LCGC Column Technology Supplement*, June 2004, p. 8K. Courtesy Agilent Technologies.]

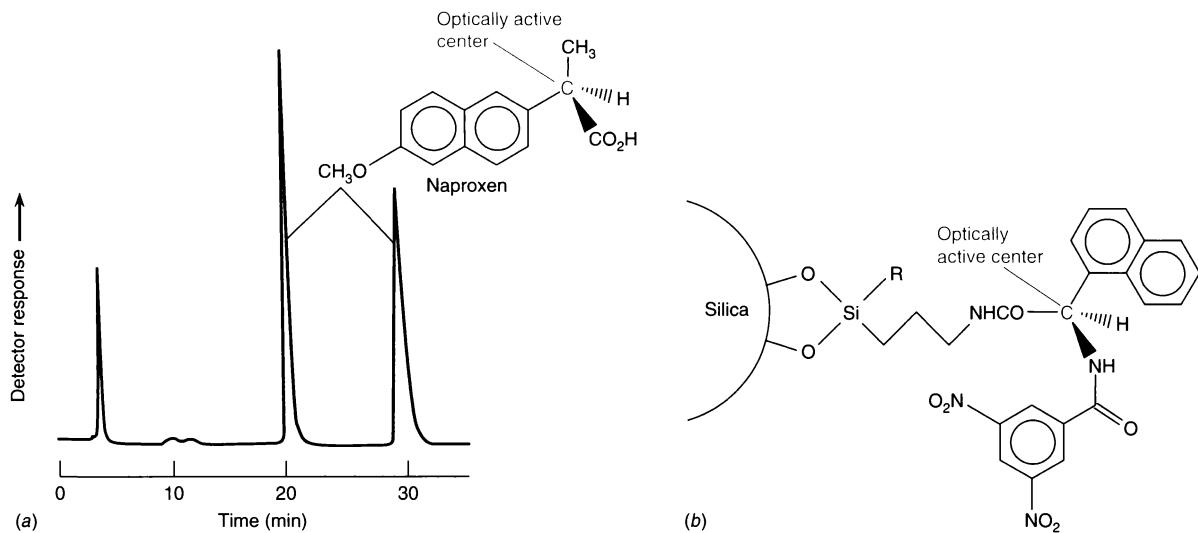


Figure 22-20 (a) HPLC separation of the two optical isomers (mirror image isomers) of the drug Naproxen eluted with 0.05 M ammonium acetate in methanol. Naproxen is the active ingredient of the anti-inflammatory drug Aleve®. (b) Structure of the bonded stationary phase. [Courtesy Phenomenex, Torrance, CA.]

physiological effect and its mirror image caused birth defects. Figure 22-20 shows separation of the two optical isomers of the anti-inflammatory drug Naproxen.

The Column

High-performance liquid chromatography columns are expensive and easily degraded by irreversible adsorption of impurities from samples and solvents. Therefore we place a short, disposable *guard column* containing the same stationary phase as that of the main column at the entrance to the main column (lower right in Figure 22-14). Substances that would bind irreversibly to the main column are instead bound in the guard column, which is periodically discarded.

Columns are commonly made of stainless steel. The stationary phase is retained by porous, stainless steel frits at both ends of the column. The entrance frit acts as a filter for particles and helps distribute liquid evenly over the column diameter. Samples should be passed through a 0.5- to 2-μm filter *prior* to injection to prevent contaminating the column with particles, plugging tubing, and damaging the pump. Deterioration of peak shape is a common indication of clogging of the frit, which can be reverse-flushed with solvent or replaced.

Because the column is under high pressure, a special technique is required to inject sample. The *injection valve* in Figure 22-21 has interchangeable steel sample loops that hold fixed volumes from 2 μL to 1 000 μL. In the load position, a syringe is used to wash and load the loop with fresh sample at atmospheric pressure. When the valve is rotated 60° counterclockwise, the content of the sample loop is injected into the column at high pressure.

For liquid chromatography, use a *blunt nose syringe*, not the pointy needle from gas chromatography. There should also be a 0.5-μm frit between the injection valve or autosampler and the chromatography column to provide additional protection against small particles that can ruin an expensive column. In-line filters should be used between solvent reservoirs and the pump.

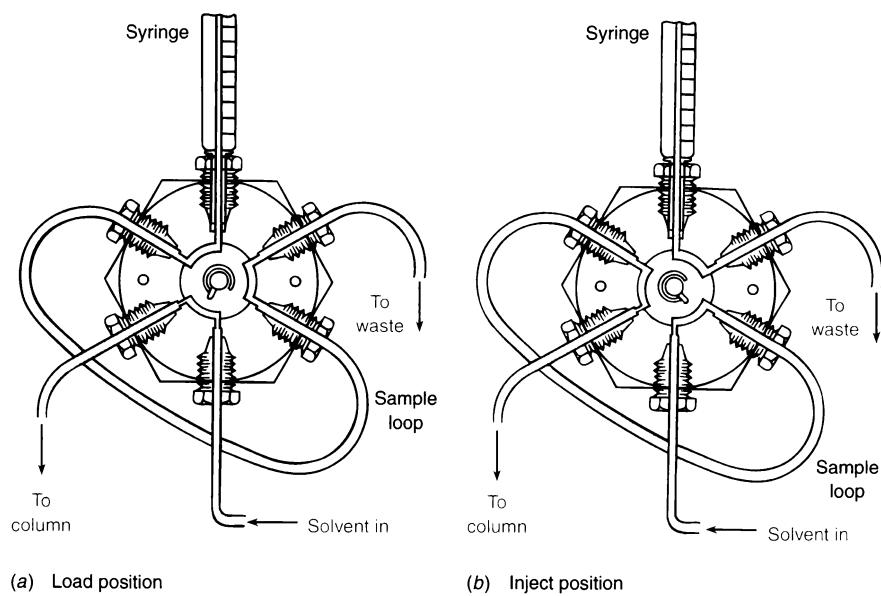


Figure 22-21 Injection valve for HPLC. Replaceable sample loop comes in various fixed-volume sizes. Similar valves can be used to inject gaseous samples for gas chromatography.

Solvents

Elution with a single solvent or a constant solvent mixture is called **isocratic elution**. If one solvent does not discriminate adequately between the components of a mixture or if the solvent does not provide sufficiently rapid elution of all components, then **gradient elution** can be used. In gradient elution, solvent is changed continuously from weak to stronger eluent strength by mixing more strong solvent into the weak solvent during chromatography.

Figure 22-22 shows the effect of eluent strength in the *isocratic* elution of eight compounds from a reversed-phase column. In a reversed-phase separation, eluent strength increases as the solvent becomes *less* polar. The first chromatogram (upper left) was obtained with a solvent consisting of 90 vol% acetonitrile and 10 vol% aqueous buffer. Acetonitrile is an organic solvent that is less polar than water. Acetonitrile has a high eluent strength, and all compounds are eluted rapidly. In fact, only three peaks are observed because of overlap. It is customary to call the aqueous solvent A and the organic solvent B. The first chromatogram was obtained with 90% B. When eluent strength is *reduced* by changing the solvent to 80% B, there is slightly more separation and five peaks are observed. At 60% B, we begin to see a sixth peak. At 40% B, there are eight clear peaks, but compounds 2 and 3 are not fully resolved. At 30% B, all peaks are resolved, but the separation takes too long. Backing up to 35% B (the bottom trace) separates all peaks in ~ 2 h (which is still too long for many purposes).

From the isocratic elutions in Figure 22-22, the *gradient* in Figure 22-23 was selected to resolve all peaks while reducing the time from 2 h to 38 min. First, 30% B was run for 8 min to separate components 1, 2, and 3. The eluent strength was then increased steadily over 5 min to 45% B and held there for 15 min to elute peaks 4 and 5. Finally, the solvent was changed to 80% B over 2 min and held there to elute the last peaks.

Pure HPLC solvents are expensive, and most organic solvents are expensive to dispose of in an environmentally safe manner. To reduce waste, choose a 2.1-mm-diameter column instead of a 4.6-mm-diameter column. If you can use a smaller particle size, you can employ a shorter column to obtain the same resolution and reduce solvent use.

To prepare a mixture of aqueous buffer with an organic solvent, adjust the pH of the buffer to the desired value *prior* to mixing it with organic solvent. Once you mix in the organic solvent, the meaning of "pH" is not well defined.

Aqueous solvent ≡ A
Organic solvent ≡ B

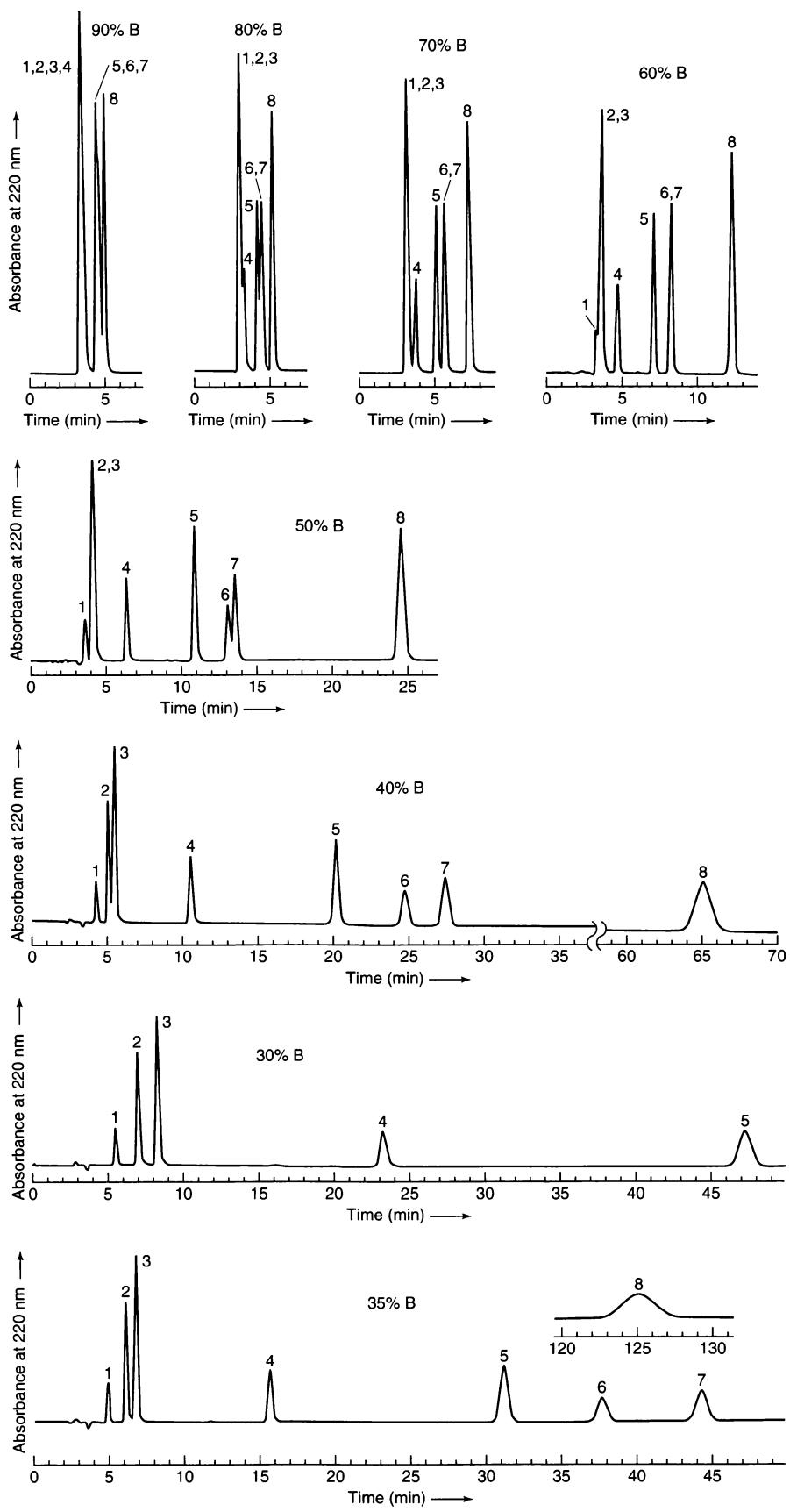


Figure 22-22 Isocratic HPLC separation of a mixture of aromatic compounds at 1.0 mL/min on a 25 × 0.46-cm Hypersil ODS column (C₁₈ on 5-μm silica) at ambient temperature (~22°C): (1) benzyl alcohol; (2) phenol; (3) 3',4'-dimethoxyacetophenone; (4) benzoin; (5) ethyl benzoate; (6) toluene; (7) 2,6-dimethoxytoluene; (8) *o*-methoxybiphenyl. Eluent consisted of aqueous buffer (designated A) and acetonitrile (designated B). The notation "90% B" in the first chromatogram means 10 vol% A and 90 vol% B. The buffer contained 25 mM KH₂PO₄ plus 0.1 g/L sodium azide adjusted to pH 3.5 with HCl.

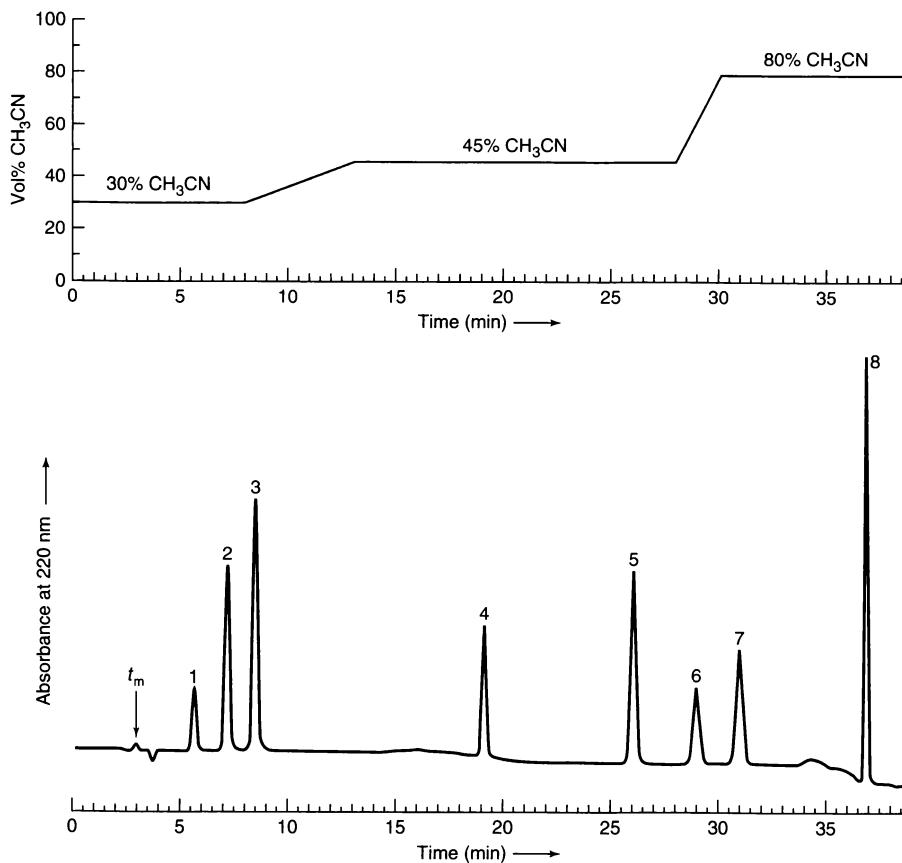


Figure 22-23 Gradient elution of the same mixture of aromatic compounds shown in Figure 22-22 with the same column, flow rate, and solvents. The upper trace is the *segmented gradient* profile, so named because it is divided into several different segments.

Detectors

An **ultraviolet detector** measures the absorbance of eluate at one wavelength or over a range of wavelengths. Analyte must absorb ultraviolet radiation to be detected. A flow cell such as that in Figure 22-24 functions as a waveguide with light rays reflected at the walls to increase the effective pathlength. Some systems employ the intense 254-nm emission of a mercury lamp. Other instruments use a deuterium or xenon lamp and monochromator, so you can choose the optimum wavelength for your analytes. In some systems, the ultraviolet-visible absorption spectrum of each solute can be recorded with a photodiode array (Figure 19-12) as it is eluted.

The *electrochemical detector* in Figure 17-7 responds to analytes that can be oxidized or reduced at an electrode over which eluate passes. Electric current is proportional to solute concentration. *Fluorescence detectors* are especially sensitive but respond only to analytes that fluoresce (Figure 19-24) or can be made fluorescent by *derivatization*. A fluorescence detector works by irradiating eluate at one wavelength and monitoring emission at a longer wavelength. Emission intensity is proportional to solute concentration. In an *evaporative light-scattering detector*, eluate is evaporated to make an aerosol of fine particles of nonvolatile solutes that can be detected by light scattering. A *refractive index detector* responds to essentially all analytes, but it is not very sensitive and, unlike other detectors, is not compatible with gradient elution.

The *charged aerosol detector* in Figure 22-25 is a sensitive universal detector that responds to almost all analytes and is compatible with gradient elution. Eluate evaporates to leave an aerosol of nonvolatile solute. The fine particles mix with a stream of N₂⁺ ions formed in a high-voltage discharge, and the aerosol particles

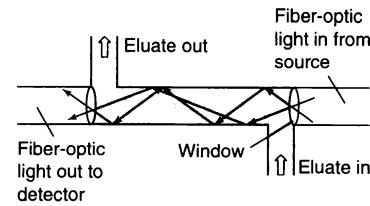


Figure 22-24 Light path in an optical waveguide flow cell. Some cells now available have a 5-mm effective pathlength containing only 0.25 μL of liquid or a 10-mm pathlength with 1 μL .

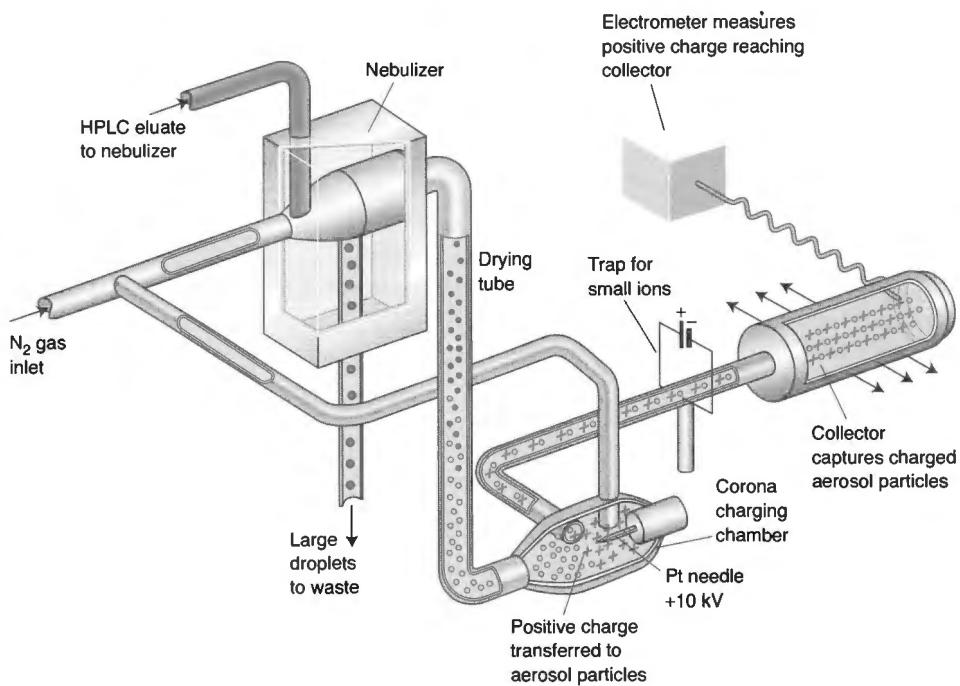


Figure 22-25 Operation of charged aerosol detector. [Courtesy ESA, Inc., Chelmsford MA.]

become positively charged. Excess N_2^+ is separated from the aerosol by an electric field, and the charged aerosol flows to a collector. The chromatogram displays charge reaching the collector as a function of time (Figure 23-4 in the next chapter). The *dynamic range* (Figure 5-1) spans analyte mass of $\sim 5 \text{ ng}$ to 10^5 ng . Equal masses of different analytes give equal response within $\sim 15\%$.

Liquid Chromatography–Mass Spectrometry

As in gas chromatography, a *mass spectrometer* is generally the most powerful detector for liquid chromatography because of its capability for both quantitative and qualitative analysis. The challenge in liquid chromatography is to remove solvent from analyte so as not to overwhelm the vacuum system of the mass spectrometer. **Electrospray** shown at the opening of this chapter creates a fine mist from which solvent evaporates and leaves ionic solutes in the gas phase. Nonvolatile buffers, such as phosphate, cannot be used with mass spectrometric detection because they clog the entrance to the mass spectrometer. To obtain acidic pH, ammonium formate and ammonium acetate buffers can be used. For alkaline pH, ammonium bicarbonate is a volatile buffer.

The other common means of introducing eluate from liquid chromatography into a mass spectrometer is **atmospheric pressure chemical ionization** (Figure 22-26). Heat and a coaxial flow of N_2 convert eluate into a fine aerosol from which solvent and analyte evaporate. The distinguishing feature of this technique is that high voltage is applied to a metal needle in the aerosol. An electric corona (a plasma containing charged particles) forms around the needle, injecting electrons into the aerosol, where a sequence of reactions can create both positive and negative ions. Common ionic products include protonated (MH^+) and deprotonated (M^-) analyte. Voltages in a mass spectrometer can be reversed to measure either cations or anions.

In electrospray and atmospheric pressure chemical ionization, there is usually little fragmentation to provide structural information. However, in both techniques,

Electrospray: Ions observed in mass spectrometer were already present in solution on chromatography column; neutral analytes are not converted into ions by electrospray

Atmospheric pressure chemical ionization: New ions such as protonated analyte are created by chemical ionization

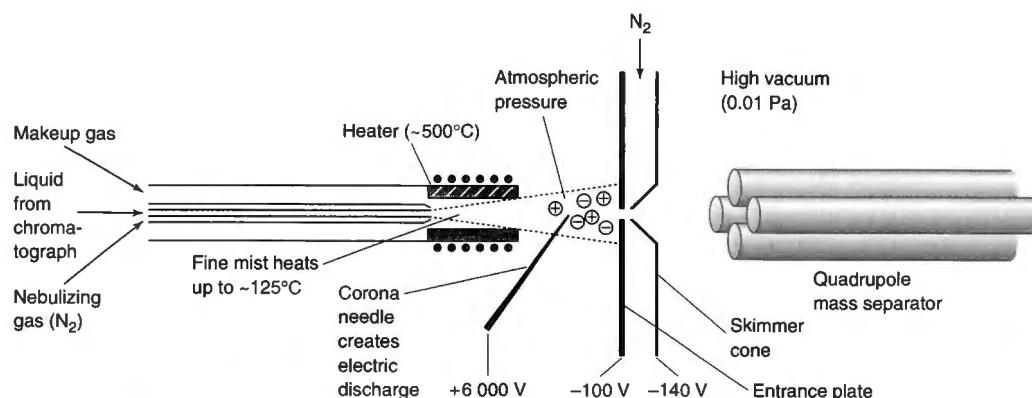


Figure 22-26 Atmospheric pressure chemical ionization interface between liquid chromatography column and mass spectrometer. Aerosol is produced by the nebulizing gas flow and the heater. Electric discharge from the corona needle creates gaseous ions from analyte. [Adapted from E. C. Huang, T. Wachs, J. J. Conboy, and J. D. Henion, *Anal. Chem.* **1990**, 62, 713A.]

the voltage between the entrance plate and the skimmer cone in Figure 22-26 can be varied to accelerate ions entering the mass separator. Fast-moving ions collide with background N_2 gas and break into smaller fragments that aid in qualitative identification. This widely used process is called *collisionally activated dissociation*.

Liquid chromatography with mass spectrometric detection is a method of choice to measure how much bisphenol A (BPA described at the opening of Chapter 21) leaches from a water bottle. Boiling water was added to a plastic hiker's bottle and cooled to room temperature. The pH was lowered to 3 with formic acid, and the water was passed through a solid-phase extraction cartridge (page 503) that retains BPA and hydrophobic substances. Many water-soluble substances pass through the cartridge and are eliminated. BPA and other hydrophobic substances were then washed from the cartridge with an alcohol-ether solvent, which was evaporated and replaced by a small volume of solvent for injection into a chromatograph. Figure 22-27 shows

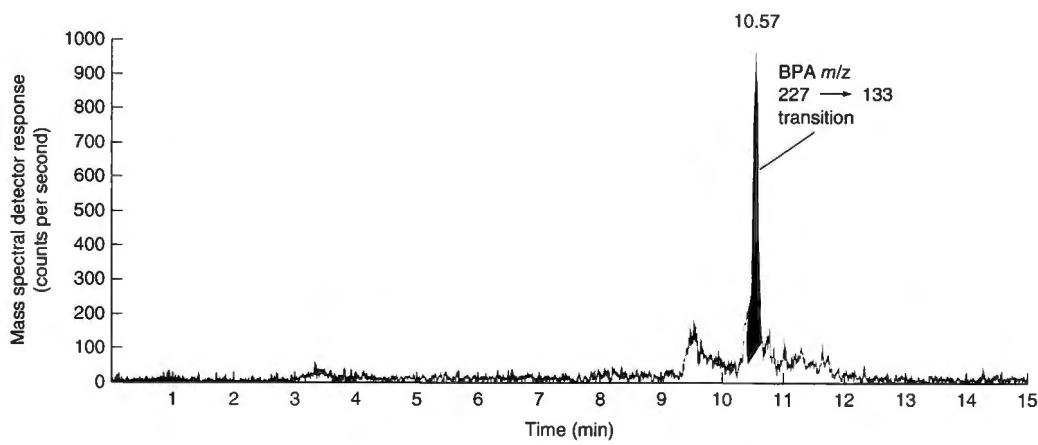


Figure 22-27 Chromatogram showing 20 ppb BPA leached into water from a polycarbonate hiking bottle. Reversed-phase chromatography on $150 \times 3.9\text{-mm}$ column of 5- μm RP18 with gradient elution over 15 min going from 40% to 100% solvent B. Solvent A = 0.1 wt% formic acid in H_2O . Solvent B = acetonitrile. Mass spectral detection of negative ions with electrospray ionization and selected reaction monitoring of m/z 227 \rightarrow 133 transition. [From M. Swartz, *LCGC*, January 2010, p. 42.]

the chromatogram obtained with mass spectrometric detection using selected reaction monitoring described on page 487. The ion m/z 227 selected by Q1 in Figure 22-11 is fragmented by collisions in Q2. The product ion at m/z 133 is selected by Q3 and measured at the detector. The highlighted peak represents 20 parts per billion BPA (20 μg BPA/kg H_2O). If you drink a liter of this water, you ingest 20 μg of BPA.

Hydrophilic Interaction Chromatography (HILIC)

Hydrophilic: “water loving”—soluble in water, surface is wetted by water
Hydrophobic: “water hating”—insoluble in water, surface is not wetted by water

Hydrophilic substances are soluble in water or attract water to their surfaces. Polar organic molecules have hydrophilic regions. **Hydrophilic interaction chromatography (HILIC)** is used to separate molecules that are too polar to be retained by reversed-phase columns. In biochemistry, HILIC is useful for separating peptides and saccharides (sugars).

Stationary phases for hydrophilic interaction chromatography, such as the amino and ZIC-HILIC® phases in Table 22-3, are strongly polar. They are thought to be coated with a thin layer of water inside the column. Polar solutes are retained by the polar bonded phase and by the thin aqueous layer. The mobile phase typically contains (25–97 vol%) CH_3CN or other polar organic solvent mixed with aqueous buffer. The higher the concentration of organic solvent, the less soluble is the polar solute in the mobile phase.

Figure 22-28 shows the separation of ascorbic acid (vitamin C) and isoascorbic acid, which differ by the configuration about one tetrahedral carbon atom (C_5). These molecules are not mirror images of each other, so they are not *optical isomers* (*enantiomers*). They have opposite configurations at C_5 but the same configuration at C_4 . Dissymmetric molecules that are not mirror images are called *diastereoisomers*. Ascorbic and isoascorbic acid are too polar to be retained and separated in reversed-phase chromatography, but they are readily separated in HILIC. In Figure 22-28, the stationary phase has a bonded amino group (Table 22-3), which is fully protonated at the solvent pH of 7. The solvent is 90:10 (vol/vol) acetonitrile: H_2O , in

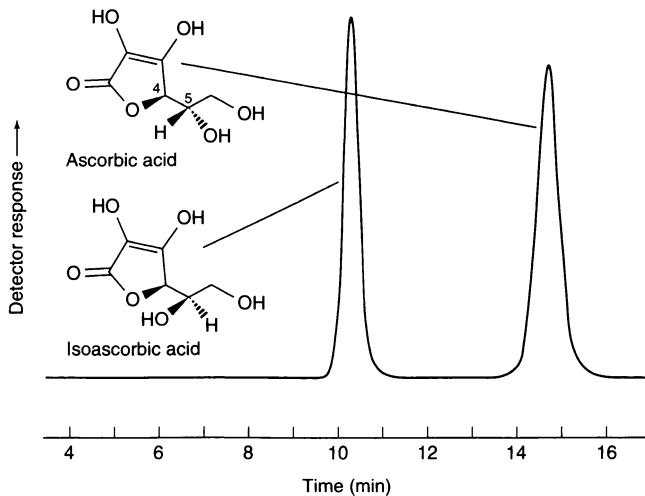


Figure 22-28 Separation of ascorbic acid and isoascorbic acid by hydrophilic interaction chromatography (HILIC) using the amino bonded phase in Table 22-3. Isocratic solvent is 90:10 acetonitrile: H_2O (containing 0.1 M ammonium acetate). [From S. Drivelos, M. E. Dasenaki, and N. S. Thomaidis, *Anal. Bioanal. Chem.* **2010**, 397, 2199.]

which the water contains 0.1 M ammonium acetate. Figure 22-29 shows that the retention time in HILIC *increases* as the fraction of acetonitrile in the mixture increases. This behavior is the opposite of that of reversed-phase chromatography, in which retention time *decreases* with increasing acetonitrile.

Let's summarize the effect of solvent polarity in normal-phase chromatography, reversed-phase chromatography, and hydrophilic interaction chromatography (HILIC). In classical normal-phase chromatography with stationary phases such as bare silica or alumina, the solvent is nonaqueous. To increase eluent strength, we increase the polarity of the nonaqueous solvent. In reversed-phase chromatography, the solvent is aqueous, and eluent strength is increased by *decreasing* the fraction of water in the mobile phase. Gradient elution goes from high aqueous content to low aqueous content. In HILIC with a bonded, strongly polar phase, eluent strength is increased by *increasing* the fraction of water in the mobile phase. Gradient elution goes from low aqueous content to high aqueous content.

Ask Yourself

- 22-C. (a) What is the difference between normal-phase and reversed-phase chromatography?
- (b) What is the difference between reversed-phase chromatography and hydrophilic interaction chromatography (HILIC)?
- (c) What is the difference between isocratic and gradient elution?
- (d) Why does eluent strength increase in normal-phase chromatography when a more polar solvent is added?
- (e) Why does eluent strength increase in reversed-phase chromatography when the fraction of organic solvent is increased?
- (f) Why does eluent strength increase in hydrophilic interaction chromatography (HILIC) when the fraction of organic solvent is decreased?
- (g) What is the purpose of a guard column?
- (h) Which chromatography–mass spectrometry interface, electrospray or atmospheric pressure chemical ionization, creates new ions in the gas phase and which just introduces existing solution-phase ions into the gas phase?
- (i) Why does the superficially porous particle with a diameter of 2.7 μm in Figure 22-17 have a similar van Deemter curve to the microporous particle with a diameter of 1.8 μm ?

22-4 Sample Preparation for Chromatography

Sample preparation is the process of transforming a sample into a form that is suitable for analysis. This process might entail extracting analyte from a complex matrix, *preconcentrating* dilute analyte to get a concentration high enough to measure, removing or masking interfering species, or chemically transforming (*derivatizing*) analyte into a form that is easier to separate or to detect. *Solid-phase microextraction* and *purge and trap* are sample preparation techniques that are especially important for gas chromatography. *Solid-phase extraction* is useful for both liquid and gas chromatography.

Solid-phase microextraction extracts compounds for gas chromatography from liquids, air, or even sludge without using any solvent. The key component is a fused-silica fiber coated with a 10- to 100- μm -thick film of nonvolatile liquid

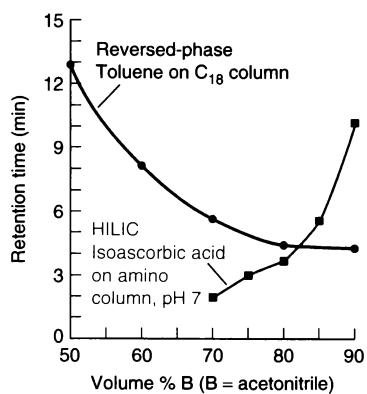


Figure 22-29 Effect of organic solvent on retention time in HILIC is the opposite of the effect in reversed-phase HPLC. Reversed-phase data for toluene (peak 6) in Figure 22-22. HILIC data for isoascorbic acid in Figure 22-28. [Data from supplement to S. Drivelos, M. E. Dasenaki, and N. S. Thomaidis, *Anal. Bioanal. Chem.* **2010**, 397, 2199.]

Example of derivatization:

The alcohol RCH_2OH is converted to $\text{RCH}_2\text{OSi}(\text{CH}_3)_3$ to make it more volatile for gas chromatography and to give characteristic mass spectral peaks that aid in identification.

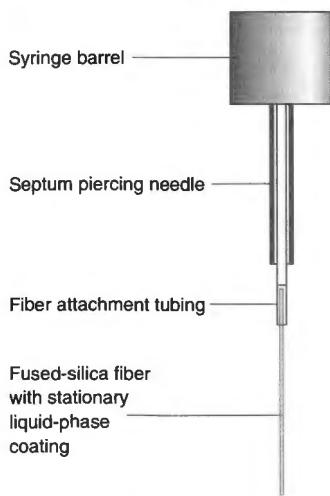


Figure 22-30 Syringe for solid-phase microextraction. The fused-silica fiber is withdrawn inside the steel needle after sample collection and whenever the syringe is used to pierce a septum.

stationary phase similar to those used in gas chromatography. Figure 22-30 shows the fiber attached to a syringe with a fixed metal needle. The fiber can be extended from the needle or retracted inside the needle. Figure 22-31 demonstrates the procedure of exposing the fiber to a sample solution (or the gaseous headspace above the liquid) for a fixed length of time while stirring and, perhaps, heating. Only a fraction of the analyte in the sample is extracted into the fiber.

After sampling, the fiber is retracted and the needle is introduced into a narrow injection port (0.7-mm inner diameter) of a gas chromatograph. The fiber is extended inside the hot injection liner, where analyte is thermally desorbed from the fiber in splitless mode for a fixed time. *Cold trapping* (page 485) focuses desorbed analyte at the head of the column prior to chromatography. Solid-phase microextraction can be adapted to liquid chromatography by using a special injection port in which the fiber is washed with a strong solvent.

A modification of solid-phase microextraction, called *stir-bar sorptive extraction*, uses a magnetic stirring bar enclosed in a thin glass capsule coated with a 0.5- to 1-mm-thick layer of sorbent. The bar is stirred in a sample solution to extract analyte from the sample. The amount of sorbent is ~100 times greater than the amount in solid-phase microextraction with a syringe needle. Therefore ~100 times more analyte can be collected, making stir-bar sorptive extraction an excellent way to collect traces of analyte for analysis.

Purge and trap is a method for removing volatile analytes from liquids or solids (such as groundwater or soil), concentrating the analytes, and introducing them into a gas chromatograph. In contrast with solid-phase microextraction, which removes only a portion of analyte from the sample, the goal in purge and trap is to remove 100% of the analyte from the sample. Quantitative removal of polar analytes from polar matrices can be difficult.

Figure 22-32 shows apparatus for extracting volatile flavor components from beverages. Helium is bubbled through the beverage in the sample vial, which is heated

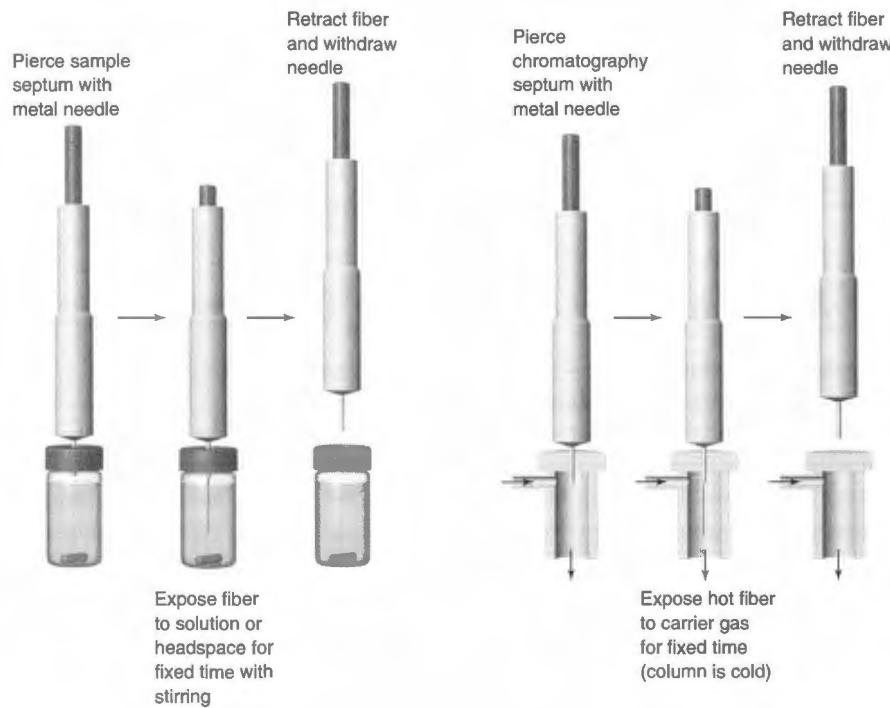


Figure 22-31 Sampling by solid-phase microextraction and thermal desorption of analyte from the coated fiber into a gas chromatograph.
[Adapted from Supelco Chromatography Products catalog, Bellefonte, PA.]

to 50°C to aid evaporation of analytes. Gas exiting the sample vial passes through an adsorption tube containing three layers of adsorbents with increasing adsorbent strength. For example, the moderate adsorbent could be a nonpolar phenylmethylpolysiloxane, the stronger adsorbent could be the polymer Tenax, and the strongest adsorbent could be carbon molecular sieves.

During purge and trap, gas flows through the adsorbent tube from end A to end B in Figure 22-32. After analyte has been collected, gas flow is reversed to go from B to A and the trap is purged at 25°C to remove as much water or other solvent as possible from the adsorbents. Outlet A of the adsorbent tube is then directed to the injection port of a gas chromatograph operating in splitless mode and the trap is heated to ~200°C. Desorbed analytes flow into the chromatography column, where they are concentrated by cold trapping. After complete desorption from the trap, the chromatography column is warmed up to initiate the separation.

Solid-phase extraction uses a liquid chromatography stationary phase in a short, open column to collect and *preconcentrate* analyte. For example, in Chapter 0 (pages 2–3) Denby and Scott extracted fat from chocolate and then extracted caffeine and theobromine with hot water and removed fine particles by repeated, laborious centrifugation and filtration. Solid-phase extraction replaces solvent extraction, centrifugation, and filtration.³ Crushed whole chocolate (0.5 g) is suspended in 20 mL of H₂O at 80°C for 15 min to extract caffeine, theobromine, and other water-soluble components. A solid-phase extraction column containing 0.5 g of C₁₈-coated silica particles is *conditioned* (cleaned and prepared) with 1 mL of methanol followed by 1 mL of H₂O (Figure 22-33). When 0.5 mL of aqueous extract is applied to the column, theobromine and caffeine adhere to C₁₈-silica. Many water-soluble components, such as sugar, are washed through with 1 mL of H₂O. Caffeine and theobromine are then eluted with 2.5 mL of methanol. After evaporating the methanol to dryness, the residue is dissolved in 1 mL of H₂O and is ready for chromatography. For solid-phase extraction, sample loading and elution at a slow flow rate such as 1–2 drops/s improves recovery and reproducibility.

Sample cleanup refers to the removal of undesirable components of an unknown that interfere with measurement of analyte. In chocolate analysis, polar molecules such as sugars that are not retained by C₁₈-silica wash right through the column and are separated from caffeine and theobromine. Fats that could interfere are probably retained on the C₁₈ column and therefore also separated from caffeine and theobromine (Figure 22-33).

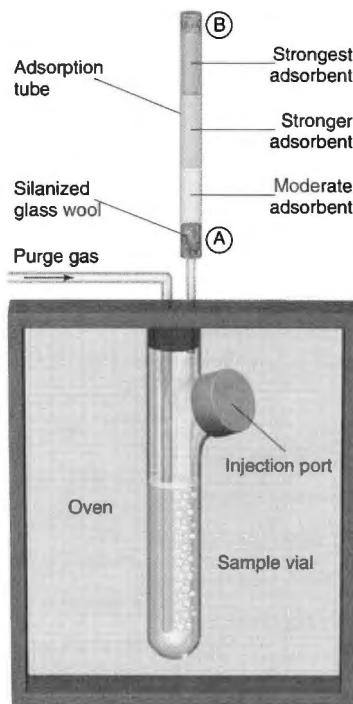


Figure 22-32 Purge and trap apparatus for extracting volatile substances from a liquid or solid by flowing gas. You need to establish the time and temperature required to purge 100% of the analyte from the sample in separate control experiments.

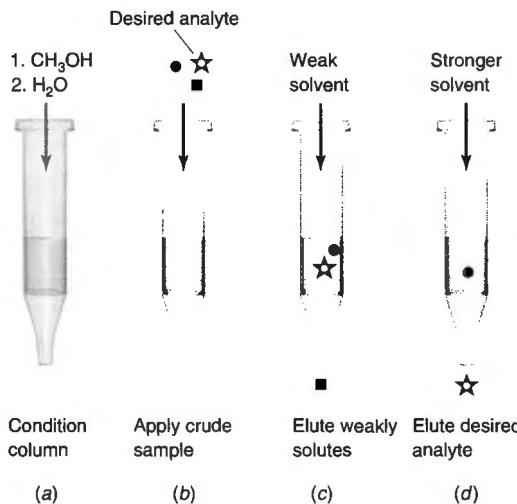
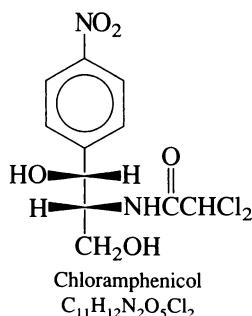


Figure 22-33 Solid-phase extraction separates caffeine and theobromine from sugars (more polar) and fats (less polar) found in chocolate.



Molecularly imprinted polymers are the newest media for solid-phase extraction. For example, a commercial imprinted polymer is available for extraction and preconcentration of the antibiotic chloramphenicol from milk. This antibiotic causes aplastic anemia and is a suspected carcinogen. Therefore it was banned from use in farm animals in North America and Europe. It is still used elsewhere, so certain foods are screened for chloramphenicol. The molecularly imprinted polymer is made by polymerizing monomeric building blocks in the presence of chloramphenicol (Figure 22-34). For extracting chloramphenicol from milk, the milk is centrifuged to separate fat from the aqueous phase, which is passed through a small cartridge containing molecularly imprinted polymer that had been washed with methanol and water. The polymer is then eluted with a sequence of solvents that remove most absorbed molecules, but not chloramphenicol. The antibiotic is finally removed by a small volume of methanol:acetic acid:water (89:1:10 vol/vol/vol). Eluate is evaporated to dryness and dissolved in 150 μL of HPLC mobile phase for chromatography.

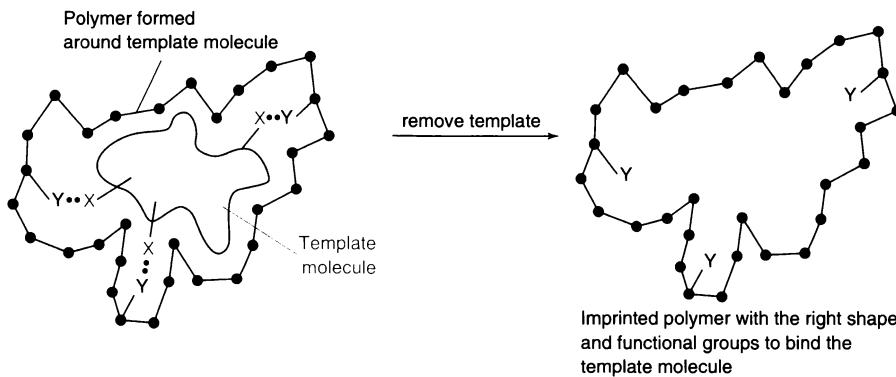


Figure 22-34 A molecularly imprinted polymer has a binding pocket for a specific template molecule.

Ask Yourself

- Why is it necessary to use cold trapping on the gas chromatography column if sample is introduced from a solid-phase microextraction syringe or from a purge and trap absorption tube?
- What is the purpose of solid-phase extraction? Why is it advantageous to use large particles (50 μm) for solid-phase extraction, but small particles (5 μm) for chromatography?
- What kind of sample cleanup does solid-phase extraction accomplish in the analysis of caffeine and theobromine in chocolate?
- Chloramphenicol, shown above, is measured by HPLC-electrospray mass spectrometry, observing negative ions. The base peak is at m/z 321. The second strongest peak, 60% as intense as the base peak, is m/z 323. Assign a molecular formula to each peak.

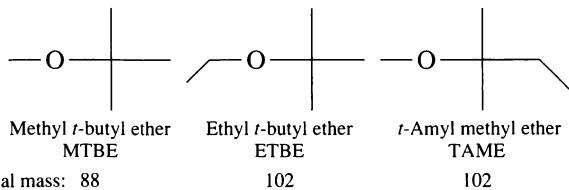
Important Terms

atmospheric pressure chemical ionization	hydrophilic interaction chromatography	solid-phase microextraction
bonded stationary phase	isocratic elution	solvent trapping
cold trapping	microporous particle	split injection
electron capture detector	molecularly imprinted polymer	splitless injection
electrospray	normal-phase chromatography	superficially porous particle
elucent strength	on-column injection	temperature programming
flame ionization detector	purge and trap	thermal conductivity detector
gas chromatography	reversed-phase chromatography	ultraviolet detector
gradient elution	sample cleanup	
guard column	sample preparation	
high-performance liquid chromatography	selected reaction monitoring	
	solid-phase extraction	

Problems

- 22-1. (a) Explain the difference between wall-coated, support-coated, and porous-layer open tubular columns for gas chromatography.
(b) What is the advantage of a bonded stationary phase in gas chromatography?
(c) Why do we use a makeup gas for some gas chromatography detectors?
(d) Explain how solvent trapping and cold trapping work in splitless injection.
- 22-2. (a) Referring to Section 21-3, explain why plate height increases at very low and very high flow rates for 5- μm and 3.5- μm particles in Figure 22-17.
(b) Plate height does not increase for high flow rates for the 1.8- μm particles in Figure 22-17. What does this observation imply about the rate of equilibration of solute between the mobile and stationary phases?
- 22-3. Why does a thermal conductivity detector respond to all analytes except the carrier gas? Why isn't a flame ionization detector universal?
- 22-4. Consider a narrow-bore (0.25 mm diameter) open tubular gas chromatography column coated with a thin film (0.10 μm thick) of stationary phase that provides 5 000 plates per meter. Consider also a wide-bore (0.53 μm diameter) column with a thick film (5.0 μm thick) of stationary phase that provides 1 500 plates per meter.
(a) Why does the thin film provide more theoretical plates per meter than the thick film?
(b) The density of stationary phase is 1.0 g/mL. What mass of stationary phase in each column is in a length equivalent to one theoretical plate?
- 22-5. (a) Calculate the number of theoretical plates (N in Equation 21-1) and the plate height (H in Equation 21-2) for peak 11 in Figure 22-4. Find the resolution (Equation 21-3) between peaks 16 and 17.
(b) Plate height for liquid chromatography in Figure 22-17 is $\sim 10 \mu\text{m}$. How many times greater is the plate height that you just calculated for gas chromatography? Why is the plate height so much greater for gas chromatography?
- 22-6. The adjusted retention time (t'_r) for a chromatographic peak is the observed retention time (t_r) minus the retention time of an unretained substance. Many gas chromatography columns do not retain methane, so a little methane is added to a sample to produce an unretained peak. Then $t'_r = t_r - t_r(\text{methane})$. For isothermal elution of a homologous series of compounds (those with similar structures, but differing by the number of CH_2 groups in a chain), $\log t'_r$ is usually a linear function of the number of carbon atoms. A compound was known to be a member of the family $(\text{CH}_3)_2\text{CH}(\text{CH}_2)_n\text{CH}_2\text{OSi}(\text{CH}_3)_3$. For the following retention times, use a spreadsheet to prepare a graph of $\log t'_r$ versus n . Use the Excel function LINEST (Section 4-8) to find the slope and intercept of the least-squares straight line through the three known points. From the equation of the line, calculate the value of n for the unknown.
- | | | | |
|----------|----------|---------------|----------|
| $n = 7$ | 4.0 min | CH_4 | 1.1 min |
| $n = 8$ | 6.5 min | Unknown | 42.5 min |
| $n = 14$ | 86.9 min | | |
- 22-7. The gasoline additive methyl *t*-butyl ether (MTBE) has been leaking into groundwater ever since its introduction

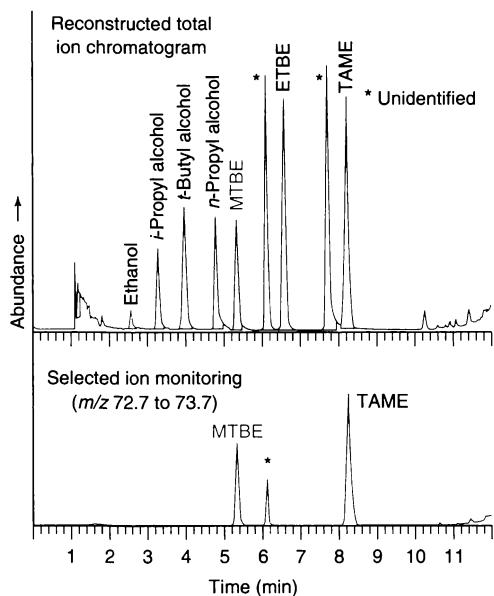
in the 1990s. MTBE can be measured at parts per billion levels by solid-phase microextraction from groundwater to which 250 g/L NaCl has been added to lower the solubility of MTBE. After microextraction, analytes are thermally desorbed from the fiber in the port of a gas chromatograph. The figure shows a reconstructed total ion chromatogram and selected ion monitoring of substances desorbed from the extraction fiber.



(a) What nominal mass is being observed in selected ion monitoring? Why are only three peaks observed?

(b) Here is a list of major ions above m/z 50 in the positive ion electron ionization mass spectra. Given that MTBE and TAME have an intense peak at m/z 73, and there is no significant peak at m/z 73 for ETBE, suggest a structure for m/z 73. Suggest a structure for m/z 87 for ETBE and TAME.

MTBE	ETBE	TAME
73	87	87
57	59	73
	57	71
		55



Reconstructed total ion chromatogram and selected ion monitoring of solid-phase microextract of groundwater. [From D. A. Cassada, Y. Zhang, D. D. Snow, and R. F. Spalding, *Anal. Chem.* **2000**, 72, 4654.]

22-8. (a) In selected reaction monitoring of caffeine in Figure 22-12, the transition m/z 194 \rightarrow 109 was used. What is the nominal mass of the molecular ion of caffeine? Why was the transition m/z 197 \rightarrow 111 used for the internal standard ($^{13}\text{CH}_3)_3\text{-caffeine}$?

(b) Why is an isotopic variant of the analyte an excellent internal standard?

(c) The relative intensities for caffeine in Figure 22-12 are m/z 195/194 = 10.3%. What is the isotopic composition of the ion at m/z 195? What is the expected intensity ratio m/z 195/194? (Hint: See Equation 21-8.)

22-9. (a) Nonpolar aromatic compounds were separated by HPLC on a bonded phase containing octadecyl groups $[-(\text{CH}_2)_{17}\text{CH}_3]$ covalently attached to silica. Eluent was 65 vol% methanol in water. How would retention times be affected if 90% methanol were used instead?

(b) Polar solutes were separated by HPLC (not HILIC) with a bonded phase containing polar diol substituents $[-\text{CH}(\text{OH})\text{CH}_2\text{OH}]$. How would retention times be affected if eluent were changed from 40 vol% to 60 vol% acetonitrile in water? Acetonitrile ($\text{CH}_3\text{C}\equiv\text{N}$) is less polar than water.

(c) Polar solutes were separated by hydrophilic interaction chromatography (HILIC) with a strongly polar bonded phase. How would retention times be affected if eluent were changed from 80 vol% to 90 vol% acetonitrile in water?

(d) Polar solutes were separated by normal-phase chromatography on bare silica, using methyl *t*-butyl ether and 2-propanol solvent. How would retention times be affected if eluent were changed from 40 vol% to 60 vol% 2-propanol? (Hint: See Table 22-2.)

22-10. Draw the chemical structures of two bonded nonpolar phases and two bonded polar phases in HPLC. Begin with a silicon atom at the surface of a silica particle.

22-11. (a) Why is high pressure needed in HPLC?

(b) Why does the efficiency (decreased plate height) of liquid chromatography increase as the stationary phase particle size is reduced?

(c) What are some advantages and disadvantages of using sub-2- μm particles (UPLC)?

(d) What is the advantage of using superficially porous particles?

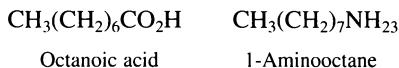
22-12. A 15-cm-long HPLC column packed with 5- μm particles has an optimum plate height of 10.0 μm in Figure 22-17. What will be the half-width of a peak eluting at 10.0 min? If the particle size were 3 μm and the plate height were 5.0 μm , what would be the half-width?

22-13. (a) UPLC can provide exquisite resolution when it is run slowly or it can provide rapid separations with reasonable resolution if it is run faster. The drug acetaminophen run on a 50-mm-long \times 2.1-mm-diameter C_{18} UPLC column has a retention time of 0.63 min and a width at half-height of 2.3 s.

Find the number of plates and the plate height. How many 1.7- μm -diameter particles placed side-by-side are equal to one theoretical plate?

(b) From Figure 22-17, we expect an optimum plate height of 4 μm . How many particles placed side-by-side are equal to one theoretical plate? Do you think the column in (a) is being run for maximum resolution or maximum speed?

22-14. Octanoic acid and 1-aminoctane were separated by HPLC on a C₁₈ bonded phase. The eluent was 20 vol% methanol in water adjusted to pH 3.0 with formic acid.



(a) Draw the predominant form (neutral or ionic) of a carboxylic acid and an amine at pH 3.0.

(b) State which compound is expected to be eluted first and why.

22-15. (a) When you try separating an unknown mixture by reversed-phase chromatography with 50% acetonitrile-50% water, the peaks are eluted between 1 and 3 min and they are too close together to be well resolved for quantitative analysis. Should you use a higher or lower percentage of acetonitrile in the next run?

(b) Suppose that you try separating an unknown mixture by normal-phase chromatography with the solvent mixture 50% hexane-50% methyl *t*-butyl ether (which is more polar than hexane). The peaks are too close together and are eluted rapidly. Should you use a higher or lower percentage of hexane in the next run?

22-16. After an isocratic elution has been optimized with several solvents, the chromatogram has a resolution of 1.2 between the two closest peaks. How might you increase the resolution without changing solvents or the type of stationary phase?

22-17. (a) Sketch a graph of the van Deemter equation (plate height versus flow rate). What would the curve look like if the multiple path term were 0? If longitudinal diffusion were 0? If the finite equilibration time were 0?

(b) When we run a UPLC column much faster than the optimum speed, which mechanism causes band broadening?

22-18. *Internal standard.* Compounds C and D gave the following HPLC results:

Compound	Concentration (mg/mL) in mixture	Peak area (cm ²)
C	1.03	10.86
D	1.16	4.37

A solution was prepared by mixing 12.49 mg of D plus 10.00 mL of unknown containing just C, and diluting to 25.00 mL. Peak areas of 5.97 and 6.38 cm² were observed for C and D, respectively. Find the concentration (mg/mL) of C in the unknown.

22-19. Spherical, microporous silica particles used in chromatography have a density of 2.20 g/mL, a diameter of 10.0 μm , and a measured surface area of 300 m²/g.

(a) The volume of a spherical particle is $\frac{4}{3}\pi r^3$, where r is the radius. The mass of the sphere is volume \times density (= mL \times g/mL). How many particles are in 1.00 g of silica?

(b) The surface area of a sphere is $4\pi r^2$. Calculate the surface area of 1.00 g of solid, spherical silica particles.

(c) By comparing the calculated and measured surface areas, what can you say about the porosity of the particles?

22-20. Here are guidelines for flushing a C₁₈ column and changing mobile phase. Explain the rationale for each.

1. To remove strongly retained solutes, flush the column initially with 5–10 column volumes of the most recent mobile phase (such as 60:40 H₂O-acetonitrile) *without* buffer. That is, replace buffer with pure water.

2. Then flush with 10–20 volumes of strong solvent (such as 10:90 H₂O-acetonitrile).

3. Store the column in the strong solvent.

4. Equilibrate with 10–20 volumes of new, desired, buffered mobile phase.

5. Use a standard mixture to check for correct retention times and plate numbers.

22-21. *Chromatography-mass spectrometry.* Cocaine metabolism in rats can be studied by injecting the drug and periodically withdrawing blood to measure levels of metabolites by HPLC-mass spectrometry. For quantitative analysis, isotopically labeled internal standards are mixed with the blood sample. Blood was analyzed by reversed-phase chromatography with an acidic eluent and atmospheric pressure chemical ionization mass spectrometry for detection. The mass spectrum of the collisionally activated dissociation products from the *m/z* 304 positive ion is shown in panel *a* on page 508. Selected reaction monitoring (*m/z* 304 from mass filter Q1 and *m/z* 182 from Q3 in Figure 22-11) gave a single chromatographic peak at 9.22 min for cocaine (panel *b*). The internal standard ²H₅-cocaine gave a single peak at 9.19 min for *m/z* 309 (Q1) \rightarrow 182 (Q3).

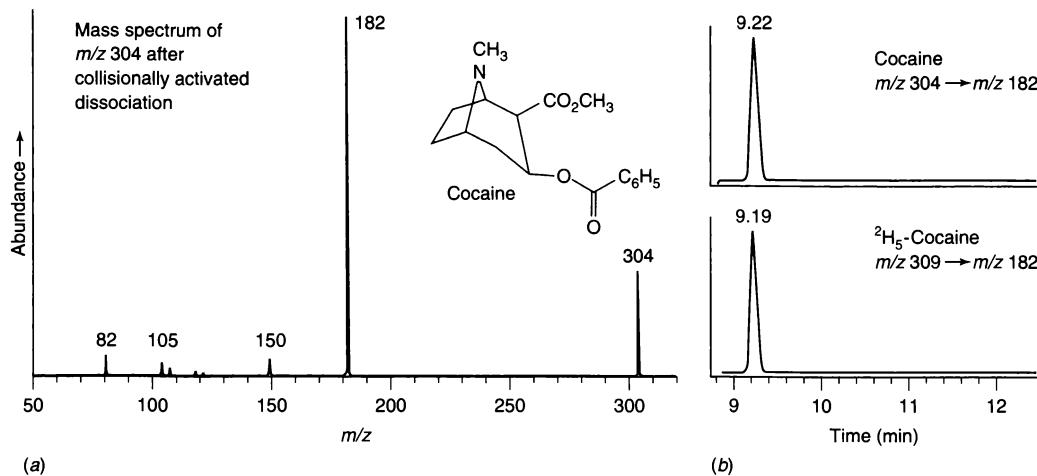
(a) Draw the structure of the ion at *m/z* 304 and suggest a structure for *m/z* 182.

(b) Intense peaks at *m/z* 182 and 304 do not have isotopic partners at *m/z* 183 and 305. Explain why.

(c) Rat plasma is exceedingly complex. Why does the chromatogram show just one clean peak?

(d) Given that ²H₅-cocaine has only two major mass spectral peaks at *m/z* 309 and 182, which atoms are labeled with deuterium?

(e) Explain how you would use ²H₅-cocaine for measuring cocaine in blood.



(a) Mass spectrum of collisionally activated dissociation products from m/z 304 positive ion from atmospheric pressure chemical ionization mass spectrum of cocaine. (b) Chromatograms obtained by selected reaction monitoring. [From G. Singh, V. Arora, P. T. Fenn, B. Mets, and I. A. Blair, *Anal. Chem.* **1999**, 71, 2021.]

22-22. The structure of bisphenol A was shown at the opening of Chapter 21, and a chromatogram with electrospray ionization and negative ion mass spectral detection is shown in Figure 22-27. The transition m/z 227 \rightarrow 133 was monitored. Suggest a structure for the ions at m/z 227 and 133.

22-23. Why is splitless injection used with purge and trap sample preparation?

22-24. What is the purpose of derivatization in chromatography? Give an example.

22-25. Explain how solid-phase microextraction works. Why is cold trapping necessary during injection with this technique? Is all the analyte in an unknown extracted into the fiber in solid-phase microextraction?

22-26. Why does a molecularly imprinted polymer selectively bind a desired analyte?

22-27. Here is a student procedure to measure nicotine in urine. A 1.00-mL sample of biological fluid was placed in a 12-mL vial containing 0.7 g Na_2CO_3 powder. After 5.00 μg of the internal standard 5-aminoquinoline were injected, the vial was capped with a Teflon-coated silicone rubber septum. The vial was heated to 80°C for 20 min, and then a solid-phase microextraction needle was passed through the septum and left in the headspace for 5.00 min. The fiber was retracted and then inserted into the injection port of a gas chromatograph. Volatile substances were desorbed from the fiber at 250°C for 9.5 min in the injection port of a chromatograph whose column was at 60°C. The column temperature was then raised to 260°C at 25°C/min, and eluate was monitored by electron ionization mass spectrometry with selected ion monitoring at m/z 84 for nicotine and m/z 144 for internal standard. Calibration data from replicate standard mixtures taken through the entire procedure are given in the table.

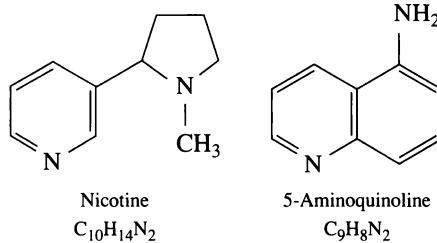
Nicotine in urine ($\mu\text{g/L}$)	Area ratio m/z 84/144
12	0.05 ₆ , 0.05 ₉
51	0.40 ₂ , 0.39 ₁
102	0.68 ₄ , 0.66 ₉
157	1.01 ₁ , 1.06 ₃
205	1.27 ₈ , 1.35 ₅

Based on A. E. Wittner, D. M. Klinger, X. Fan, M. Lam, D. T. Mathers, and S. A. Mabury, *J. Chem. Ed.* **2002**, 79, 1257.

(a) Why was the vial heated to 80°C before and during extraction?

(b) Why was the chromatography column kept at 60°C during thermal desorption of the extraction fiber?

(c) Suggest a structure for m/z 84 from nicotine. What is the m/z 144 ion from the internal standard, 5-aminoquinoline?



(d) Urine from an adult female nonsmoker had an area ratio m/z 84/144 = 0.51 and 0.53 in replicate determinations. Urine from a nonsmoking girl whose parents are heavy smokers had an area ratio 1.18 and 1.32. Find the nicotine concentration ($\mu\text{g/L}$) and its uncertainty in the urine of each person.

How Would You Do It?

22-28. The compound reserpine has the formula C₃₃H₄₀N₂O₉ with a nominal mass of 608 Da. The following positive ion mass spectra were recorded:

(i) Electron ionization of reserpine from gas chromatography column.

(ii) Electrospray of reserpine from liquid chromatography column.

(iii) Electrospray followed by tandem mass spectroscopy. The base peak from (ii) went through a collision cell, and a full-scan mass spectrum of the resulting fragments was obtained.

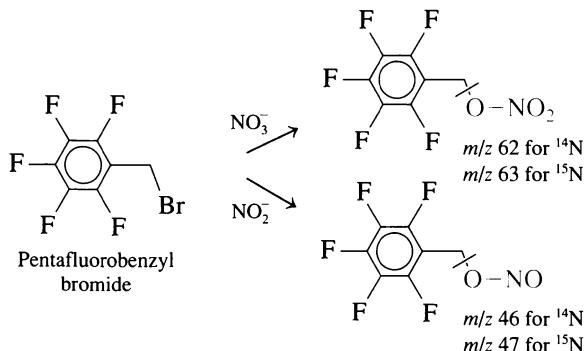
Here are descriptions of the three spectra in random order. Which description belongs to which spectrum—(i), (ii), and (iii)? Explain your reasoning.

a. Base peak at m/z 609 with 40% intensity at m/z 610. No other major peaks.

b. Base peak at m/z 195 with many other significant peaks. The two highest mass peaks are m/z 608 = 19% and m/z 609 = 6%. There is no peak at m/z 610.

c. Base peak at m/z 609 with nothing significant at m/z 610. There are several significant peaks at lower m/z .

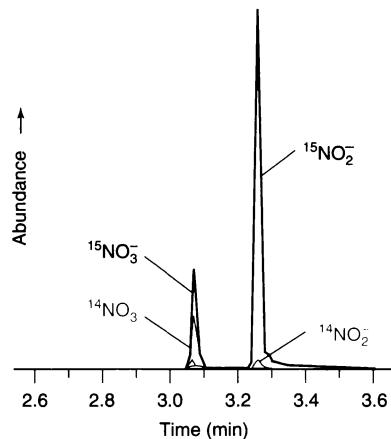
22-29. Nitric oxide (NO) is a cell signaling agent in physiologic processes such as vasodilation, inhibition of clotting, and inflammation. A sensitive chromatography–mass spectrometry method was developed to measure two of its metabolites, nitrite (NO_2^-) and nitrate (NO_3^-), in biological fluids. Internal standards $^{15}\text{NO}_2^-$ and $^{15}\text{NO}_3^-$ were added to the fluid at concentrations of 80.0 and 800.0 μM , respectively. The naturally occurring $^{14}\text{NO}_2^-$ and $^{14}\text{NO}_3^-$ plus the internal standards were then converted into volatile derivatives in aqueous acetone:



Because biological fluids are so complex, the derivatives were first isolated by high-performance liquid chromatography. For quantitative analysis, liquid chromatography peaks corresponding to the two products were injected into a gas chromatograph, ionized by *negative ion* chemical ionization (giving major peaks for NO_2^- and NO_3^-), and the products were measured by selected ion monitoring. Results are shown in the figure. If the ^{15}N internal standards undergo the same reactions and same separations at the same rate as the ^{14}N analytes, then the concentrations of analytes are simply

$$[{}^{14}\text{NO}_x^-] = [{}^{15}\text{NO}_x^-](R - R_{\text{blank}})$$

where R is the measured peak area ratio (m/z 46/47 for nitrite and m/z 62/63 for nitrate) and R_{blank} is the measured ratio of peak areas in a blank prepared from the same buffers and reagents with no added nitrate or nitrite. In the figure, the ratios of peak areas are m/z 46/47 = 0.062 and m/z 62/63 = 0.538. The ratios for the blank were m/z 46/47 = 0.040 and m/z 62/63 = 0.058. Find the concentrations of nitrite and nitrate in the urine.



Selected ion chromatogram showing *negative ions* at m/z 46, 47, 62, and 63 obtained by derivatizing nitrite and nitrate plus internal standards ($^{15}\text{NO}_2^-$ and $^{15}\text{NO}_3^-$) in urine. [From D. Tsikas, *Anal. Chem.* **2000**, 72, 4064; *Anal. Chem.* **2010**, 82, 2585.]

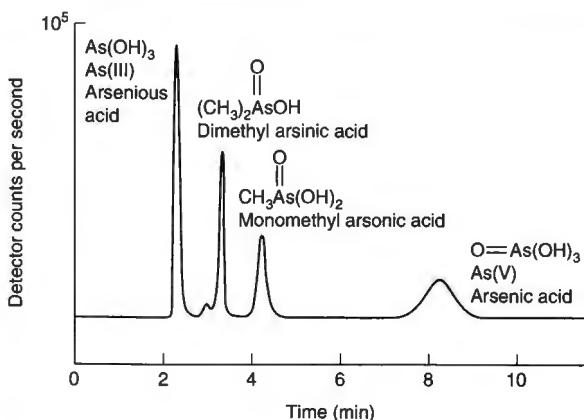
Notes and References

1. D. J. Frederick, "John Fenn: Father of Electrospray Ionization," *Chemistry*, Winter 2003, p. 13.
2. Training modules for chromatography, electrophoresis, mass spectrometry, and spectroscopy are available from www.academysavant.com. See also D. C. Stone, "Teaching Chromatography Using Virtual Laboratory Exercises," *J. Chem. Ed.* **2007**, 84, 1488.
3. A. Carlin-Sinclair, I. Marc, L. Menguy, and D. Prim, "The Determination of Methylxanthines in Chocolate and Cocoa by Different Separation Techniques: HPLC, Instrumental TLC, and MECC," *J. Chem. Ed.* **2009**, 86, 1307.

Chromated Copper Arsenate Wood Preservative



Residential outdoor wooden structure. [Colin Buckland/Fotolia.com.]



Anion chromatography (Hamilton PRP-X100 column) of arsenic compounds (each at 10 ppb) at pH 5.8 in 15 mM K₂HPO₄/KH₂PO₄ with atomic fluorescence detection of As. Structures of the parent acids are shown. Arsenic compounds were converted to AsH₃, which decomposes to As atoms in the detector. [Courtesy Y. Cai and L. Yehiyan, Florida International University.]

Chromated copper arsenate, made from a mixture of copper, chromium, and arsenic compounds, has been used to protect wood for outdoor applications from decay and insects since the 1930s. Treated wood has a characteristic light green color. Environmental concerns led to restrictions on the use of this preservative for residential timber in the U.S. and Europe beginning in 2004. Treated wood is still legal for many applications, and a great deal of treated wood remains in structures built before the ban.

A recent study measured the rate at which arsenic is leached by rainwater from treated lumber in Florida.¹ Rainwater runoff from a backyard wooden deck in the first year after its construction had an average total arsenic concentration of 600 µg/L (600 ppb), compared with 2 µg/L from a deck made of untreated lumber. (The U.S. limit for As in drinking water is 10 ppb.) About 90% of the arsenic was As(V) (arsenate) and 10% was As(III) (arsenite). No organoarsenic species were detected. At the end of a year, 2.2 g of arsenic had been leached, amounting to 5% of the arsenic in the deck. Arsenic concentration in the water infiltrating the sand beneath the deck through a depth of 0.7 m was 18 µg/L at the end of the year. Most of the arsenic leached from the wood was adsorbed by the sand. When chromated copper arsenate-treated wood is discarded, it should be sent to a lined landfill to protect the surroundings from contamination.

Chromatographic Methods and Capillary Electrophoresis

The liquid chromatography discussed in Chapter 22 separates solutes by *adsorption* or *partition* mechanisms. In this chapter, we consider separations by *ion-exchange*, *molecular exclusion*, and *affinity* chromatography, which were illustrated in Figure 21-2. We also consider *capillary electrophoresis*, which separates species on the basis of their different rates of migration in an electric field.

23-1 Ion-Exchange Chromatography

Ion-exchange chromatography is based on the attraction between solute ions and charged sites in the stationary phase (Figure 21-2). **Anion exchangers** have positively charged groups on the stationary phase that attract solute anions. **Cation exchangers** contain negatively charged groups that attract solute cations.

The stationary phase for ion-exchange chromatography is usually a *resin*, such as polystyrene, which consists of amorphous (noncrystalline) particles. Polystyrene is made into a cation exchanger when negative sulfonate ($-\text{SO}_3^-$) or carboxylate ($-\text{CO}_2^-$) groups are attached to the benzene rings (Figure 23-1). Polystyrene is an

Anion exchangers contain bound positive groups.

Cation exchangers contain bound negative groups.

Poly(acrylic acid) in superabsorbent disposable baby diapers is a commonly encountered cation exchanger that you can demonstrate in your classroom.²

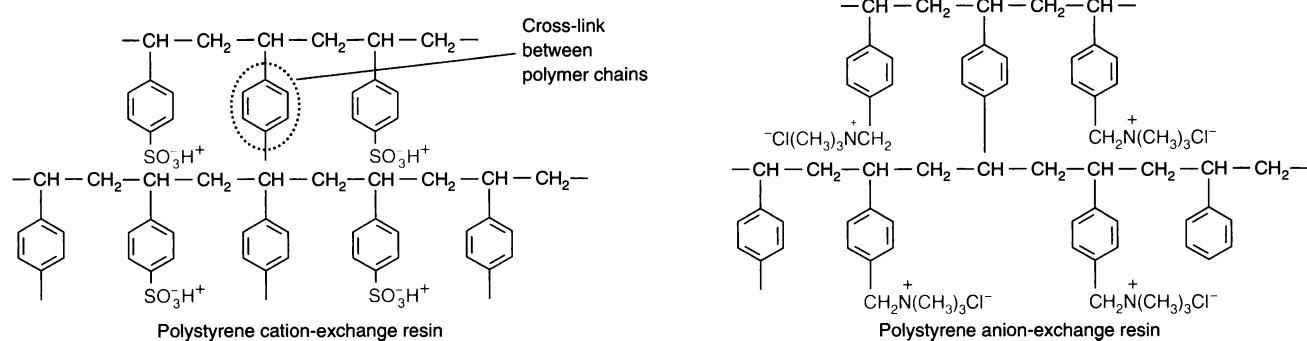


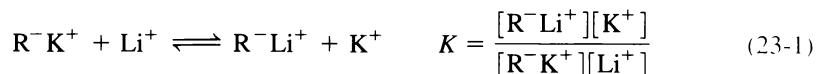
Figure 23-1 Structures of polystyrene ion-exchange resins. Cross-links are covalent bridges between polymer chains.

anion exchanger if ammonium groups ($-\text{NR}_3^+$) are attached. Cross-links (covalent bonds) between polystyrene chains in Figure 23-1 control the pore sizes into which solutes can diffuse.

Gel particles are softer than resin particles. Cellulose and dextran ion-exchange gels, which are polymers of the sugar glucose, possess larger pore sizes and lower charge densities. Gels are better suited than resins for ion exchange of macromolecules, such as proteins. For high-performance liquid chromatography, proprietary organic- and inorganic-based ion exchangers are designed to have appropriate pore size and charge density for protein interactions, combined with rigidity for operation at high pressure.

Ion-Exchange Selectivity

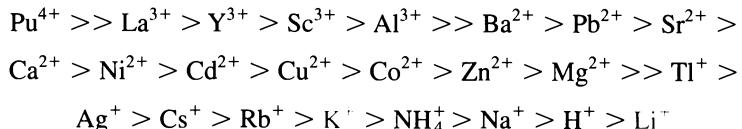
Consider the competition of K^+ and Li^+ for sites on the cation-exchange resin, R^- :



The equilibrium constant is called the *selectivity coefficient*, because it describes the relative affinities of the resin for Li^+ and K^+ . Discrimination between different ions tends to increase with the extent of cross-linking, because the resin pore size shrinks as cross-linking increases.

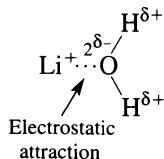
The **hydrated radius** of an ion is the effective size of the ion plus its tightly bound sheath of water molecules, which are attracted by the positive or negative charge of the ion (Figure 12-3). The large species $\text{Li}(\text{H}_2\text{O})_x^+$ does not have as much access to the resin as the smaller species $\text{K}(\text{H}_2\text{O})_x^+$.

More highly charged ions bind more tightly to ion-exchange resins. For ions of the same charge, the larger the hydrated radius, the less tightly the ion is bound. An approximate order of selectivity for some cations is



A large excess of one ion will displace another ion from the resin.

“Quantitative” is chemists’ jargon for “complete.”



Reaction 23-1 can be driven in either direction. Washing a column containing K^+ with a substantial excess of Li^+ will replace K^+ with Li^+ . Washing a column in the Li^+ form with excess K^+ will convert the resin into the K^+ form.

An ion exchanger loaded with one ion will bind a small amount of a different ion nearly quantitatively (completely). A resin loaded with K^+ binds small amounts of Li^+ quantitatively, even though the selectivity is greater for K^+ . The same resin binds large quantities of Ni^{2+} because the selectivity for Ni^{2+} is greater than that for K^+ . Even though Fe^{3+} is bound more tightly than H^+ , Fe^{3+} can be quantitatively removed from the resin by washing with excess acid.

To separate one ion from another by ion-exchange chromatography, *gradient elution* with increasing ionic strength (ionic concentration) in the eluent is extremely valuable. In Figure 23-2, a gradient of $[\text{H}^+]$ was used to separate lanthanide cations (M^{3+}). The more strongly bound metal ions require a higher concentration of H^+ for elution. An ionic strength gradient is analogous to a solvent gradient in HPLC or a temperature gradient in gas chromatography. Box 23-1 shows some applications of ion-exchange chromatography.

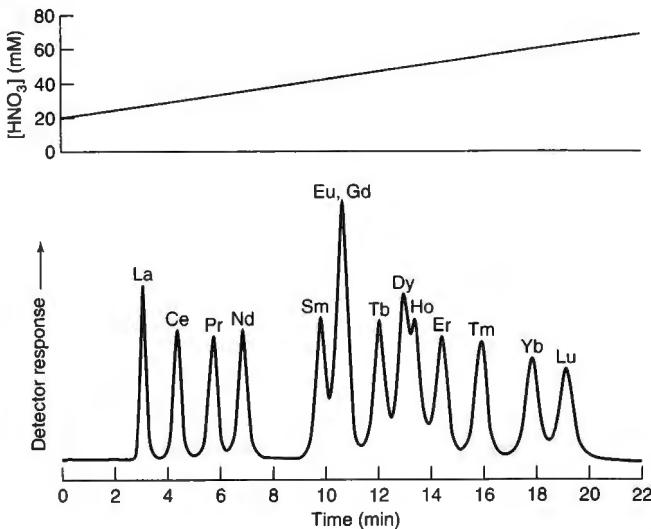


Figure 23-2 Elution of lanthanide(III) ions from a cation exchanger, by using a gradient of H^+ (20–80 mM HNO_3 over 25 min) to drive off more strongly retained cations. The higher the atomic number of the lanthanide, the smaller its ionic radius and the more strongly it binds to chelating groups on the resin. Lanthanides were detected spectrophotometrically by reaction with a color-forming reagent after elution. [Y. Inoue, H. Kumagai, Y. Shimomura, T. Yokoyama, and T. M. Suzuki, *Anal. Chem.* 1996, 68, 1517.]

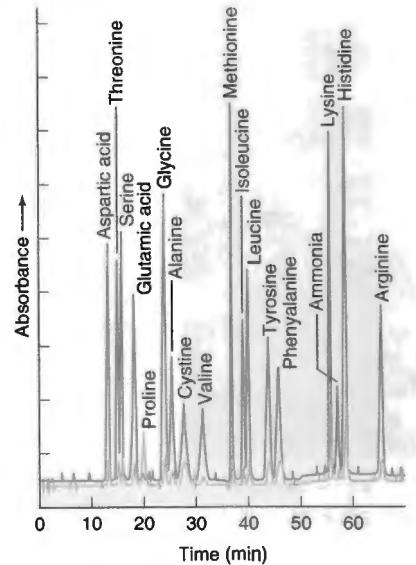
Box 23-1 Applications of Ion Exchange

In the rush to create an atomic bomb during World War II, it was necessary to isolate significant quantities of pure lanthanide elements (rare earth elements 57 to 71).³ The photograph shows some of 12 ion-exchange columns (3.0 m long \times 10 cm diameter) in a pilot plant at Iowa State College. Each column took several weeks to separate a 50- to 100-g mixture of rare earth chlorides.



Preparative-scale ion-exchange columns used to separate rare earths for the Manhattan Project during World War II. [Courtesy Iowa State University Library Special Collections Department.]

In the 1950s, W. H. Stein and S. Moore conducted pioneering work at Rockefeller Institute to understand the structure of the enzyme ribonuclease, for which they were awarded the Nobel Prize in 1972. Continual need to measure amino acids led them to explore amino acid separations by ion exchange.⁴ By 1958, their design led to the first commercial automated amino acid analyzer—a revolutionary advance for biochemistry.



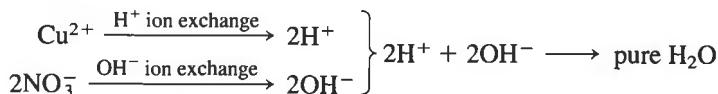
Ion-exchange chromatogram from Beckman-Spinco model 121MB amino acid analyzer introduced in 1969. After separation, amino acids were derivatized with ninhydrin to form colored products detected by visible absorption. [Courtesy Beckman-Coulter, Fullerton CA.]

What Is Deionized Water?

Home water softeners use ion exchange to replace Ca^{2+} and Mg^{2+} from "hard" water with Na^+ (Box 13-2).

Charge is conserved during ion exchange. One Cu^{2+} displaces 2H^+ from a cation-exchange column. It takes 3H^+ to displace one Fe^{3+} . One SO_4^{2-} displaces 2OH^- from an anion-exchange column.

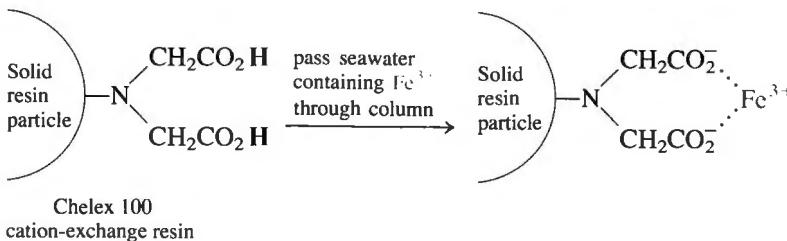
Deionized water is prepared by passing water through an anion-exchange resin loaded with OH^- and a cation-exchange resin loaded with H^+ . Suppose, for example, that $\text{Cu}(\text{NO}_3)_2$ is present in the water. The cation-exchange resin binds Cu^{2+} and replaces it with 2H^+ . The anion-exchange resin binds NO_3^- and replaces it with OH^- . The H^+ and OH^- combine, so the eluate is pure H_2O :



Preconcentration

Measuring extremely low levels of analyte is called **trace analysis**. Trace analysis is especially important for environmental problems in which low concentrations of substances, such as mercury in fish, can become concentrated over many years in people who eat large quantities of fish. For trace analysis, analyte concentration may be so low that it cannot be measured without **preconcentration**, a process in which analyte is brought to a higher concentration prior to analysis.

Metals in natural waters can be preconcentrated with a cation-exchange column:



When a large volume of water is passed through a small volume of resin, cations are concentrated into the small column. Cations can then be displaced into a small volume of solution by eluting the column with concentrated acid:

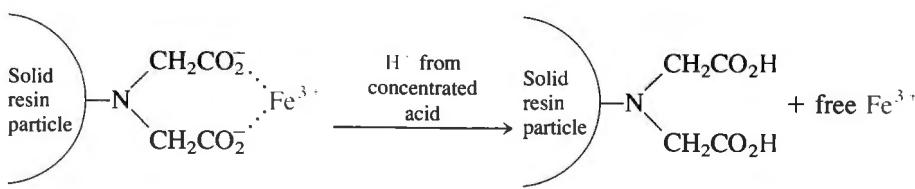


Figure 23-3 shows a column containing 1.2 mL of cation-exchange resin used to preconcentrate traces (11–330 ng) of Fe^{3+} from 10 L of filtered seawater. Elution of Fe^{3+} from the resin with 1.5 M HNO_3 into a final volume of 10 mL gives a 1 000-fold increase in concentration. When done with *extreme* care in a shipboard clean-room, a blank has only ~1 ng of Fe.

Simultaneous Separation of Cations and Anions

If the stationary phase has fixed positive *and* negative groups, then it can act as an anion and cation exchanger simultaneously (Figure 23-4). The ZIC-HILIC® bonded

Figure 23-3 Cation-exchange column containing 1.2 mL of resin used for shipboard preconcentration of traces of Fe^{3+} from 10 L of filtered seawater. [F. Lacan, A. Radic, M. Labatut, C. Jeandel, F. Poitrasson, G. Sarthou, C. Pradoux, J. Chmeleff, and R. Freydier, *Anal. Chem.* 2010, 82, 7103. Figure courtesy F. Lacan, Observatoire Midi Pyrenees.]

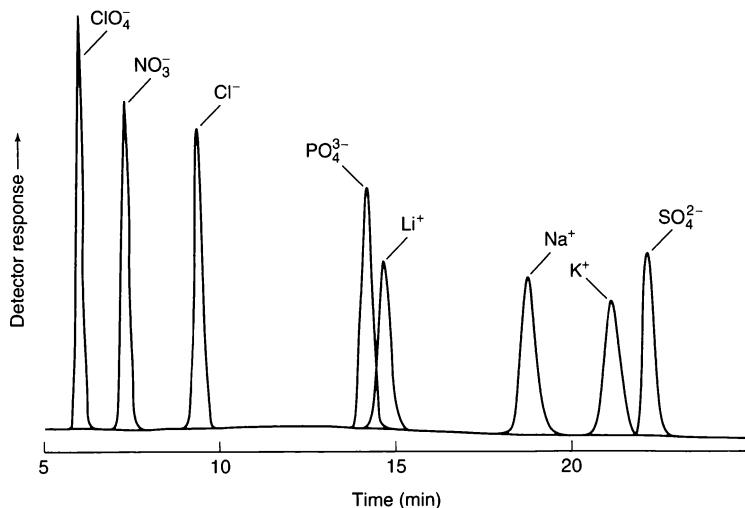


Figure 23-4 Simultaneous separation of anions and cations on a ZIC-HILIC® zwitterionic stationary phase with charged aerosol detection. The $150 \times 4.6\text{-mm}$ column with $5\text{-}\mu\text{m}$ -diameter stationary phase was eluted with a gradient of 20–70% B over 26 min at a flow rate of $0.5\text{ mL}/\text{min}$ at 30°C . Solvent A: 15 vol% 100 mM ammonium acetate ($\text{pH } 4.68$) in water, 5% methanol, 20% 2-propanol, 60% acetonitrile. Solvent B: 50 vol% 30 mM ammonium acetate ($\text{pH } 4.68$) in water, 5% methanol, 20% 2-propanol, 25% acetonitrile. [M. Swartz, *LCGC*, July 2010, p. 530.]

stationary phase in Table 22-3 is an example of such a phase. The charged aerosol detector used to obtain the chromatogram was shown in Figure 22-25. This detector responds to almost all analytes and is compatible with gradient elution.



Ask Yourself

- 23-A. (a) What is deionized water? What kind of impurities are not removed by deionization?
- (b) Why is gradient elution used in Figure 23-2?
- (c) Explain how preconcentration of cations with an ion exchanger works. Why must the concentrated acid eluent be very pure?
- (d) How can an ion-exchange column separate both anions and cations in Figure 23-4?
- (e) Explain how the charged aerosol detector used in Figure 23-4 works.

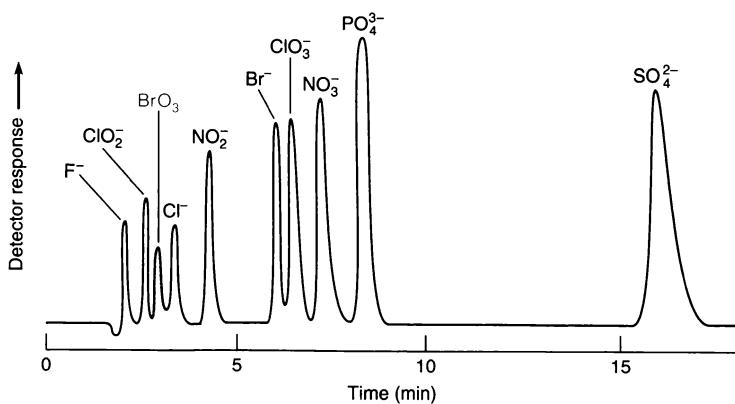
23-2 Ion Chromatography

Ion chromatography is a high-performance version of ion-exchange chromatography, with a key modification that removes eluent ions before detecting analyte ions. Ion chromatography is the method of choice for anion analysis. It is used in the semiconductor industry to monitor anions and cations at 0.1-ppb levels in deionized water. Figure 23-5 shows an example of anion chromatography in environmental analysis.

In ion chromatography, anions are separated by ion exchange and detected by their electrical conductivity. The conductivity of the electrolyte in the eluent is ordinarily high enough to make it difficult or impossible to detect the conductivity change when analyte ions are eluted. Therefore the key feature of *suppressed-ion* chromatography is removal of unwanted electrolyte prior to conductivity measurement.

Figure 23-5 Converting one human hazard into another. Chlorination of drinking water converts some organic compounds into potential carcinogens, such as CHCl_3 . To reduce this risk, ozone (O_3) has replaced Cl_2 in some municipal purification systems. Unfortunately, O_3 converts bromide (Br^-) into bromate (BrO_3^-), another carcinogen that must be monitored. The figure shows an anion chromatographic separation of ions found in drinking water. With preconcentration of the water, the detection limit for bromate is ~ 2 ppb. [R. J. Joyce, *Am. Environ. Lab.*, May 1994, p. 1.]

The separator column separates analytes, and the suppressor replaces ionic eluent with a nonionic species.



In Figure 23-6, a sample containing NaNO_3 and CaSO_4 is injected into the *separator column*, which is an anion-exchange column with OH^- at the anion-exchange sites. Upon elution with KOH , NO_3^- and SO_4^{2-} equilibrate with the resin and are slowly displaced by OH^- . Na^+ and Ca^{2+} are not retained and simply wash through. Eventually, KNO_3 and K_2SO_4 are eluted from the separator column. These species cannot be easily detected, however, because eluate contains KOH , whose high conductivity obscures that of the analytes.

To remedy this problem, the solution next passes through an electrolytic suppressor, in which cations (K^+ , in this example) are replaced by H^+ . The net result is that

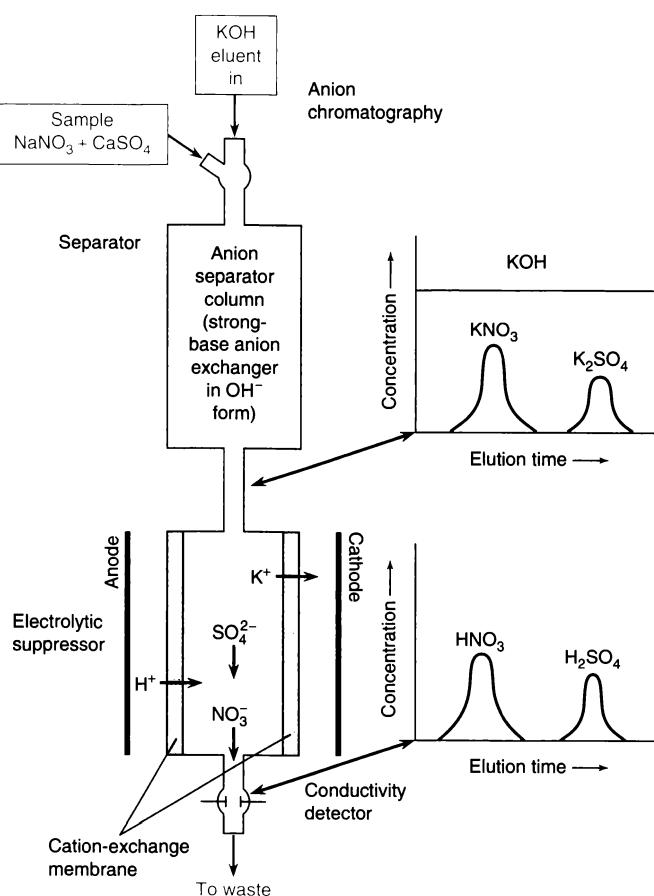


Figure 23-6 Suppressed-ion anion chromatography.

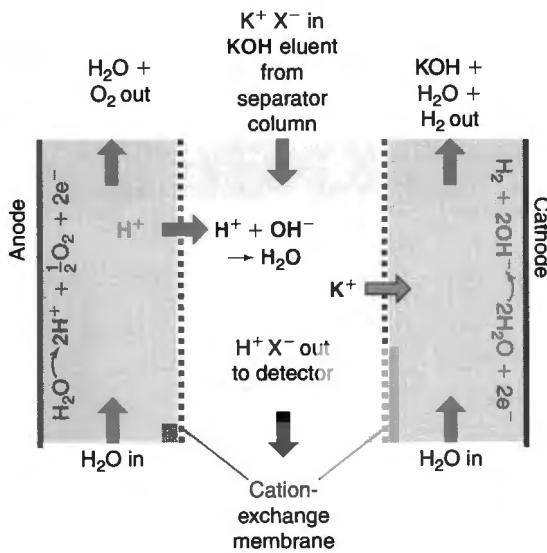


Figure 23-7 Electrolytic suppressor for anion chromatography replaces KOH eluent with H₂O.

KOH eluent, which has high conductivity, is converted into H₂O, which has low conductivity. When analyte is present, HNO₃ or H₂SO₄ with high conductivity is produced and detected.

Details of the electrolytic suppressor are shown in Figure 23-7. The anode at the left generates H⁺ and O₂ from flowing H₂O. The H⁺ diffuses into the eluate, where it reacts with OH⁻ to make H₂O. The cathode at the right generates OH⁻ and H₂. For charge balance, K⁺ migrates into the cathode compartment, where it is swept away in a flowing stream of water. The net result is that KOH in the eluate is converted to H₂O.

Automated systems also generate KOH eluent electrolytically. It is only necessary to add deionized water to compensate for evaporation to allow an automated system with a 4-mm-diameter column to run for a month.

Ask Yourself

- What do the separator and suppressor do in ion chromatography?
- What causes nitrate and sulfate to be separated in Figure 23-6?
- Why do we need to convert KOH to H₂O in the suppressor?

23-3 Molecular Exclusion Chromatography

In **molecular exclusion chromatography** (also called *gel filtration*, *gel permeation*, or *size exclusion chromatography*), molecules are separated according to their size. Small molecules enter the small pores in the stationary phase, but large molecules do not (Figure 21-2). Because small molecules must pass through an effectively larger volume in the column, large molecules are eluted first (Figure 23-8). This technique is widely used in biochemistry and polymer chemistry to purify macromolecules and to measure molecular mass (Figure 23-9). Figure 23-10 shows an application in materials chemistry.

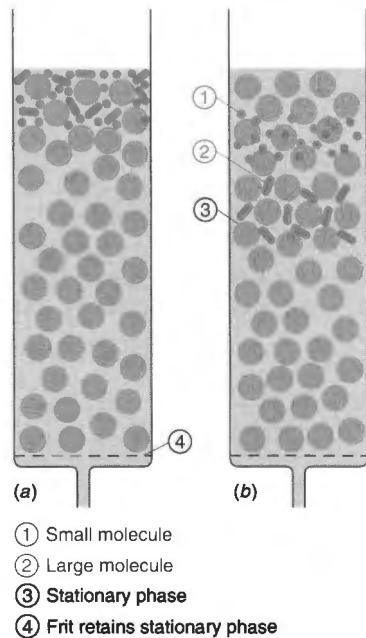


Figure 23-8 (a) A mixture of large and small molecules is applied to the top of a molecular exclusion chromatography column. (b) Large molecules cannot penetrate the pores of the stationary phase, but small molecules can. Therefore less of the volume is available to large molecules and they move down the column faster.

Large molecules pass through the column *faster* than small molecules do.

1	Glutamate dehydrogenase (290 000 Da)
2	Lactate dehydrogenase (140 000 Da)
3	Enolase kinase (67 000 Da)
4	Adenylate kinase (32 000 Da)
5	Cytochrome c (12 400 Da)

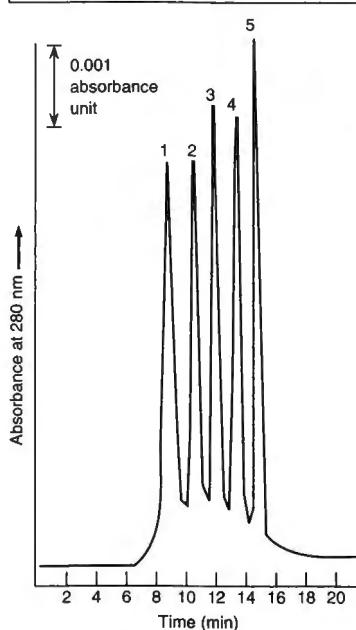


Figure 23-9 Separation of proteins by molecular exclusion chromatography, using a TSK 3000SW HPLC column. The highest molecular masses are eluted first. [Courtesy Varian Associates, Palo Alto, CA.]

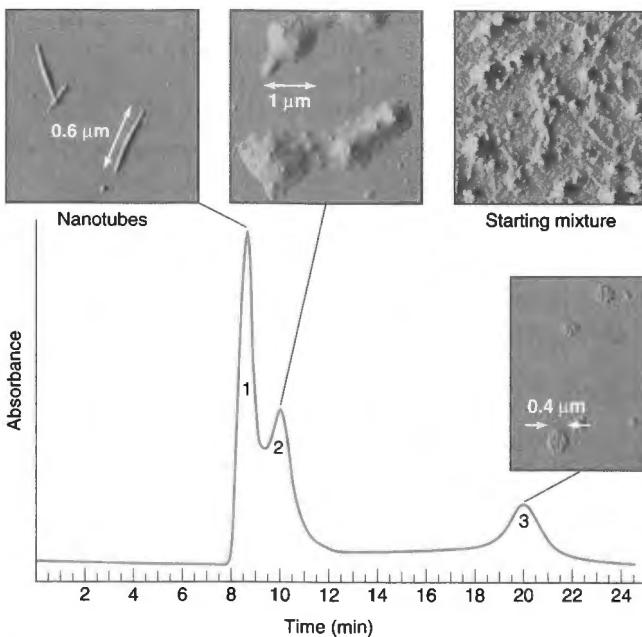


Figure 23-10 Purification of carbon nanotubes by molecular exclusion chromatography. An electric arc struck between graphite rods creates nanometer-size carbon products, including tubes with extraordinary strength and possible use in electronic devices. Molecular exclusion chromatography separates nanotubes (fraction 1) from other forms of carbon in fractions 2 and 3. The stationary phase is PLgel MIXED-A, a polystyrene-divinylbenzene resin with pore sizes corresponding to a molecular mass range of 2 000 to 40 000 000 Da. Images of carbon in each fraction were made by atomic force microscopy. [B. Zao, H. Hu, S. Niyogi, M. E. Itkis, M. A. Hamon, P. Bhowmik, M. S. Meier, and R. C. Haddon, *J. Am. Chem. Soc.* **2001**, 123, 11673.]

In molecular exclusion chromatography, the volume of mobile phase (the solvent) in the column *outside* the stationary phase is called the *void volume*, V_0 . Large molecules that are excluded from the stationary phase are eluted in the void volume. Void volume is measured by passing through the column a molecule that is too large to enter the pores. The dye Blue Dextran (2×10^6 Da) is commonly used.

Molecular Mass Determination

Retention volume is the volume of mobile phase required to elute a particular solute from the column. Each stationary phase has a range over which there is a logarithmic relation between molecular mass and retention volume. We can estimate the molecular mass of an unknown by comparing its retention volume with those of standards. For proteins, it is important to use eluent with an ionic strength high enough (such as 0.05 M NaCl) to eliminate electrostatic adsorption of solute by occasional charged sites on the gel.

Example Molecular Mass Determination by Gel Filtration

Proteins were passed through a gel filtration column and retention volumes (V_r) were measured. Estimate the molecular mass (MM) of the unknown.

Compound	V_r (mL)	Molecular mass	$\log(\text{molecular mass})$
Blue Dextran 2000	17.7	2×10^6	6.301
aldolase	35.6	158 000	5.199
catalase	32.3	210 000	5.322
ferritin	28.6	440 000	5.643
thyroglobulin	25.1	669 000	5.825
unknown	30.3	?	

SOLUTION Figure 23-11 plots V_r versus $\log(\text{MM})$. Putting the retention volume of unknown into the least-squares fit to the five calibration standards allows us to solve for the molecular mass of the unknown:

$$V_r (\text{mL}) = -15.75[\log(\text{MM})] + 117.0$$

$$30.3 = -15.75[\log(\text{MM})] + 117.0$$

$$\Rightarrow \log(\text{MM}) = 5.505 \Rightarrow \text{MM} = 10^{5.505} = 320 000$$

 **Test Yourself** What is the expected retention volume of a protein with a molecular mass of 888 000? (**Answer:** 23.3 mL)

Ask Yourself

- 23-C. A gel filtration column has a radius (r) of 0.80 cm and a length (l) of 20.0 cm.
- Calculate the total volume of the column, which is equal to $\pi r^2 l$.
 - Blue Dextran was eluted in a volume of 18.2 mL. What volume is occupied by the stationary phase plus the solvent inside the pores of the stationary phase?
 - Suppose that the pores occupy 60.0% of the stationary phase volume. Over what volume range (from x mL for the largest molecules to y mL for the smallest molecules) are all solutes expected to be eluted?

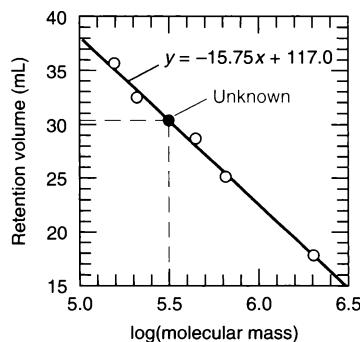


Figure 23-11 Calibration curve used to estimate molecular mass of an unknown protein by molecular exclusion chromatography.

23-4 Affinity Chromatography

Affinity chromatography is used to isolate a single compound from a complex mixture. The technique is based on specific binding of that one compound to the stationary phase (Figure 21-2). When sample is passed through the column, only one solute is bound. After everything else has washed through, the one adhering solute is eluted by changing conditions such as pH or ionic strength to weaken its binding. Affinity chromatography is especially applicable in biochemistry and is based on specific interactions between enzymes and substrates, antibodies and antigens, or receptors and hormones.

Figure 23-12 shows the isolation of the protein immunoglobulin G (IgG) by affinity chromatography on a column containing covalently bound *protein A*. Protein A binds to one specific region of IgG at $\text{pH} \geq 7.2$. When a crude mixture containing IgG and other proteins was passed through the column at $\text{pH } 7.6$, everything except IgG was eluted within 0.3 min. At 1 min, the eluent pH was lowered to 2.6 and IgG was cleanly eluted at 1.3 min.

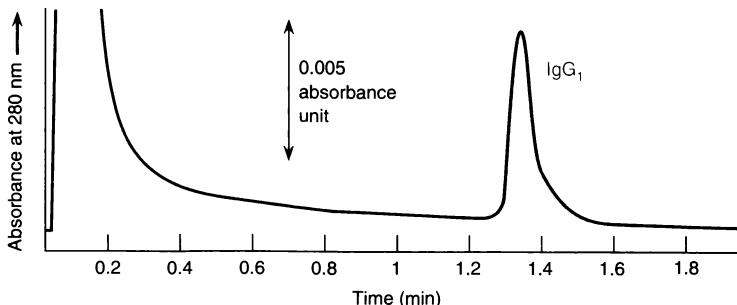


Figure 23-12 Purification of monoclonal antibody IgG by affinity chromatography on a column (5 cm long \times 4.6 mm diameter) containing protein A covalently attached to a polymer support. Other proteins in the sample are eluted from 0 min to 0.3 min at pH 7.6. When the eluent pH is lowered to 2.6, IgG is freed from protein A and emerges from the column. [B. J. Compton and L. Kreilgaard, *Anal. Chem.* 1994, 66, 1175A.]

23-5 What Is Capillary Electrophoresis?

Late in 2007, more than 200 people receiving the anticoagulant *heparin* suffered acute, allergic reactions and died.⁵ Heparin is a mixture of sulfate-substituted polysaccharides (polymers made of sugar units) that have molecular masses of 2 to 50 kDa and are isolated from pig intestines. As soon as the problem was recognized in January 2008, U.S. distributors recalled heparin and the U.S. Food and Drug Administration launched an investigation. Heparin is administered thousands of times every day to manage life-threatening conditions, so an immediate understanding and solution to the problem were required.

The enzyme heparinase cleaves heparin into disaccharide units (containing two sugars). Tainted heparin contained 20 to 50 wt% of macromolecular components that did not react with heparinase. *Capillary electrophoresis* was the initial tool of choice to observe two contaminants (Figure 23-13).^{6,7} One was dermatan sulfate, which was not known to cause allergic reactions. The other was identified by nuclear magnetic resonance as oversulfated chondroitin sulfate. An animal study verified that oversulfated chondroitin sulfate caused the allergic reaction. By March 2008, deaths from

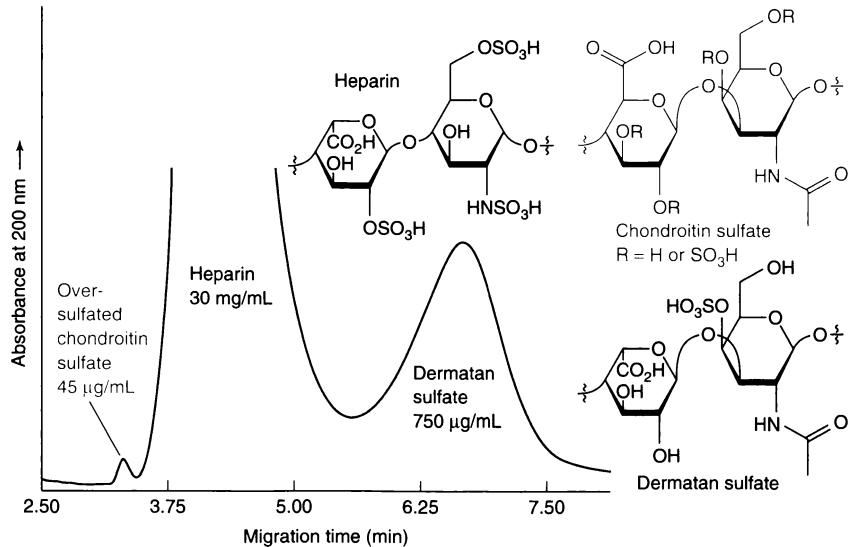


Figure 23-13 Electropherogram of heparin (30 mg/mL) spiked with oversulfated chondroitin sulfate and dermatan sulfate. Tainted heparin had \sim 200 times more oversulfated chondroitin sulfate than shown here. [Courtesy Robert Weinberger, CE Technologies, and Todd Wielgos, Baxter Healthcare; T. Wielgos, K. Havel, N. Ivanova, and R. Weinberger, *J. Pharma. Biomed. Anal.* 2009, 49, 319.]

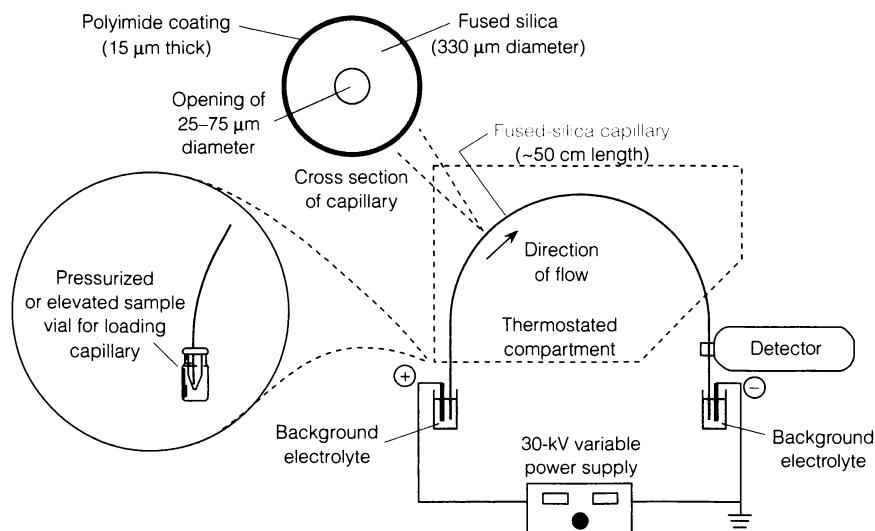


Figure 23-14 Capillary electrophoresis. Sample is injected by elevating or applying pressure to the sample vial or by applying suction at the outlet of the capillary.

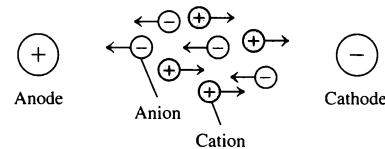
contaminated heparin had ceased and emergency regulations were issued to incorporate capillary electrophoresis and nuclear magnetic resonance into required testing of heparin imported into the U.S. Contaminated heparin had been prepared in China. Oversulfated chondroitin sulfate might have been added because it has anticoagulant activity and costs less than heparin.

Electrophoresis is the migration of ions in an electric field. Anions are attracted to the anode and cations are attracted to the cathode. Different ions migrate at different speeds. **Capillary electrophoresis** is a high-resolution separation technique conducted with solutions of ions in a narrow capillary tube. A clever modification of the technique allows us to separate neutral analytes also. Capillary electrophoresis applies with equal ease to the separation of macromolecules, such as proteins and DNA, and small species, such as Na^+ and benzene. Capillary electrophoresis can analyze the contents of a single cell.

The typical experiment in Figure 23-14 features a fused-silica (SiO_2) capillary that is 50 cm long and has an inner diameter of 25–75 μm . The capillary is immersed in *background electrolyte* solution at each end. At the start of the experiment, one end is dipped into a sample vial and $\sim 10 \text{ nL}$ (nanoliters, 10^{-9} L) of liquid are introduced by applying pressure to the sample vial or by applying an electric field between the sample vial and the column. After the capillary is placed back into the electrolyte solution, a potential difference of 20–30 kV is applied to the electrodes to cause ions in the capillary to migrate. Different ions migrate at different speeds, so they separate from one another as they travel through the capillary. Ions are detected inside the capillary near the far end with an ultraviolet absorbance monitor or other detector. The graph of detector response versus time in Figure 23-15 is called an *electropherogram*. (In chromatography, we call the same graph a *chromatogram*.) Capillary electrophoresis is not as sensitive as ion chromatography, but it is more sensitive than many currently available ion-selective electrodes.

Capillary electrophoresis can provide extremely narrow bands. Three mechanisms of band broadening in chromatography are longitudinal diffusion (B in the van Deemter equation 21-7), the finite rate of mass transfer between the stationary and mobile phases (C in the van Deemter equation), and multiple flow paths around particles (A in the van Deemter equation). An open tubular column in chromatography or electrophoresis reduces band broadening (relative to that of a packed column) by eliminating multiple flow paths (the A term). Capillary electrophoresis further

Cations are attracted to the negative terminal (the cathode). Anions are attracted to the positive terminal (the anode).



Background electrolyte, also called *run buffer*, is the solution in the electrode reservoirs. It controls pH and ionic composition in the capillary.

The greater the charge on the ion, the faster it migrates in the electric field. The greater the size of the ion, the slower it migrates.

Working ranges for chloride analysis:

Ion-selective electrode

Capillary electrophoresis

Ion chromatography

1 ppb	1 ppm	1 000 ppm
1 ng/mL	1 $\mu\text{g/mL}$	1 mg/mL

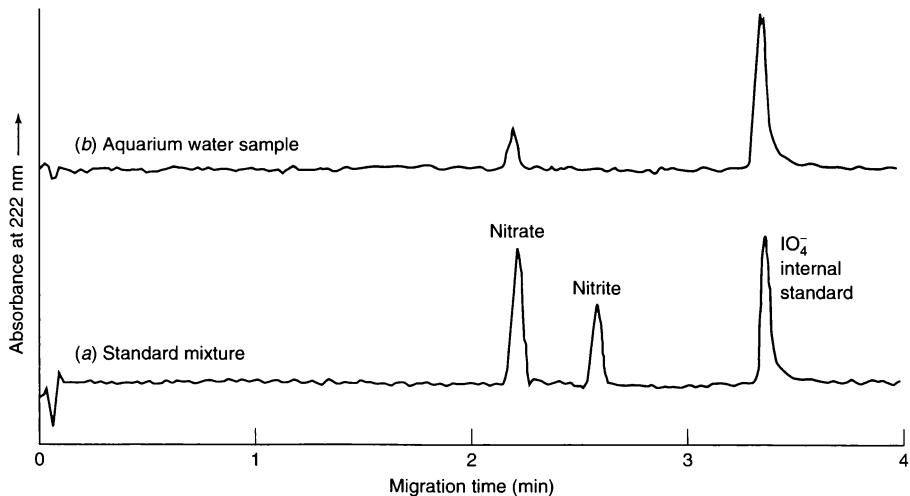


Figure 23-15 Measurement of nitrate in aquarium water (Box 6-1) by capillary electrophoresis. At the detection wavelength of 222 nm, many species in the water have too little absorbance to be observed. (a) Standard mixture containing 15 $\mu\text{g/mL}$ nitrate (NO_3^-), 5 $\mu\text{g/mL}$ nitrite (NO_2^-), and 10 $\mu\text{g/mL}$ of the internal standard, periodate (IO_4^-). (b) 1:100 dilution of aquarium water with distilled water containing internal standard. This aquarium has NO_3^- but no detectable NO_2^- . [D. S. Hage, A. Chattopadhyay, C. A. C. Wolfe, J. Grundman, and P. B. Kelter, *J. Chem. Ed.* **1998**, 75, 1588.] Periodate is a questionable standard because it is an oxidizing agent that might react with organic matter in the aquarium water.

reduces broadening by eliminating the mass transfer problem (the *C* term) because *there is no stationary phase*. The only source of broadening under ideal conditions is longitudinal diffusion of solute as it migrates through the capillary. The routine separation efficiency of 50 000 to 500 000 theoretical plates in capillary electrophoresis is an order of magnitude greater than that of chromatography.

Ask Yourself

23-D. Capillary electrophoresis is noteworthy for analyzing small sample volumes and for producing high-resolution separations.

(a) A typical injected sample occupies a 5-mm length of the capillary. What volume of sample is this if the inside diameter of the capillary is 25 μm ? 50 μm ? (The volume of a cylinder of radius r is $\pi r^2 \times \text{length}$.)

(b) Which mechanisms of band broadening that operate in chromatography are absent in capillary electrophoresis?

23-6 How Capillary Electrophoresis Works

Capillary electrophoresis involves two simultaneous processes called *electrophoresis* and *electroosmosis*. Electrophoresis is the migration of ions in an electric field. Electroosmosis pumps the entire solution through the capillary from the anode toward the cathode. Superimposed on this one-way flow are the flow of cations, which are attracted to the cathode, and the flow of anions, which are attracted to the anode. In Figure 23-14, cations migrate from the injection end at the left toward the detector at the right. Anions migrate toward the left. Both cations and anions are

Two processes operate in capillary electrophoresis:

- *electrophoresis*: migration of cations to the cathode and anions to the anode
- *electroosmosis*: migration of bulk fluid toward the cathode

swept from left to right by electroosmosis. Cations arrive at the detector before anions. Neutral molecules swept along by electroosmosis arrive at the detector after the cations and before the anions.

Electroosmosis

Electroosmosis is the propulsion of fluid inside a fused-silica capillary from the anode toward the cathode caused by the applied electric field. To understand electroosmosis, consider what happens at the inside wall of the capillary. The wall is covered with silanol ($\text{Si}-\text{OH}$) groups that are negatively charged ($\text{Si}-\text{O}^-$) above pH 2. Figure 23-16a shows that the capillary wall and the solution immediately adjacent to the wall form an *electric double layer*. The double layer is composed of (1) a negative charge fixed to the wall and (2) an equal positive charge in solution adjacent to the wall. The thickness of the positive layer, called the *diffuse part of the double layer*, is approximately 1 nm. When an electric field is applied, cations are attracted to the cathode and anions are attracted to the anode. Excess cations in the diffuse part of the double layer drive the entire solution in the capillary toward the cathode (Figure 23-16b). The greater the applied electric field, the faster the flow.

Color Plate 23 shows a critical distinction between electroosmotic flow induced by an electric field and ordinary hydrodynamic flow induced by a pressure difference. Because it is driven by ions on the walls of the capillary, electroosmotic flow is uniform across the diameter of the liquid, as shown schematically in Figure 23-16c. The

Ions in the diffuse part of the double layer adjacent to the capillary wall are the “pump” that drives electroosmotic flow.

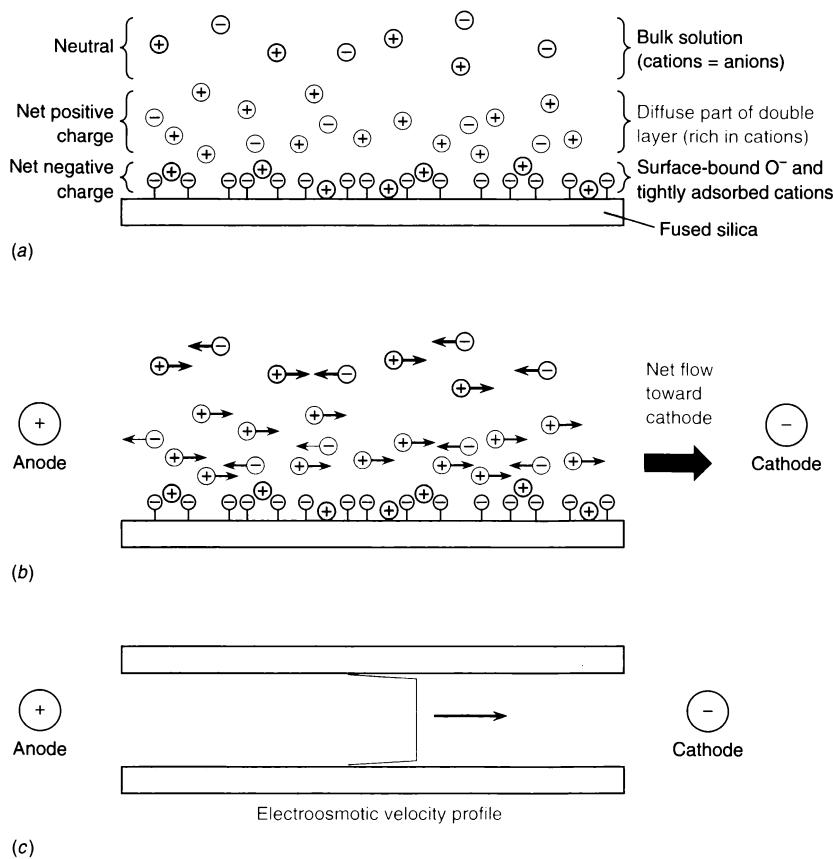


Figure 23-16 (a) Electric double layer is created by negative silica surface and excess cations in the diffuse part of the double layer in solution near the wall. The wall is negative and the diffuse part of the double layer is positive. (b) Predominance of cations in diffuse part of the double layer produces net electroosmotic flow toward the cathode when an external field is applied. (c) Electroosmotic velocity profile is uniform over more than 99.9% of the cross section of the capillary. A capillary is required to maintain constant temperature in the liquid. Temperature variation in larger-diameter tubes causes bands to broaden.

Migration time in electrophoresis is analogous to retention time in chromatography.

For quantitative analysis, use
normalized peak area = $\frac{\text{peak area}}{\text{migration time}}$

only mechanism that broadens the moving band is diffusion. In contrast, hydrodynamic flow has a parabolic velocity profile, with fastest motion at the center of the capillary and little velocity at the wall. A parabolic profile creates broad bands.

Electroosmosis decreases at low pH because the wall loses its negative charge when Si—O⁻ is converted into Si—OH and the number of cations in the double layer diminishes. Electroosmotic velocity is measured by adding an ultraviolet-absorbing neutral solute, such as methanol, to the sample and measuring the time it takes (called the *migration time*) to reach the detector. In one experiment with 30 kV across a 50-cm capillary, the electroosmotic velocity was 4.8 mm/s at pH 9 and 0.8 mm/s at pH 3.

In Figures 23-14 and 23-16, electroosmosis is from left to right because cations in the double layer are attracted to the cathode. Superimposed on electroosmosis of the bulk fluid, electrophoresis transports cations to the right and anions to the left. At neutral or high pH, electroosmosis is faster than electrophoresis and the net flow of anions is to the right. At low pH, electroosmosis is weak and anions may flow to the left and never reach the detector. To separate anions at low pH, you can reverse the polarity to make the sample side negative and the detector side positive.

For quantitative analysis by electrophoresis, *normalized peak areas* are required. The normalized peak area is the measured peak area divided by the migration time. In chromatography, each analyte passes through the detector at the same rate, so peak area is proportional to the quantity of analyte. In electrophoresis, analytes with different apparent mobilities pass through the detector at different rates. The higher the apparent mobility, the shorter the migration time and the less time the analyte spends in the detector. To correct for time spent in the detector, divide the peak area for each analyte by its migration time.

Detectors

The most common detector is an *ultraviolet absorbance monitor* set to a wavelength near 200 nm, where many solutes absorb. It is not possible to use such short wavelengths with larger-diameter columns, because the solvent absorbs too much radiation. A *fluorescence detector* works for fluorescent analytes or fluorescent derivatives. To measure either absorbance or fluorescence, the protective polyimide coating on the capillary in Figure 23-14 is removed at the location of the detector. *Electrochemical detection* is sensitive to analytes that can gain or lose electrons at an electrode. Eluate can be directed into a *mass spectrometer* with an electrospray interface to provide information on the quantity and molecular structure of analyte. *Conductivity detection* with ion-exchange suppression of the background electrolyte (as in ion chromatography, Figure 23-7) gives a sensitivity of 1–10 ppb for small ions.

In contrast with direct detection of analyte discussed so far, **indirect detection** relies on measuring a strong signal from background electrolyte and a weak signal from analyte as it passes the detector. Figure 23-17 illustrates indirect fluorescence

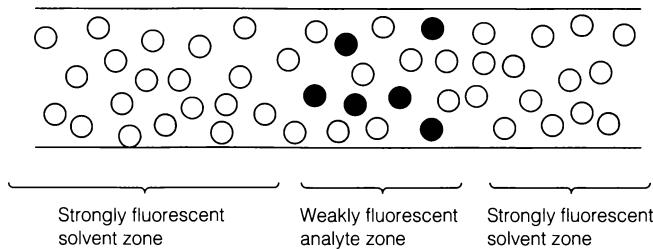


Figure 23-17 Principle of indirect detection: When analyte emerges from the capillary, the strong background signal decreases.

detection, but the same principle applies to any type of detection. A fluorescent ion with the same sign of charge as the analyte is added to background electrolyte to provide a steady background signal. In the analyte zone, there is less background ion because electroneutrality must be preserved. If the analyte ion is not fluorescent, the fluorescence level decreases when analyte emerges. We observe a *negative* signal.

Figure 23-18 shows indirect ultraviolet detection of Cl^- in the presence of ultraviolet-absorbing chromate, CrO_4^{2-} . In the absence of analyte, CrO_4^{2-} gives a steady absorbance at 254 nm. When Cl^- reaches the detector, there is less CrO_4^{2-} present and Cl^- does not absorb; therefore the detector signal *decreases*. $^{35}\text{Cl}^-$ and $^{37}\text{Cl}^-$ differ in electrophoretic mobility by only $\sim 0.1\%$. To obtain the exquisite separation seen in Figure 23-18, it was necessary to give the two species maximum time to migrate apart. This goal was achieved by selecting a high pH of 9.2, at which the electroosmotic flow toward the cathode is just barely greater than electrophoretic flow of Cl^- toward the anode, and by allowing 40 minutes for the two isotopes to separate.

Ask Yourself

- 23-E. (a) Capillary electrophoresis was conducted at pH 9, at which electroosmotic velocity is greater than electrophoretic velocity for a particular anion. Draw a picture of the capillary, showing the anode, cathode, injector, and detector. Show the directions of electroosmotic and electrophoretic flow of a cation and an anion. Show the direction of net flow for each ion.
 (b) If the pH is reduced to 3, electroosmotic velocity is less than electrophoretic velocity. In what directions will cations and anions migrate?
 (c) Explain why the detector signal is negative in Figure 23-18.

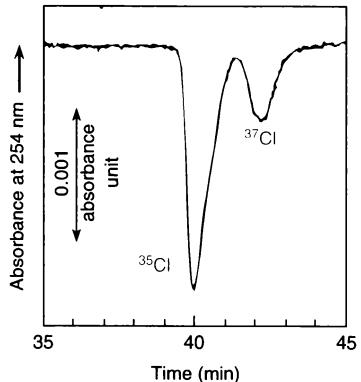


Figure 23-18 Separation of natural isotopes of $0.56 \text{ mM } \text{Cl}^-$ by capillary electrophoresis with indirect spectrophotometric detection at 254 nm. The background electrolyte contains $5 \text{ mM } \text{CrO}_4^{2-}$ to provide absorbance at 254 nm. There are few ways to separate isotopes so cleanly. This impressive separation was done by an undergraduate student at the University of Calgary. [C. A. Lucy and T. L. McDonald, *Anal. Chem.* **1995**, *67*, 1074.]

23-7 Types of Capillary Electrophoresis

The type of electrophoresis discussed so far is called **capillary zone electrophoresis**, in which separation is based on different electrophoretic velocities of different ions. Electroosmotic flow of the bulk fluid is toward the cathode (Figure 23-16b). Cations migrate faster than the bulk fluid and anions migrate slower than the bulk fluid. Therefore the order of elution is cations before neutrals before anions. If electrode polarity is reversed, the order of elution is anions before neutrals before cations. Neither scheme separates neutral molecules from one another.

Capillary zone electrophoresis can separate optical isomers if a suitable *optically active* complexing agent is added to the background electrolyte. An optically active substance, also called a *chiral* substance, is one that is not superimposable on its mirror image. The chiral crown ether in Figure 23-19 can bind to the ammonium group of an amino acid through $\text{NH} \cdots \text{O}$ hydrogen bonds. The crown ether has greater affinity for D-amino acids than for L-amino acids (see structures on page 493). Amino acids migrating through an electrophoresis column spend part of the time complexed to the crown ether, during which time they migrate at a rate different from that of the free amino acid. Migration times of D- and L-amino acids differ if one spends more time bound to the crown ether. Figure 23-19 shows the separation of D- and L-amino acids in the presence of the chiral crown ether.

Order of elution in capillary zone electrophoresis:

1. cations (highest mobility first)
2. all neutrals (unseparated)
3. anions (highest mobility last)

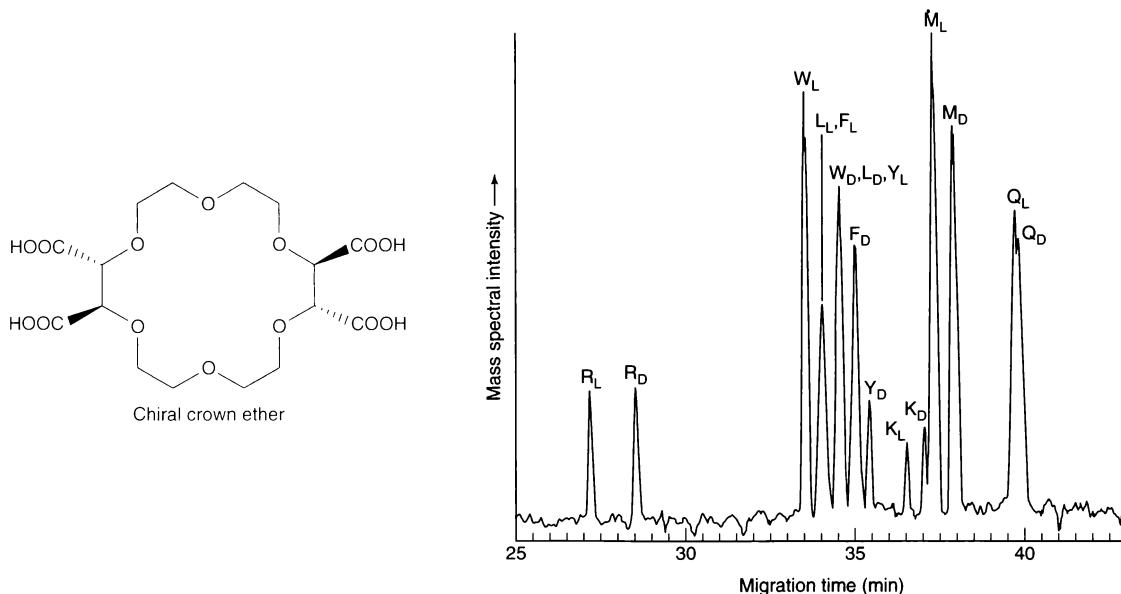


Figure 23-19 Capillary zone electrophoretic separation of D- and L-amino acids by adding a *chiral* crown ether to background electrolyte. Amino acids are designated by one-letter abbreviations listed in Table 11-1. The crown ether binds D-amino acids more strongly than it binds L-amino acids and therefore changes the migration time of the D-amino acid more than that of the L-amino acid. Wide-scan (m/z 74.5–250) mass spectra of liquid exiting the column were recorded continuously. The electropherogram was reconstructed by displaying the sum of ion intensities at m/z 132, 147, 150, 166, 175, 182, and 205. For example, m/z 150 responds only to protonated methionine, designated M in the figure. [C. L. Schultz and M. Moini, *Anal. Chem.* **2003**, *75*, 1508.]

Capillary electrophoresis also is being applied to the separation and identification of bacterial strains for medical diagnosis and in cases of food contamination (Figure 23-20). The surface of a bacterium has many charged groups, so it migrates in an electric field. The high density of charged groups in bacteria or proteins makes them prone to stick to the charged surface of a capillary. Therefore

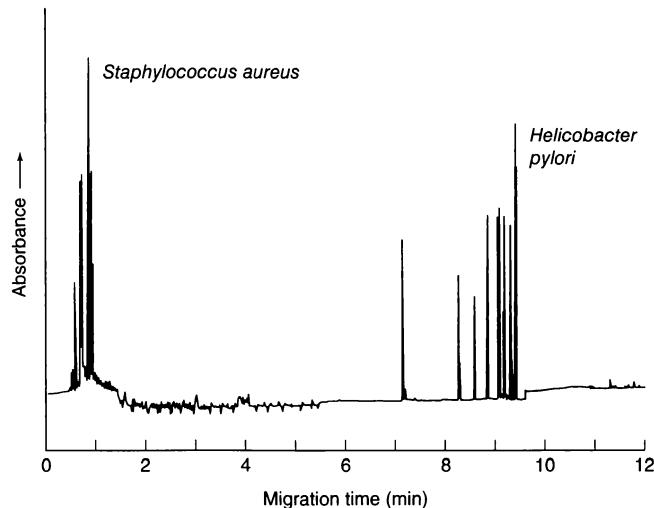


Figure 23-20 Capillary electrophoretic separation of whole bacteria found in wounds and bedsores. [E. Kłodzńska and B. Buszewski, *Anal. Chem.* **2009**, *81*, 8.]

capillary walls must be chemically modified or coated with polymers to reduce interactions with the walls.

Micellar Electrokinetic Capillary Chromatography

This mouthful of words describes a form of capillary electrophoresis that separates neutral molecules as well as ions (Figure 23-21). The key modification in **micellar electrokinetic capillary chromatography** is the use of *micelles* in the capillary solution. Micelles are described in Box 23-2, which you should read now.

To understand how neutral molecules are separated, suppose that the background electrolyte contains negatively charged micelles. In Figure 23-22, electroosmotic flow is to the right. Electrophoretic migration of the negatively charged micelles is to the left, but net motion is to the right because electroosmotic flow is faster than electrophoretic flow.

In the absence of micelles, all neutral molecules reach the detector together at a time we designate t_0 . Micelles injected with the sample reach the detector at time t_{mc} , which is longer than t_0 because micelles are negative and migrate upstream. If a neutral molecule equilibrates between free solution and the inside of the micelles, its migration time is increased, because it migrates at the slower rate of the micelle part of the time. In this case, the neutral molecule reaches the detector at a time between t_0 and t_{mc} .

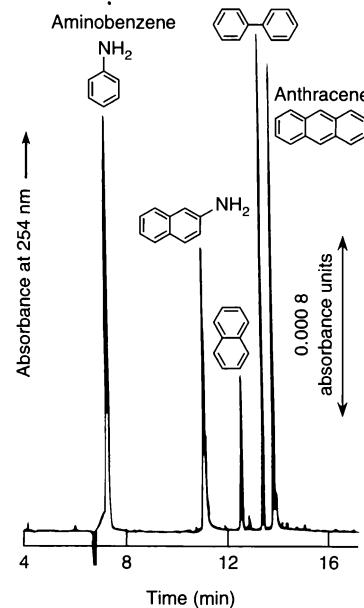
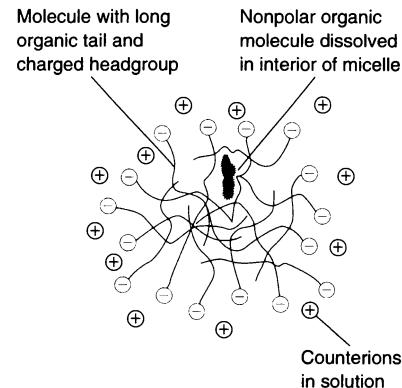
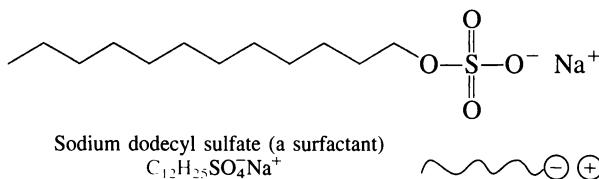


Figure 23-21 Separation of neutral molecules by micellar electrokinetic capillary chromatography. The average plate count in this experiment is 250 000 in 50 cm of capillary length. [J. T. Smith, W. Nashabeh, and Z. E. Rassi, *Anal. Chem.* **1994**, *66*, 1119.]

Box 23-2 What Is a Micelle?

A **micelle** is an aggregate of molecules with ionic headgroups and long, nonpolar tails. Such molecules are called *surfactants*, an example of which is sodium dodecyl sulfate:

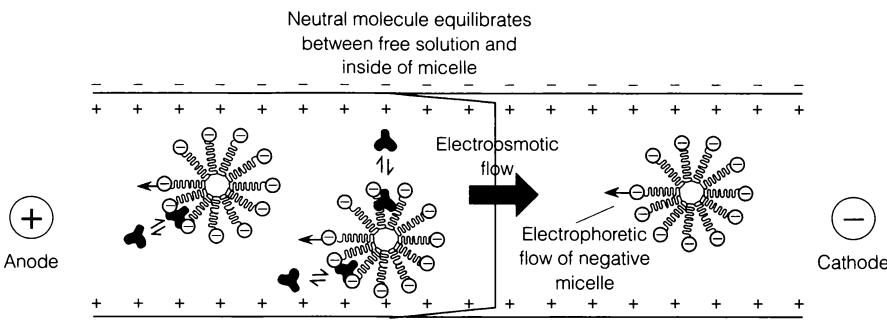


The polar headgroups of a micelle face outward, where they are surrounded by polar water molecules. The nonpolar tails face inward, where they form a little pocket resembling a nonpolar hydrocarbon solution. *Nonpolar solutes are soluble inside the micelle.*

At low concentrations, surfactant molecules do not form micelles. When the concentration exceeds the *critical micelle concentration*, spontaneous aggregation into micelles occurs. Isolated surfactant molecules exist in equilibrium with micelles.

Figure 23-22 Negatively charged sodium dodecyl sulfate micelles migrate upstream against electroosmotic flow. Neutral molecules are in dynamic equilibrium between free solution and the inside of the micelle. The more time spent in the micelle, the more the neutral molecule lags behind the electroosmotic flow.

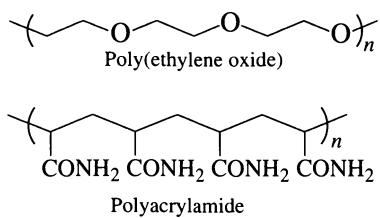
Micellar electrokinetic capillary electrophoresis: The more time a solute spends inside the micelle, the longer its migration time.



The more soluble the neutral molecule is in the micelle, the more time it spends inside the micelle and the longer is its migration time. The nonpolar interior of a sodium dodecyl sulfate micelle dissolves nonpolar solutes best. Polar solutes are not as soluble in the micelles and have a shorter retention time than nonpolar solutes do. Migration times of cations and anions also can be affected by micelles because ions might associate with micelles. Micellar electrokinetic capillary chromatography is truly a form of chromatography because micelles behave like a pseudostationary phase. Solutes partition between the mobile phase (the aqueous solution) and the pseudostationary micelles.

Capillary Gel Electrophoresis

In **capillary gel electrophoresis**, macromolecules are separated by *sieving* as they migrate through a gel inside a capillary tube. Large molecules become entangled in the gel, and their motion is slowed. Small molecules travel faster than large molecules through the gel. This behavior is the opposite of that in molecular exclusion chromatography, in which large molecules are excluded from stationary phase particles and move faster than small ones. For DNA sequencing, capillary electrophoresis separates 500 different lengths of DNA in <20 min (Color Plate 24). In capillary gel electrophoresis, gels are not cross-linked polymers but are just solutions of polymers such as poly(ethylene oxide) or polyacrylamide, whose long chains are entangled and therefore behave as a gel.



- 23-F.** (a) Explain why neutral solutes are eluted between times t_0 and t_{mc} in micellar electrokinetic capillary chromatography, where t_0 is the elution time of neutral molecules in the absence of micelles and t_{mc} is the elution time of the micelles.
 (b) Micellar electrokinetic capillary chromatography in Figure 23-21 was conducted at pH 10 with anionic micelles and the anode on the sample side, as in Figure 23-14. (i) Is aminobenzene ($C_6H_5NH_2$) a cation, an anion, or a neutral molecule in this experiment? (ii) Explain how Figure 23-21 allows you to decide which compound, aminobenzene or anthracene, is more soluble in the micelles.

23-8 Lab-on-a-Chip: DNA Profiling

An exciting and rapidly developing area of analytical chemistry is the “lab-on-a-chip,” also called a *microfluidic chip*. Glass or plastic chips—often the size of a microscope slide—employ electroosmosis (Figure 23-16) or pressure to move liquid

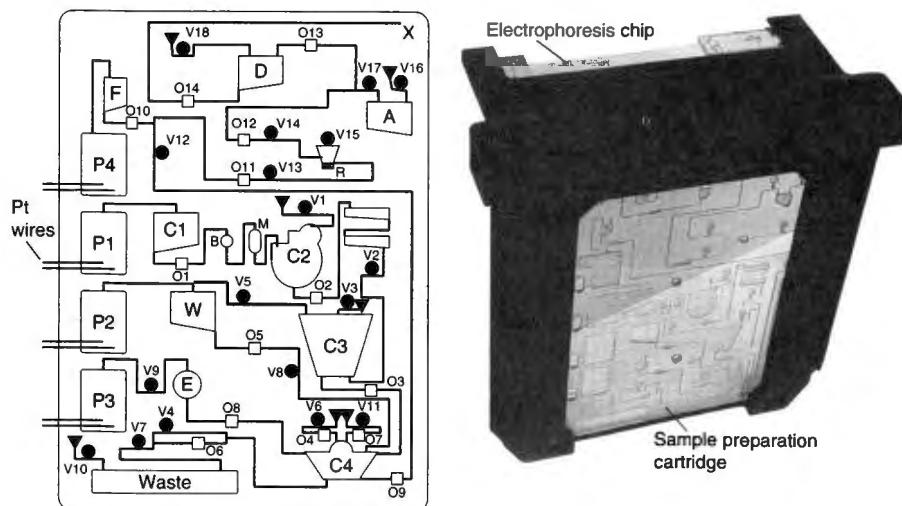


Figure 23-23 Microfluidic device for automated forensic DNA analysis being developed by the University of Arizona and the British Forensic Science Service. Features labeled V and O in the diagram of the sample preparation cartridge are valves. Other items are mentioned in the text. [Courtesy F. Zenhausern, University of Arizona. From A. J. Hopwood, C. Hurth, J. Yang, Z. Cai, N. Moran, J. G. Lee-Edghill, A. Nordquist, R. Lenigk, M. D. Estes, J. P. Haley, C. R. McAlister, X. Chen, C. Brooks, S. Smith, K. Elliott, P. Koumi, F. Zenhausern, and G. Tully, *Anal. Chem.* **2010**, *82*, 6991.]

with precise control through micrometer-size channels. Chemical reactions can be conducted by moving microliters or picoliters of fluid from different reservoirs, mixing and heating them, and analyzing products by electrophoresis in a narrow channel etched into glass or polymer.

Forensic DNA (deoxyribonucleic acid) analysis creates a profile of DNA from a sample such as blood or saliva to assign that sample to an individual person with a high probability and to exclude other individuals.⁸ The process often takes two weeks, during which a suspect in custody has a good chance of being released. The microfluidic device in Figure 23-23 is being developed to permit DNA profiling in 2 h while a suspect is in custody. The $23 \times 18\text{-cm}$ polycarbonate plastic sample preparation cartridge is designed for a single use to avoid contamination of a sample by previous samples.

In the first step of creating a DNA profile, cells swabbed from inside a person's cheek are *lysed* (broken open) with 1 mL of liquid, centrifuged, and 150 μL of liquid from the centrifuge tube are injected into chamber C1 in Figure 23-23. Pump P1 creates pressure by electrolyzing NaCl solution with Pt electrodes to generate H₂ and O₂ gas that pushes liquid from the pump. Upon passage through chambers B, M, C2, and C3, the sample has been mixed with magnetic ion-exchange beads that bind negatively charged DNA. In chamber C4, the beads with their DNA are captured in a magnetic field. Liquid containing other cellular constituents is washed from the captured DNA into the waste compartment. Pump P2 then sends buffer from chamber W to raise the pH and convert the charge of the beads from positive to neutral. DNA is released from the beads and 10 μL of DNA solution are sent to chamber R by pump P3.

In chamber R, the *polymerase chain reaction* (PCR) is conducted. This ingenious reaction, which won a Nobel Prize for Kary Mullis, who invented the process in 1984, *amplifies* (makes many copies) chosen sections of DNA. The microfluidic system conducts 27 cycles of amplification to make about 10^7 copies of each of 16 *short tandem repeat* sections of DNA found in the human genome. Each cycle of PCR amplification takes 4 min and requires changing the temperature of chamber R to 94°, 59°, and 72°C. Every repetition of the temperature sequence doubles the amount of the selected DNA. Primers used to start each DNA replication are labeled with one of four different fluorescent dyes. Each of the 16 different kinds of DNA that is replicated is uniquely characterized by the combination of the color of its fluorescence and the number of base pairs in the DNA.

Chamber F contains formamide solvent and a set of DNA standards used to calibrate the rate at which different lengths of DNA migrate in gel electrophoresis. These standards

are labeled with a fluorescent dye that can be distinguished from the four dyes used in amplifying the unknown DNA. Pump P4 sends formamide solution from chamber F through chamber R to carry replicated DNA into chamber D, where it is denatured at 95°C. *Denaturation* means that hydrogen bonds are broken and the double helix unwinds into two separate chains. Denatured DNA is pumped from exit X through Teflon capillary tubing onto the glass electrophoresis chip at the top right of Figure 23-23.

The electrophoresis chip has a thin (25 μm deep \times 50 μm wide) channel that is 13 cm long etched into the glass. During gel electrophoresis on this chip, short strands of DNA migrate more rapidly than long strands. DNA migrating past a fluorescence detector located 11 cm from the injection point is identified by the wavelength of its fluorescence and its migration time. Comparison with the migration times of the internal standards tells us the number of nucleotide bases in each unknown DNA. The resulting DNA profile, such as that in Figure 23-24, can be compared

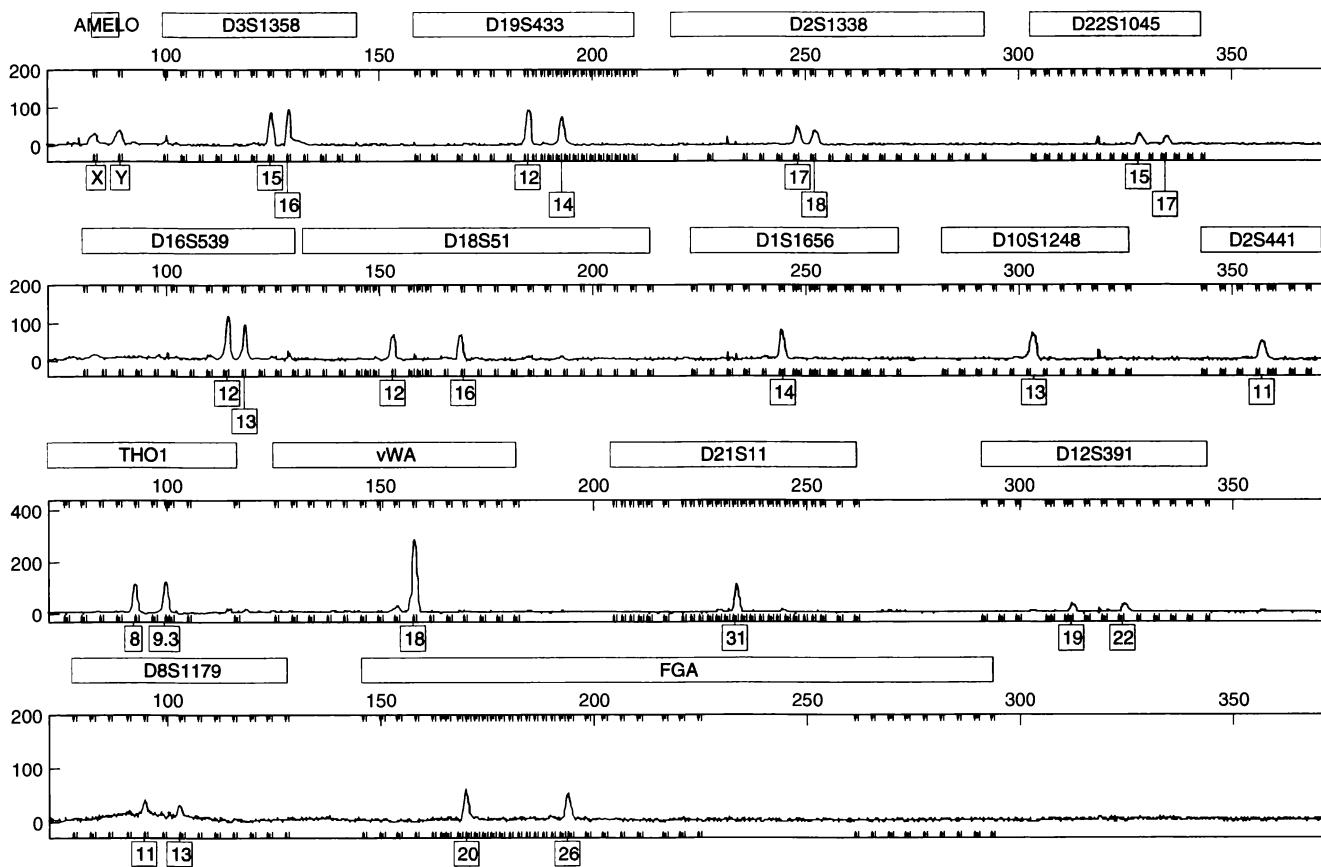


Figure 23-24 DNA profile created with the microfluidic device in Figure 23-23. Each row is the electropherogram of DNA labeled with one of four fluorescent dyes. Migration times were converted to the number of nucleotide bases from DNA calibration standards run simultaneously with the unknown. The 16 different kinds of DNA that were amplified are labeled in gray boxes above each row. In the top row, DNA D3S1358 could have 100, 104, 108, 112, . . . 140, or 144 nucleotide bases, indicated by 12 tick marks. Each different length of this DNA is called an *allele*. The 12 possible alleles of D3S1358 are numbered 9 through 20. The person who provided the saliva has alleles 15 and 16—one from his mother and one from his father. If the mother and father both happened to have the same allele, then only that one allele would appear in the profile. DNA fragment D1S1656 in the second row has only allele 14. The number of possible combinations of alleles of the 16 kinds of DNA is so great that the profile can be assigned to one person with high probability. [Courtesy C. Hurth, University of Arizona. See C. Hurth, S. D. Smith, A. R. Nordquist, R. Lenigk, B. Duane, D. Nguyen, A. Surve, A. J. Hopwood, M. D. Estes, J. Yang, Z. Cai, X. Chen, J. G. Lee-Edghill, N. Moran, K. Elliott, G. Tully, and F. Zenhausern, *Electrophoresis* 2010, 31, 3510.]

with the profile of authentic DNA from a suspect or used to interrogate an international database (such as FBI-CODIS⁹) to identify unknown DNA.

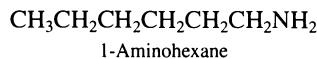
DNA evidence is good at excluding innocent people, but sloppy laboratory work could incriminate the wrong person. Forensic analysis requires meticulous execution without preconception of who is guilty. The automated microfluidic device not only reduces the time for DNA profiling, but also removes many sources of human error from the results.

Important Terms

affinity chromatography	cation exchanger	ion chromatography	molecular exclusion chromatography
anion exchanger	deionized water	ion-exchange chromatography	preconcentration
capillary electrophoresis	electroosmosis	micellar electrokinetic chromatography	retention volume
capillary gel electrophoresis	electrophoresis	capillary chromatography	trace analysis
capillary zone electrophoresis	hydrated radius	micelle	
	indirect detection		

Problems

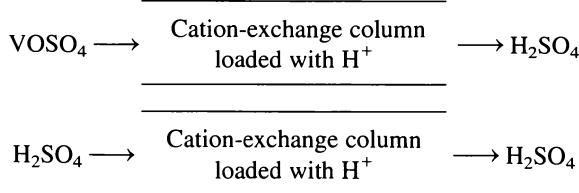
23-1. (a) Hexanoic acid and 1-aminohexane, adjusted to pH 12 with NaOH, were passed through a cation-exchange column loaded with NaOH at pH 12. State the principal species that will be eluted and the order in which they are expected.



(b) Hexanoic acid and 1-amino hexane, adjusted to pH 3 with HCl, were passed through a cation-exchange column loaded with HCl at pH 3. State the principal species that will be eluted and the order in which they are expected.

23-2. The exchange capacity of an ion-exchange resin is defined as the number of moles of charged sites per gram of dry resin. Describe how you would measure the exchange capacity of an anion-exchange resin by using standard NaOH, standard HCl, or any other reagent you wish.

23-3. Commercial vanadyl sulfate (VOSO₄, FM 163.00) is contaminated with H₂SO₄ and H₂O. A solution was prepared by dissolving 0.2447 g of impure VOSO₄ in 50.0 mL of water. Spectrophotometric analysis indicated that the concentration of the blue VO²⁺ ion was 0.0243 M. A 5.00-mL sample was passed through a cation-exchange column loaded with H⁺. VO²⁺ is exchanged for 2H⁺ by this process. H₂SO₄ is unchanged by the cation-exchange column.



H⁺ eluted from the column required 13.03 mL of 0.02274 M NaOH for titration. Find the weight percents of VOSO₄, H₂SO₄, and H₂O in the vanadyl sulfate.

23-4. Consider a negatively charged protein adsorbed on anion-exchange gel at pH 8.

(a) How will a gradient from pH 8 to some lower pH be useful for eluting the protein?

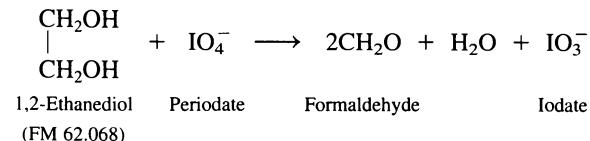
(b) How would a gradient of increasing NaCl concentration (at constant pH) be useful for eluting the protein?

23-5. Look up the pK_a values for trimethylamine, dimethylamine, methylamine, and ammonia. Predict the order of elution of these compounds from a cation-exchange column eluted with a gradient of increasing pH, beginning at pH 7.

23-6. (a) Why is a suppressor used in ion chromatography?

(b) Explain how electrolytic suppression works in Figure 23-7.

23-7. One mole of 1,2-ethanediol consumes one mole of periodate in the reaction



To analyze 1,2-ethanediol, oxidation with excess IO₄⁻ is followed by passage of the reaction solution through an anion-exchange resin that binds both IO₄⁻ and IO₃⁻. IO₃⁻ is then quantitatively removed from the resin by elution with NH₄Cl. The absorbance of eluate is measured at 232 nm to find the quantity of IO₃⁻ (molar absorptivity (ϵ) = 900 M⁻¹ cm⁻¹) produced by the reaction. In one experiment, 0.2139 g of aqueous 1,2-ethanediol was dissolved in 10.00 mL. Then 1.000 mL of the solution was treated with 3 mL of 0.15 M KIO₄ and subjected to ion-exchange

separation of IO_3^- from unreacted IO_4^- . The eluate (diluted to 250.0 mL) had an absorbance of $A_{232} = 0.521$ in a 1.000-cm cell, and a blank had $A_{232} = 0.049$. Find the weight percent of 1,2-ethanediol in the original sample.

23-8. The table gives the mean ionic composition of clouds at a mountaintop in Germany, measured by ion chromatography.

Ion	Concentration (μM)	Ion	Concentration (μM)
Cl^-	101	H^+	131
NO_3^-	360	Na^+	100
SO_4^{2-}	156	NH_4^+	472
		K^+	1.3
		Ca^{2+}	26
		Mg^{2+}	12

Data from K. Acker, D. Möller, W. Wieprecht, D. Kalafä, and R. Auel, *Fresenius J. Anal. Chem.* **1998**, 361, 59.

(a) What is the pH of the cloud water?

(b) Do the anion charges equal the magnitude of the cation charges? What does your answer suggest about the quality of the analysis?

(c) What is the total mass of dissolved ions in each milliliter of water?

23-9. In *ion-exclusion chromatography*, ions are separated from nonelectrolytes (uncharged molecules) by an ion-exchange column. Nonelectrolytes penetrate the stationary phase, whereas ions with charge of the same sign as that of the stationary phase are repelled by the stationary phase. Because electrolytes have access to less of the column volume, they are eluted before nonelectrolytes. A mixture of trichloroacetic acid (TCA, $\text{p}K_a = -0.5$), dichloroacetic acid (DCA, $\text{p}K_a = 1.1$), and monochloroacetic acid (MCA, $\text{p}K_a = 2.86$) was separated by passage through a cation-exchange resin eluted with 0.01 M HCl. The order of elution was TCA < DCA < MCA. Explain why the three acids are separated and the order of elution.

23-10.  Polystyrene standards of known molecular mass gave the following calibration data in a molecular exclusion column. Prepare a plot of $\log(\text{molecular mass})$ versus retention time (t_r) and find the equation of the line. Find the molecular mass of an unknown with a retention time of 13.00 min.

Molecular mass	Retention time, t_r (min)
8.50×10^6	9.28
3.04×10^6	10.07
1.03×10^6	10.88
3.30×10^5	11.67
1.56×10^5	12.14
6.60×10^4	12.74
2.85×10^4	13.38
9.20×10^3	14.20
3.25×10^3	14.96
5.80×10^2	16.04

23-11. A molecular exclusion column has a diameter of 7.8 mm and a length of 30 cm. The solid portion of the particles occupies 20% of the volume, the pores occupy 40%, and the volume between particles occupies 40%.

(a) At what volume would totally excluded molecules be expected to emerge?

(b) At what volume would the smallest molecules be expected?

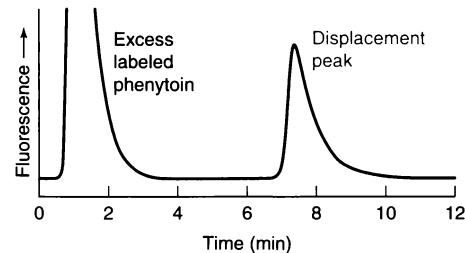
(c) A mixture of polymers of various molecular masses is eluted between 23 and 27 mL. What does this imply about the retention mechanism for these solutes on the column?

23-12. Immunoaffinity measurement of drug concentration.

At therapeutic levels in blood, ~90% of the antiepileptic drug phenytoin is bound to the protein serum albumin. The unbound 10% is thought to be the active form of the drug. Free phenytoin can be measured with a thin-layer (0.94 mm tall \times 2.1 mm diameter) affinity column at 37°C containing antiphenytoin antibodies covalently bound to silica.

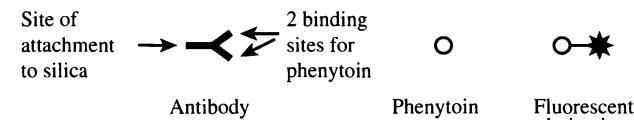
1. A fluorescent phenytoin derivative was applied to the column at time $t = 0$ to saturate the antibodies. Excess phenytoin was washed off with pH 7.4 buffer.

2. At $t = 6$ min, 5 μL of serum were injected. As it flowed through the column, free phenytoin displaced some fluorescently labeled phenytoin from the silica. Fluorescence was measured at 820 nm with laser excitation at 785 nm.



Fluorescence signal observed during phenytoin analysis. [From C. M. Ohnmacht, J. E. Schiel, and D. S. Hage, *Anal. Chem.* **2006**, 78, 7547.]

(a) With the following symbols, draw what happens on the column before and after step 1 and during analysis in step 2.



(b) Why are there two peaks in the chromatogram?

(c) The analysis was designed so that the residence time of serum on the column is short enough that insignificant dissociation of phenytoin from albumin occurs. If liquid occupies ~50% of the column volume, and the elution rate is 1.2 mL/min, what is the residence time of sample in the column?

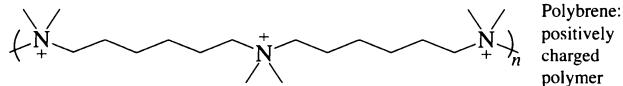
(d) *Comparison of means.* A calibration curve was constructed by plotting the area of the peak near 8 min versus concentration of free phenytoin applied to the column. Serum with 40.0 μM

total phenytoin had $5.99 \pm 0.14 \mu\text{M}$ free phenytoin, according to the calibration curve. Uncertainty is the standard deviation for three replicates. A different assay (ultrafiltration) gave $6.11 \pm 0.44 \mu\text{M}$ free phenytoin for three replicates. Are the standard deviations significantly different? Do the two means differ at the 95% confidence level?

23-13. (a) A capillary is set up as in Figure 23-14, with the injector end positive and the detector end negative. In what order will cations, anions, and neutral molecules be eluted?

(b) The electropherograms in Figure 23-15 were obtained with the injector end *negative* and a run buffer of pH 4.0. Will there be high or low electroosmotic flow? Are the anions moving with or against the electroosmotic flow? Will cations injected with the sample reach the detector before or after the anions?

(c) When polybrene is injected into a silica capillary, positively charged ammonium groups adhere to the $-\text{SiO}^-$ groups, converting the net charge on the wall from negative to positive. A capillary is set up with the injector end negative and the detector end positive. In what order will cations, anions, and neutral molecules be eluted?



23-14. From Figure 23-15, *estimate* the concentration of nitrate in the aquarium water. Aquarium water was diluted from 1 mL to 100 mL, and the final solution contains the same 10 ppm of internal standard IO_4^- as in the standard mixture. For an estimate, use peak height instead of peak area. There is no need to use normalized peak area = peak area/migration time because migration times are nearly the same in both electropherograms.

23-15. (a) What is electroosmosis?

(b) Why is the electroosmotic flow in a silica capillary five times faster at pH 9 than at pH 3?

(c) When the Si—OH groups on a silica capillary wall are converted into Si—O(CH_2)₁₇ CH_3 groups, the electroosmotic flow is small and nearly independent of pH. Explain why.

23-16. Why is the detector response *negative* in indirect spectrophotometric detection?

23-17. Explain how neutral molecules can be separated by micellar electrokinetic capillary chromatography. Why is this a form of chromatography?

23-18. A van Deemter plot for capillary electrophoresis is a graph of plate height versus migration velocity, where migration velocity is governed by the net sum of electroosmotic flow and electrophoretic flow.

(a) What is the principal source of band broadening in ideal capillary zone electrophoresis? Sketch what you expect the van Deemter curve to look like.

(b) What are the sources of band broadening in micellar electrokinetic capillary chromatography? Sketch what you expect the van Deemter curve to look like.

23-19. (a) Measure the migration time and peak width of $^{35}\text{Cl}^-$ in Figure 23-18 and calculate the number of theoretical plates.

(b) The distance from injection to the detector is 40 cm. From your answer to (a), find the plate height.

(c) Why are the peaks negative?

23-20. The water-soluble vitamins niacinamide (a neutral compound), riboflavin (a neutral compound), niacin (an anion), and thiamine (a cation) were separated by micellar electrokinetic capillary chromatography in 15 mM borate buffer (pH 8.0) with 50 mM sodium dodecyl sulfate. The migration times were niacinamide, 8.1 min; riboflavin, 13.0 min; niacin, 14.3 min; and thiamine, 21.9 min. What would the order have been in the absence of sodium dodecyl sulfate? Which compound is most soluble in the micelles?

23-21. *Molecular mass by capillary gel electrophoresis.* Protein molecular mass can be estimated by sodium dodecyl sulfate (SDS)-gel electrophoresis. Proteins are first *denatured* (unfolded) by sodium dodecyl sulfate (Box 23-2), which binds to hydrophobic regions and gives the protein a negative charge that is approximately proportional to the length of the protein. Also, disulfide bonds ($-\text{S}-\text{S}-$) are reduced to sulfhydryl ($-\text{SH}$) by excess 2-mercaptoethanol ($\text{HSCH}_2\text{CH}_2\text{OH}$). Denatured proteins are separated by electrophoresis through a gel that behaves as a sieve. Large molecules are retarded more than small molecules—behavior opposite that of size exclusion chromatography. The logarithm of molecular mass of the SDS-coated protein is proportional to $1/(\text{migration time})$ of the protein through the gel. Absolute migration times are somewhat variable from run to run, so relative migration times are measured. The relative migration time is the migration time of a protein divided by the migration time of a fast-moving small dye molecule. Migration times for protein standards and unknowns are given in the table.

Protein	Molecular mass (Da)	Migration time (min)
orange G marker dye	small molecule	13.17
α -lactalbumin	14 200	16.46
carbonic anhydrase	29 000	18.66
ovalbumin	45 000	20.16
bovine serum albumin	66 000	22.36
phosphorylase B	97 000	23.56
β -galactosidase	116 000	24.97
myosin	205 000	28.25
ferritin light chain		17.07
ferritin heavy chain		17.97

Data from J. K. Grady, J. Zang, T. M. Laue, P. Arosio, and N. D. Chasteen, *Anal. Biochem.* **2002**, 302, 263.

Unknowns are the light and heavy chains of ferritin, the iron-storage protein found in animals, plants, and microbes.

Ferritin is a hollow shell containing 24 subunits that are a mixture of heavy (H) and light (L) chains, arranged in octahedral symmetry. The hollow core has a diameter of 8 nm and can hold as many as 4 500 iron atoms in the approximate form of the mineral ferrihydrite ($5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$). Iron(II) enters the protein through any of six pores located on the threefold symmetry axes of the octahedron. Oxidation to Fe(III) takes place at catalytic sites on the H chains. Sites on the inside of L chains appear to nucleate crystallization of ferrihydrite.

Prepare a graph of $\log(\text{molecular mass})$ versus $1/(\text{relative migration time})$, where relative migration time = (migration time)/(migration time of marker dye). Compute the molecular mass of the ferritin light and heavy chains. True masses computed from amino acid sequences are 19 766 and 21 099 Da.

23-22. Limits of detection and quantitation. An ion-chromatographic method was developed to measure sub-part-per-billion levels of the disinfectant by-products iodate (IO_3^-), chlorite (ClO_2^-), and bromate (BrO_3^-) in drinking water. As the oxyhalides are eluted, they react with Br^- to make Br_3^- , which is measured by its strong absorption at 267 nm (absorptivity = $40\,900\, \text{M}^{-1}\,\text{cm}^{-1}$). For example, each mole of BrO_3^- makes three moles of Br_3^- : $\text{BrO}_3^- + 8\text{Br}^- + 6\text{H}^+ \rightarrow 3\text{Br}_3^- + 3\text{H}_2\text{O}$.

(a) Bromate near its detection limit gave the following chromatographic peak heights and standard deviations. For each concentration, estimate the limit of detection and the limit of quantitation. Find the mean of the four values of detection and quantitation limit. The blank is 0 because chromatographic peak height is measured from the baseline adjacent to the peak. Because blank = 0, relative standard deviation applies to both peak height and concentration, which are proportional to each other. Detection limit is $3s$ and quantitation limit is $10s$ for peak height or concentration.

Bromate concentration ($\mu\text{g/L}$)	Peak height (arbitrary units)	Relative standard deviation (%)	Number of measurements
0.2	17	14.4	8
0.5	31	6.8	7
1.0	56	3.2	7
2.0	111	1.9	7

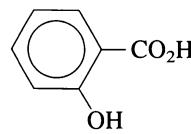
Data from H. S. Weinberg and H. Yamada, *Anal. Chem.* **1998**, *70*, 1.

(b) What is the absorbance of Br_3^- in the 6.00-mm-pathlength detection cell of the chromatograph if the bromate concentration is at its mean detection limit?

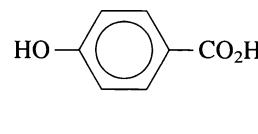
How Would You Do It?

23-23. (a) To obtain the best separation of two weak acids by electrophoresis, it makes sense to use the pH at which their charge difference is greatest. Explain why.

(b) Prepare a spreadsheet to examine the charges of *ortho*- and *para*-hydroxybenzoic acids as a function of pH. At what pH is the difference greatest?

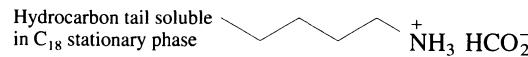


o-Hydroxybenzoic acid
 $\text{pK}_{\text{a}} = 2.97$



p-Hydroxybenzoic acid
 $\text{pK}_{\text{a}} = 4.54$

23-24. An aqueous solution of NaCl , NaNO_3 , and Na_2SO_4 was passed through a C_{18} -silica reversed-phase liquid chromatography column eluted with water. None of the cations or anions is retained by the C_{18} stationary phase, so all three salts were eluted in a single, sharp band with a retention time of 0.9 min. Then the column was equilibrated with aqueous 10 mM pentylammonium formate, whose hydrophobic tail is soluble in the C_{18} stationary phase.



When the mixture of NaCl , NaNO_3 , and Na_2SO_4 was passed through the column and eluted with 10 mM pentylammonium formate, the cations all came out in a single peak with a retention time of 0.9 min. However, anions were separated, with retention times of 1.9 min (Cl^-), 2.1 min (NO_3^-), and 4.1 min (SO_4^{2-}). Explain why this column behaves as an anion exchanger. Why is sulfate eluted last?

23-25. Cosmogenic ^{35}S . 10 Radioactive ^{35}S is produced in the atmosphere by the action of cosmic rays on Ar atoms. ^{35}S atoms are oxidized to SO_4^{2-} and fall to the ground in rain or as dry solids. Counting ^{35}S radioactive disintegrations allows us to measure the removal time for sulfur in the atmosphere and the residence time for sulfur species in different parts of the environment. To analyze minute quantities of ^{35}S in rain or lake water, 30-L volumes were passed through a 0.45- μm filter, acidified to pH 3–4 with HCl , and then 20 mg of Na_2SO_4 (containing no ^{35}S) were added. The whole volume was passed through 50 g of anion-exchange resin. SO_4^{2-} was quantitatively eluted with 300 mL of 3 M NaCl . After adjusting eluate to pH 3–4 with HCl , 5 mL of 10 wt% $\text{BaCl}_2 \cdot 3\text{H}_2\text{O}$ were added. After 5 h, precipitate was quantitatively recovered on a filter and ^{35}S was measured by scintillation counting. Explain the purpose of (a) initial filtration through a 0.45- μm filter; (b) passage through anion-exchange resin; (c) addition of BaCl_2 . What is the final chemical form of ^{35}S that is measured by scintillation counting? (d) Why was Na_2SO_4 added prior to anion exchange?

23-26. A buffer containing 1 mM MgSO_4 and 1 mM CaCl_2 strongly reduces electroosmotic flow in capillary electrophoresis.¹¹ Electroosmosis is restored by adding 3 mM EDTA to the buffer. Suggest an explanation.

Notes and References

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Appendix A

Solubility Products^a

Formula	K_{sp}	Formula	K_{sp}
Azides: L = N ₃ ⁻		Chromates: L = CrO ₄ ²⁻	
CuL	4.9 × 10 ⁻⁹	BaL	2.1 × 10 ⁻¹⁰
AgL	2.8 × 10 ⁻⁹	CuL	3.6 × 10 ⁻⁶
Hg ₂ L ₂	7.1 × 10 ⁻¹⁰	Ag ₂ L	1.2 × 10 ⁻¹²
TiL	2.2 × 10 ⁻⁴	Hg ₂ L	2.0 × 10 ⁻⁹
PdL ₂ (α)	2.7 × 10 ⁻⁹	Tl ₂ L	9.8 × 10 ⁻¹³
Bromates: L = BrO ₃ ⁻		Cobalticyanides: L = Co(CN) ₆ ³⁻	
BaL · H ₂ O	7.8 × 10 ⁻⁶	Ag ₃ L	3.9 × 10 ⁻²⁶
AgL	5.5 × 10 ⁻⁵	(Hg ₂) ₃ L ₂	1.9 × 10 ⁻³⁷
TiL	1.7 × 10 ⁻⁴	Cyanides: L = CN ⁻	
PbL ₂	7.9 × 10 ⁻⁶	AgL	2.2 × 10 ⁻¹⁶
Bromides: L = Br ⁻		Hg ₂ L ₂	5 × 10 ⁻⁴⁰
CuL	5 × 10 ⁻⁹	ZnL ₂	3 × 10 ⁻¹⁶
AgL	5.0 × 10 ⁻¹³	Ferrocyanides: L = Fe(CN) ₆ ⁴⁻	
Hg ₂ L ₂	5.6 × 10 ⁻²³	Ag ₄ L	8.5 × 10 ⁻⁴⁵
TiL	3.6 × 10 ⁻⁶	Zn ₂ L	2.1 × 10 ⁻¹⁶
Hg ₂ L ₂	1.3 × 10 ⁻¹⁹	Cd ₂ L	4.2 × 10 ⁻¹⁸
PbL ₂	2.1 × 10 ⁻⁶	Pb ₂ L	9.5 × 10 ⁻¹⁹
Carbonates: L = CO ₃ ²⁻		Fluorides: L = F ⁻	
MgL	3.5 × 10 ⁻⁸	LiL	1.7 × 10 ⁻³
CaL (calcite)	4.5 × 10 ⁻⁹	Mg ₂ L	7.4 × 10 ⁻⁹
CaL (aragonite)	6.0 × 10 ⁻⁹	CaL ₂	3.2 × 10 ⁻¹¹
SrL	9.3 × 10 ⁻¹⁰	SrL ₂	2.6 × 10 ⁻⁹
BaL	5.0 × 10 ⁻⁹	BaL ₂	1.5 × 10 ⁻⁶
Y ₂ L ₃	2.5 × 10 ⁻³¹	LaL ₃	2 × 10 ⁻¹⁹
La ₂ L ₃	4.0 × 10 ⁻³⁴	ThL ₄	5 × 10 ⁻²⁹
MnL	5.0 × 10 ⁻¹⁰	PbL ₂	3.6 × 10 ⁻⁸
FeL	2.1 × 10 ⁻¹¹	Hydroxides: L = OH ⁻	
CoL	1.0 × 10 ⁻¹⁰	Mg ₂ L ₂ (amorphous)	6 × 10 ⁻¹⁰
NiL	1.3 × 10 ⁻⁷	Mg ₂ L ₂ (brucite crystal)	7.1 × 10 ⁻¹²
CuL	2.3 × 10 ⁻¹⁰	CaL ₂	6.5 × 10 ⁻⁶
Ag ₂ L	8.1 × 10 ⁻¹²	BaL ₂ · 8H ₂ O	3 × 10 ⁻⁴
Hg ₂ L	8.9 × 10 ⁻¹⁷	YL ₃	6 × 10 ⁻²⁴
ZnL	1.0 × 10 ⁻¹⁰	LaL ₃	2 × 10 ⁻²¹
CdL	1.8 × 10 ⁻¹⁴	CeL ₃	6 × 10 ⁻²²
PbL	7.4 × 10 ⁻¹⁴	UO ₂ (\rightleftharpoons U ⁴⁺ + 4OH ⁻)	6 × 10 ⁻⁵⁷
Chlorides: L = Cl ⁻		UO ₂ L ₂ (\rightleftharpoons UO ₂ ²⁺ + 2OH ⁻)	4 × 10 ⁻²³
CuL	1.9 × 10 ⁻⁷	MnL ₂	1.6 × 10 ⁻¹³
AgL	1.8 × 10 ⁻¹⁰	FeL ₂	7.9 × 10 ⁻¹⁶
Hg ₂ L ₂	1.2 × 10 ⁻¹⁸	CoL ₂	1.3 × 10 ⁻¹⁵
TiL	1.8 × 10 ⁻⁴	NiL ₂	6 × 10 ⁻¹⁶
PbL ₂	1.7 × 10 ⁻⁵	CuL ₂	4.8 × 10 ⁻²⁰

^a Solubility products generally apply at 25°C and zero ionic strength. The designations α , β , or γ after some formulas refer to particular crystalline forms.

Formula	K_{sp}	Formula	K_{sp}
VL ₃	4.0×10^{-35}	Phosphates: L = PO ₄ ³⁻	1.7×10^{-6}
CrL ₃	1.6×10^{-30}	MgHL • 3H ₂ O (\rightleftharpoons) Mg ²⁺ + HL ²⁻	2.6×10^{-7}
FeL ₃	1.6×10^{-39}	CaHL • 2H ₂ O (\rightleftharpoons) Ca ²⁺ + HL ²⁻	1.2×10^{-7}
CoL ₃	3×10^{-45}	SrHL (\rightleftharpoons) Sr ²⁺ + HL ²⁻	4.0×10^{-8}
VOL ₂ (\rightleftharpoons VO ²⁺ + 2OH ⁻)	3×10^{-24}	BaHL (\rightleftharpoons) Ba ²⁺ + HL ²⁻	3.7×10^{-23}
PdL ₂	3×10^{-29}	LaL	1×10^{-36}
ZnL ₂ (amorphous)	3.0×10^{-16}	Fe ₃ L ₂ • 8H ₂ O	4×10^{-27}
CdL ₂ (β)	4.5×10^{-15}	FeL • 2H ₂ O	8×10^{-26}
HgO (red) (\rightleftharpoons Hg ²⁺ + 2OH ⁻)	3.6×10^{-26}	(VO) ₃ L ₂ (\rightleftharpoons 3VO ²⁺ + 2L ³⁻)	2.8×10^{-18}
Cu ₂ O (\rightleftharpoons 2Cu ⁺ + 2OH ⁻)	4×10^{-30}	Ag ₃ L	4.0×10^{-13}
Ag ₂ O (\rightleftharpoons 2Ag ⁺ + 2OH ⁻)	3.8×10^{-16}	Hg ₂ HL (\rightleftharpoons Hg ₂ ²⁺ + HL ²⁻)	5×10^{-36}
AuL ₃	3×10^{-6}	Zn ₃ L ₂ • 4H ₂ O	3.0×10^{-44}
AlL ₃ (α)	3×10^{-34}	Pb ₃ L ₂	1×10^{-21}
GaL ₃ (amorphous)	10^{-37}	GaL	2.3×10^{-22}
InL ₃	1.3×10^{-37}	InL	
SnO (\rightleftharpoons Sn ²⁺ + 2OH ⁻)	6×10^{-27}	Sulfates: L = SO ₄ ²⁻	
PbO (yellow) (\rightleftharpoons Pb ²⁺ + 2OH ⁻)	8×10^{-16}	CaL	2.4×10^{-5}
PbO (red) (\rightleftharpoons Pb ²⁺ + 2OH ⁻)	5×10^{-16}	SrL	3.2×10^{-7}
Iodates: L = IO ₃ ⁻		BaL	1.1×10^{-10}
CaL ₂	7.1×10^{-7}	RaL	4.3×10^{-11}
SrL ₂	3.3×10^{-7}	Ag ₂ L	1.5×10^{-5}
BaL ₂	1.5×10^{-9}	Hg ₂ L	7.4×10^{-7}
YL ₃	7.1×10^{-11}	PbL	6.3×10^{-7}
LaL ₃	1.0×10^{-11}	Sulfides: L = S ²⁻	
CeL ₃	1.4×10^{-11}	MnL (pink)	3×10^{-11}
ThL ₄	2.4×10^{-15}	MnL (green)	3×10^{-14}
UO ₂ L ₂ (\rightleftharpoons UO ₂ ²⁺ + 2IO ₃ ⁻)	9.8×10^{-8}	FeL	8×10^{-19}
CrL ₃	5×10^{-6}	CoL (α)	5×10^{-22}
AgL	3.1×10^{-8}	CoL (β)	3×10^{-26}
Hg ₂ L ₂	1.3×10^{-18}	NiL (α)	4×10^{-20}
TIL	3.1×10^{-6}	NiL (β)	1.3×10^{-25}
ZnL ₂	3.9×10^{-6}	NiL (γ)	3×10^{-27}
CdL ₂	2.3×10^{-8}	CuL	8×10^{-37}
PbL ₂	2.5×10^{-13}	Cu ₂ L	3×10^{-49}
Iodides: L = I ⁻		Ag ₂ L	8×10^{-51}
CuL	1×10^{-12}	Tl ₂ L	6×10^{-22}
AgL	8.3×10^{-17}	ZnL (α)	2×10^{-25}
CH ₃ HgL (\rightleftharpoons CH ₃ Hg ⁺ + I ⁻)	3.5×10^{-12}	ZnL (β)	3×10^{-23}
CH ₃ CH ₂ HgL (\rightleftharpoons CH ₃ CH ₂ Hg ⁺ + I ⁻)	7.8×10^{-5}	CdL	1×10^{-27}
TIL	5.9×10^{-8}	HgL (black)	2×10^{-53}
Hg ₂ L ₂	4.6×10^{-29}	HgL (red)	5×10^{-54}
SnL ₂	8.3×10^{-6}	SnL	1.3×10^{-26}
PbL ₂	7.9×10^{-9}	PbL	3×10^{-28}
Oxalates: L = C ₂ O ₄ ²⁻		In ₂ L ₃	4×10^{-70}
CaL	1.3×10^{-8}	Thiocyanates: L = SCN ⁻	
SrL	4×10^{-7}	CuL	4.0×10^{-14}
BaL	1×10^{-6}	AgL	1.1×10^{-12}
La ₂ L ₃	1×10^{-25}	Hg ₂ L ₂	3.0×10^{-20}
ThL ₂	4.2×10^{-22}	TIL	1.6×10^{-4}
UO ₂ L (\rightleftharpoons UO ₂ ²⁺ + C ₂ O ₄ ²⁻)	2.2×10^{-9}	HgL ₂	2.8×10^{-20}

Appendix B

Acid Dissociation Constants^a

Name	Structure ^b	pK _a ^c	K _a
Acetic acid (ethanoic acid)	CH ₃ CO ₂ H	4.756	1.75 × 10 ⁻⁵
Alanine	$\begin{array}{c} \text{NH}_3^+ \\ \\ \text{CHCH}_3 \\ \\ \text{CO}_2\text{H} \end{array}$	2.344 (CO ₂ H) 9.868 (NH ₃)	4.53 × 10 ⁻³ 1.36 × 10 ⁻¹⁰
Aminobenzene (aniline)		4.601	2.51 × 10 ⁻⁵
2-Aminobenzoic acid (anthranilic acid)		2.08 (CO ₂ H) 4.96 (NH ₃)	8.3 × 10 ⁻³ 1.10 × 10 ⁻⁵
2-Aminoethanol (ethanolamine)	HOCH ₂ CH ₂ NH ₃ ⁺	9.498	3.18 × 10 ⁻¹⁰
2-Aminophenol		4.70 (NH ₃) (20°) 9.97 (OH) (20°)	2.0 × 10 ⁻⁵ 1.05 × 10 ⁻¹⁰
Ammonia	NH ₄ ⁺	9.245	5.69 × 10 ⁻¹⁰
Arginine	$\begin{array}{c} \text{NH}_3^+ \\ \\ \text{CHCH}_2\text{CH}_2\text{CH}_2\text{NHC}=\text{NH}_2 \\ \\ \text{CO}_2\text{H} \end{array}$	1.823 (CO ₂ H) 8.991 (NH ₃) (12.1) (NH ₂)	1.50 × 10 ⁻² 1.02 × 10 ⁻⁹ 8 × 10 ⁻¹³
Arsenic acid (hydrogen arsenate)		2.24 6.96 (11.50)	5.8 × 10 ⁻³ 1.10 × 10 ⁻⁷ 3.2 × 10 ⁻¹²
Arsenious acid (hydrogen arsenite)	As(OH) ₃	9.29	5.1 × 10 ⁻¹⁰
Asparagine		2.16 (CO ₂ H) 8.73 (NH ₃)	6.9 × 10 ⁻³ 1.86 × 10 ⁻⁹
Aspartic acid		1.990 (α-CO ₂ H) 3.900 (β-CO ₂ H) 10.002 (NH ₃)	1.02 × 10 ⁻² 1.26 × 10 ⁻⁴ 9.95 × 10 ⁻¹¹

^a A. E. Martell, R. M. Smith, and R. J. Motekaitis, *NIST Critically Selected Stability Constants of Metal Complexes*, NIST Standard Reference Database 46, Gaithersburg, MD, 2001.

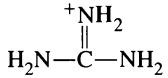
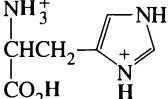
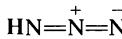
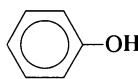
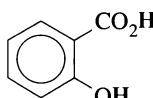
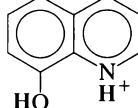
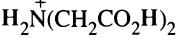
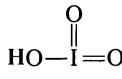
^b Each acid is written in its protonated form. The acidic protons are indicated in bold type.

^c pK_a values refer to 25°C unless otherwise indicated. Values in parentheses are considered to be less reliable.

Name	Structure ^b	pK _a ^c	K _a
Benzene-1,2,3-tricarboxylic acid (hemimellitic acid)		2.86 4.30 6.28	1.38×10^{-3} 5.0×10^{-5} 5.2×10^{-7}
Benzoic acid		4.202	6.28×10^{-5}
Benzylamine		9.35	4.5×10^{-10}
2,2'-Bipyridine		— 4.34	— 4.6×10^{-5}
Boric acid (hydrogen borate)	$\text{B}(\text{OH})_3$	9.237 (12.74) (20°) (13.80) (20°)	5.79×10^{-10} 1.82×10^{-13} 1.58×10^{-14}
Bromoacetic acid	$\text{BrCH}_2\text{CO}_2\text{H}$	2.902	1.25×10^{-3}
Butane-2,3-dione dioxime (dimethylglyoxime)		10.66 (12.0)	2.2×10^{-11} 1×10^{-12}
Butanoic acid	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$	4.818	1.52×10^{-5}
cis-Butenedioic acid (maleic acid)		1.92 6.27	1.20×10^{-2} 5.37×10^{-7}
trans-Butenedioic acid (fumaric acid)		3.02 4.48	9.5×10^{-4} 3.3×10^{-5}
Butylamine	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+$	10.640	2.29×10^{-11}
Carbonic acid (hydrogen carbonate)		6.351 10.329	4.46×10^{-7} 4.69×10^{-11}
Chloroacetic acid	$\text{ClCH}_2\text{CO}_2\text{H}$	2.865	1.36×10^{-3}
Chlorous acid (hydrogen chlorite)	$\text{HOCl}=\text{O}$	1.96	1.10×10^{-2}
Chromic acid (hydrogen chromate)		-0.2 (20°) 6.51	1.6 3.1×10^{-7}
Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid)		3.128 4.761 6.396	7.44×10^{-4} 1.73×10^{-5} 4.02×10^{-7}
Cyanoacetic acid	$\text{NCCH}_2\text{CO}_2\text{H}$	2.472	3.37×10^{-3}
Cyclohexylamine		10.567	2.71×10^{-11}

(continued)

Name	Structure ^b	pK _a ^c	K _a
Cysteine		(1.7) (CO ₂ H) 8.36 (SH) 10.74 (NH ₃)	2 × 10 ⁻² 4.4 × 10 ⁻⁹ 1.82 × 10 ⁻¹¹
Dichloroacetic acid	Cl ₂ CHCO ₂ H	(1.1)	8 × 10 ⁻²
Diethylamine	(CH ₃ CH ₂) ₂ NH ₂ ⁺	11.00	1.10 × 10 ⁻¹¹
1,2-Dihydroxybenzene (catechol)		9.45 (13.3)	3.5 × 10 ⁻¹⁰ 5.0 × 10 ⁻¹⁴
1,3-Dihydroxybenzene (resorcinol)		9.30 11.06	5.0 × 10 ⁻¹⁰ 8.7 × 10 ⁻¹²
D-2,3-Dihydroxybutanedioic acid (D-tartaric acid)		3.036 4.366	9.20 × 10 ⁻⁴ 4.31 × 10 ⁻⁵
Dimethylamine	(CH ₃) ₂ NH ₂ ⁺	10.774	1.68 × 10 ⁻¹¹
2,4-Dinitrophenol		4.114	7.69 × 10 ⁻⁵
Ethane-1,2-dithiol	HSCH ₂ CH ₂ SH	8.85 (30°) 10.43 (30°)	1.4 × 10 ⁻⁹ 3.7 × 10 ⁻¹¹
Ethylamine	CH ₃ CH ₂ NH ₂ ⁺	10.673	2.12 × 10 ⁻¹¹
Ethylenediamine (1,2-diaminoethane)	H ₃ N ⁺ CH ₂ CH ₂ NH ₃ ⁺	6.848 9.928	1.42 × 10 ⁻⁷ 1.18 × 10 ⁻¹⁰
Ethylenedinitrioltetraacetic acid (EDTA)	(HO ₂ CCH ₂) ₂ NHCH ₂ CH ₂ NH ⁺ (CH ₂ CO ₂ H) ₂	(0.0)(CO ₂ H) (1.5)(CO ₂ H) 2.00 (CO ₂ H) 2.69 (CO ₂ H) 6.13 (NH) 10.37 (NH)	1.0 0.032 0.010 0.002 0 7.4 × 10 ⁻⁷ 4.3 × 10 ⁻¹¹
Formic acid (methanoic acid)	HCO ₂ H	3.744	1.80 × 10 ⁻⁴
Glutamic acid		2.16 (α-CO ₂ H) 4.30 (γ-CO ₂ H) 9.96 (NH ₃)	6.9 × 10 ⁻³ 5.0 × 10 ⁻⁵ 1.10 × 10 ⁻¹⁰
Glutamine		2.19 (CO ₂ H) 9.00 (NH ₃)	6.5 × 10 ⁻³ 1.00 × 10 ⁻⁹
Glycine (aminoacetic acid)		2.350 (CO ₂ H) 9.778 (NH ₃)	4.47 × 10 ⁻³ 1.67 × 10 ⁻¹⁰

Name	Structure ^b	pK _a ^c	K _a
Guanidine		(13.5)	3×10^{-14}
1,6-Hexanedioic acid (adipic acid)	<chem>HO2CCH2CH2CH2CH2CO2H</chem>	4.424 5.420	3.77×10^{-5} 3.80×10^{-6}
Histidine		(1.6) (CO ₂ H) 5.97 (NH) 9.28 (NH ₃)	2.5×10^{-2} 1.07×10^{-6} 5.2×10^{-10}
Hydrazoic acid (hydrogen azide)		4.65	2.2×10^{-5}
Hydrogen cyanate	<chem>HOC#N</chem>	3.48	3.3×10^{-4}
Hydrogen cyanide	<chem>HC#N</chem>	9.21	6.2×10^{-10}
Hydrogen fluoride	<chem>HF</chem>	3.17	6.8×10^{-4}
Hydrogen peroxide	<chem>HOOH</chem>	11.65	2.2×10^{-12}
Hydrogen sulfide	<chem>H2S</chem>	7.02 14.0	9.5×10^{-8} 1×10^{-14}
Hydrogen thiocyanate	<chem>HSC#N</chem>	(-1.1)	1.3×10^1
Hydroxyacetic acid (glycolic acid)	<chem>HOCH2CO2H</chem>	3.832	1.48×10^{-4}
Hydroxybenzene (phenol)		9.997	1.01×10^{-10}
2-Hydroxybenzoic acid (salicylic acid)		2.972 (CO ₂ H) (13.7) (OH)	1.07×10^{-3} 2×10^{-14}
Hydroxylamine		5.96	1.10×10^{-6}
8-Hydroxyquinoline (oxine)		4.94 (NH) 9.82 (OH)	1.15×10^{-5} 1.51×10^{-10}
Hypochlorous acid (hydrogen hypochlorite)	<chem>HOCl</chem>	7.53	3.0×10^{-8}
Hypophosphorous acid (hydrogen hypophosphite)		(1.3)	5×10^{-2}
Imidazole (1,3-diazole)		6.993	1.02×10^{-7}
Iminodiacetic acid		(1.85) (CO ₂ H) 2.84 (CO ₂ H) 9.79 (NH ₂)	1.41×10^{-2} 1.45×10^{-3} 1.62×10^{-10}
Iodic acid (hydrogen iodate)		0.77	0.17
Iodoacetic acid	<chem>ICH2CO2H</chem>	3.175	6.68×10^{-4}

(continued)

Name	Structure ^b	pK _a ^c	K _a
Isoleucine		2.318 (CO ₂ H) 9.758 (NH ₃)	4.81 × 10 ⁻³ 1.75 × 10 ⁻¹⁰
Leucine		2.328 (CO ₂ H) 9.744 (NH ₃)	4.70 × 10 ⁻³ 1.80 × 10 ⁻¹⁰
Lysine		(1.77) (CO ₂ H) 9.07 (α-NH ₃) 10.82 (ε-NH ₃)	1.70 × 10 ⁻² 8.5 × 10 ⁻¹⁰ 1.51 × 10 ⁻¹¹
Malonic acid (propanedioic acid)		2.847 5.696	1.42 × 10 ⁻³ 2.01 × 10 ⁻⁶
Mercaptoacetic acid (thioglycolic acid)		3.64 (CO ₂ H) 10.61 (SH)	2.3 × 10 ⁻⁴ 2.5 × 10 ⁻¹¹
2-Mercaptoethanol		9.72	1.9 × 10 ⁻¹⁰
Methionine		2.18 (CO ₂ H) 9.08 (NH ₃)	6.6 × 10 ⁻³ 8.3 × 10 ⁻¹⁰
Methylamine		10.632	2.33 × 10 ⁻¹¹
4-Methylaniline (<i>p</i> -toluidine)		5.080	8.32 × 10 ⁻⁶
2-Methylphenol (<i>o</i> -cresol)		10.31	4.9 × 10 ⁻¹¹
4-Methylphenol (<i>p</i> -cresol)		10.269	5.4 × 10 ⁻¹¹
Morpholine (perhydro-1,4-oxazine)		8.492	3.22 × 10 ⁻⁹
1-Naphthoic acid		3.67	2.1 × 10 ⁻⁴
2-Naphthoic acid		4.16	6.9 × 10 ⁻⁵
1-Naphthol		9.416	3.84 × 10 ⁻¹⁰

Name	Structure ^b	pK _a ^c	K _a
2-Naphthol		9.573	2.67×10^{-10}
Nitrilotriacetic acid	$\text{H}^+(\text{CH}_2\text{CO}_2\text{H})_3$	(1.0) (CO ₂ H) (25°) 2.0 (CO ₂ H) (25°) 2.940 (CO ₂ H) (20°) 10.334 (NH) (20°)	0.10 0.010 1.15×10^{-3} 4.63×10^{-11}
4-Nitrobenzoic acid		3.442	3.61×10^{-4}
Nitroethane	$\text{CH}_3\text{CH}_2\text{NO}_2$	8.57	2.7×10^{-9}
4-Nitrophenol		7.149	7.10×10^{-8}
<i>N</i> -Nitrosophenylhydroxylamine (cupferron)		4.16	6.9×10^{-5}
Nitrous acid	$\text{HON}=\text{O}$	3.15	7.1×10^{-4}
Oxalic acid (ethanedioic acid)	$\text{HO}_2\text{CCO}_2\text{H}$	1.250 4.266	5.62×10^{-2} 5.42×10^{-5}
Oxoacetic acid (glyoxylic acid)		3.46	3.5×10^{-4}
Oxobutanedioic acid (oxaloacetic acid)		2.56 4.37	2.8×10^{-3} 4.3×10^{-5}
2-Oxopentanedioic (α -ketoglutaric acid)		(1.9) 4.44	1.3×10^{-2} 3.6×10^{-5}
2-Oxopropanoic acid (pyruvic acid)		2.48	3.3×10^{-3}
1,5-Pentanedioic acid (glutaric acid)	$\text{HO}_2\text{CCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$	4.345 5.422	4.52×10^{-5} 3.78×10^{-6}
1,10-Phenanthroline		1.8 4.91	0.016 1.23×10^{-5}
Phenylacetic acid		4.310	4.90×10^{-5}
Phenylalanine		2.20 (CO ₂ H) 9.31 (NH ₃)	6.3×10^{-3} 4.9×10^{-10}

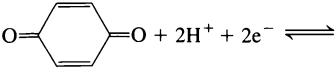
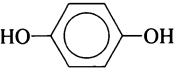
(continued)

Name	Structure ^b	pK _a ^c	K _a
Phosphoric acid (hydrogen phosphate)		2.148 7.198 12.375	7.11×10^{-3} 6.34×10^{-8} 4.22×10^{-13}
Phosphorous acid (hydrogen phosphite)		(1.5) 6.78	3×10^{-2} 1.66×10^{-7}
Phthalic acid (benzene-1,2-dicarboxylic acid)		2.950 5.408	1.12×10^{-3} 3.90×10^{-6}
Piperazine (perhydro-1,4-diazine)		5.333 9.731	4.65×10^{-6} 1.86×10^{-10}
Piperidine		11.125	7.50×10^{-12}
Proline		1.952 (CO2H) 10.640 (NH2)	1.12×10^{-2} 2.29×10^{-11}
Propanoic acid	CH ₃ CH ₂ CO ₂ H	4.874	1.34×10^{-5}
Propenoic acid (acrylic acid)	H ₂ C=CHCO ₂ H	4.258	5.52×10^{-5}
Propylamine	CH ₃ CH ₂ CH ₂ NH ₃ ⁺	10.566	2.72×10^{-11}
Pyridine (azine)		5.20	6.3×10^{-6}
Pyridine-2-carboxylic acid (picolinic acid)		(1.01) (CO ₂ H) 5.39 (NH)	9.8×10^{-2} 4.1×10^{-6}
Pyridine-3-carboxylic acid (nicotinic acid)		2.03 (CO ₂ H) 4.82 (NH)	9.3×10^{-3} 1.51×10^{-5}
Pyridoxal-5-phosphate		1.4 (POH) 3.44 (OH) 6.01 (POH) 8.45 (NH)	0.04 3.6×10^{-4} 9.8×10^{-7} 3.5×10^{-9}
Pyrophosphoric acid (hydrogen diphosphate)		0.9 2.28 6.70 9.40	0.13 5.2×10^{-3} 2.0×10^{-7} 4.0×10^{-10}

Name	Structure ^b	pK _a ^c	K _a
Serine		2.187 (CO ₂ H) 9.209 (NH ₃)	6.50 × 10 ⁻³ 6.18 × 10 ⁻¹⁰
Succinic acid (butanedioic acid)		4.207 5.636	6.21 × 10 ⁻⁵ 2.31 × 10 ⁻⁶
Sulfuric acid (hydrogen sulfate)		1.987 (pK ₂)	1.03 × 10 ⁻²
Sulfurous acid (hydrogen sulfite)		1.857 7.172	1.39 × 10 ⁻² 6.73 × 10 ⁻⁸
Thiosulfuric acid (hydrogen thiosulfate)		(0.6) (1.6)	0.3 0.03
Threonine		2.088 (CO ₂ H) 9.100 (NH ₃)	8.17 × 10 ⁻³ 7.94 × 10 ⁻¹⁰
Trichloroacetic acid		(-0.5)	3
Triethanolamine		7.762	1.73 × 10 ⁻⁸
Triethylamine		10.72	1.9 × 10 ⁻¹¹
1,2,3-Trihydroxybenzene (pyrogallol)		8.96 11.00 (14.0) (20°)	1.10 × 10 ⁻⁹ 1.00 × 10 ⁻¹¹ 10 ⁻¹⁴
Trimethylamine		9.799	1.59 × 10 ⁻¹⁰
Tris(hydroxymethyl) aminomethane (tris or tham)		8.072	8.47 × 10 ⁻⁹
Tryptophan		2.37 (CO ₂ H) 9.33 (NH ₃)	4.3 × 10 ⁻³ 4.7 × 10 ⁻¹⁰
Tyrosine		2.41 (CO ₂ H) 8.67 (NH ₃) 11.01 (OH)	3.9 × 10 ⁻³ 2.1 × 10 ⁻⁹ 9.8 × 10 ⁻¹²
Valine		2.286 (CO ₂ H) 9.719 (NH ₃)	5.18 × 10 ⁻³ 1.91 × 10 ⁻¹⁰

Appendix C

Standard Reduction Potentials

Reaction ^a	E° (volts)	Reaction ^a	E° (volts)
Aluminum		dehydroascorbic acid + 2H^+ + $2\text{e}^- \rightleftharpoons$	
$\text{Al}^{3+} + 3\text{e}^- \rightleftharpoons \text{Al}(s)$	-1.677	ascorbic acid + H_2O	0.390
$\text{Al}(\text{OH})_4^- + 3\text{e}^- \rightleftharpoons \text{Al}(s) + 4\text{OH}^-$	-2.328	$(\text{CN})_2(g) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons 2\text{HCN}(aq)$	0.373
Arsenic		$\text{H}_2\text{CO} + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{CH}_3\text{OH}$	0.237
$\text{H}_3\text{AsO}_4 + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_3\text{AsO}_3 + \text{H}_2\text{O}$	0.575	$\text{C}(s) + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons \text{CH}_4(g)$	0.131 5
$\text{H}_3\text{AsO}_3 + 3\text{H}^+ + 3\text{e}^- \rightleftharpoons \text{As}(s) + 3\text{H}_2\text{O}$	0.247 5	$\text{HCO}_2\text{H} + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2\text{CO} + \text{H}_2\text{O}$	-0.029
$\text{As}(s) + 3\text{H}^+ + 3\text{e}^- \rightleftharpoons \text{AsH}_3(g)$	-0.238	$\text{CO}_2(g) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{CO}(g) + \text{H}_2\text{O}$	-0.103 8
Barium		$\text{CO}_2(g) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{HCO}_2\text{H}$	-0.114
$\text{Ba}^{2+} + 2\text{e}^- \rightleftharpoons \text{Ba}(s)$	-2.906	$2\text{CO}_2(g) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2\text{C}_2\text{O}_4$	-0.432
Beryllium		Cerium	
$\text{Be}^{2+} + 2\text{e}^- \rightleftharpoons \text{Be}(s)$	-1.968	$\text{Ce}^{4+} + \text{e}^- \rightleftharpoons \text{Ce}^{3+}$	$\begin{cases} 1.72 \\ 1.70 & 1\text{ F HClO}_4 \\ 1.44 & 1\text{ F H}_2\text{SO}_4 \\ 1.61 & 1\text{ F HNO}_3 \\ 1.47 & 1\text{ F HCl} \end{cases}$
Boron		$\text{Ce}^{3+} + 3\text{e}^- \rightleftharpoons \text{Ce}(s)$	-2.336
$2\text{B}(s) + 6\text{H}^+ + 6\text{e}^- \rightleftharpoons \text{B}_2\text{H}_6(g)$	-0.150	Cesium	
$\text{B}_4\text{O}_7^{2-} + 14\text{H}^+ + 12\text{e}^- \rightleftharpoons 4\text{B}(s) + 7\text{H}_2\text{O}$	-0.792	$\text{Cs}^+ + \text{e}^- \rightleftharpoons \text{Cs}(s)$	-3.026
$\text{B}(\text{OH})_3 + 3\text{H}^+ + 3\text{e}^- \rightleftharpoons \text{B}(s) + 3\text{H}_2\text{O}$	-0.889	Chlorine	
Bromine		$\text{HClO}_2 + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{HOCl} + \text{H}_2\text{O}$	1.674
$\text{BrO}_4^- + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{BrO}_3^- + \text{H}_2\text{O}$	1.745	$\text{HClO} + \text{H}^+ + \text{e}^- \rightleftharpoons \frac{1}{2}\text{Cl}_2(g) + \text{H}_2\text{O}$	1.630
$\text{HOBr} + \text{H}^+ + \text{e}^- \rightleftharpoons \frac{1}{2}\text{Br}_2(l) + \text{H}_2\text{O}$	1.584	$\text{ClO}_3^- + 6\text{H}^+ + 5\text{e}^- \rightleftharpoons \frac{1}{2}\text{Cl}_2(g) + 3\text{H}_2\text{O}$	1.458
$\text{BrO}_3^- + 6\text{H}^+ + 5\text{e}^- \rightleftharpoons \frac{1}{2}\text{Br}_2(l) + 3\text{H}_2\text{O}$	1.513	$\text{Cl}_2(aq) + 2\text{e}^- \rightleftharpoons 2\text{Cl}^-$	1.396
$\text{Br}_2(aq) + 2\text{e}^- \rightleftharpoons 2\text{Br}^-$	1.098	$\text{Cl}_2(g) + 2\text{e}^- \rightleftharpoons 2\text{Cl}^-$	1.360 4
$\text{Br}_2(l) + 2\text{e}^- \rightleftharpoons 2\text{Br}^-$	1.078	$\text{ClO}_4^- + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{ClO}_3^- + \text{H}_2\text{O}$	1.226
$\text{Br}_3^- + 2\text{e}^- \rightleftharpoons 3\text{Br}^-$	1.062	$\text{ClO}_3^- + 3\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{HClO}_2 + \text{H}_2\text{O}$	1.157
$\text{BrO}^- + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{Br}^- + 2\text{OH}^-$	0.766	$\text{ClO}_3^- + 2\text{H}^+ + \text{e}^- \rightleftharpoons \text{ClO}_2 + \text{H}_2\text{O}$	1.130
$\text{BrO}_3^- + 3\text{H}_2\text{O} + 6\text{e}^- \rightleftharpoons \text{Br}^- + 6\text{OH}^-$	0.613	$\text{ClO}_2 + \text{e}^- \rightleftharpoons \text{ClO}_2^-$	1.068
Cadmium		Chromium	
$\text{Cd}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cd}(s)$	-0.402	$\text{Cr}_2\text{O}_7^{2-} + 14\text{H}^+ + 6\text{e}^- \rightleftharpoons 2\text{Cr}^{3+} + 7\text{H}_2\text{O}$	1.36
$\text{Cd}(\text{NH}_3)_4^{2+} + 2\text{e}^- \rightleftharpoons \text{Cd}(s) + 4\text{NH}_3$	-0.613	$\text{CrO}_4^{2-} + 4\text{H}_2\text{O} + 3\text{e}^- \rightleftharpoons$	
Calcium		$\text{Cr}(\text{OH})_3(s, \text{hydrated}) + 5\text{OH}^- \rightleftharpoons$	-0.12
$\text{Ca}(s) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{CaH}_2(s)$	0.776	$\text{Cr}^{3+} + \text{e}^- \rightleftharpoons \text{Cr}^{2+}$	-0.42
$\text{Ca}^{2+} + 2\text{e}^- \rightleftharpoons \text{Ca}(s)$	-2.868	$\text{Cr}^{3+} + 3\text{e}^- \rightleftharpoons \text{Cr}(s)$	-0.74
$\text{Ca}(\text{acetate})^+ + 2\text{e}^- \rightleftharpoons \text{Ca}(s) + \text{acetate}^-$	-2.891	$\text{Cr}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cr}(s)$	-0.89
$\text{CaSO}_4(s) + 2\text{e}^- \rightleftharpoons \text{Ca}(s) + \text{SO}_4^{2-}$	-2.936	Cobalt	
Carbon		$\text{Co}^{3+} + \text{e}^- \rightleftharpoons \text{Co}^{2+}$	$\begin{cases} 1.92 \\ 1.817 & 8\text{ F H}_2\text{SO}_4 \\ 1.850 & 4\text{ F HNO}_3 \end{cases}$
$\text{C}_2\text{H}_2(g) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{C}_2\text{H}_4(g)$	0.731	$\text{Co}(\text{NH}_3)_6^{3+} + \text{e}^- \rightleftharpoons \text{Co}(\text{NH}_3)_6^{2+}$	0.1
		$\text{CoOH}^+ + \text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Co}(s) + \text{H}_2\text{O}$	0.003
	0.700		
$\text{CH}_3\text{OH} + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{CH}_4(g) + \text{H}_2\text{O}$	0.583		

^a All species are aqueous unless otherwise indicated.

Reaction ^a	E° (volts)	Reaction ^a	E° (volts)
$\text{Co}^{2+} + 2\text{e}^- \rightleftharpoons \text{Co}(s)$	-0.282	$\text{FeOH}^{2+} + \text{H}^+ + \text{e}^- \rightleftharpoons \text{Fe}^{2+} + \text{H}_2\text{O}$	0.900
$\text{Co(OH)}_2(s) + 2\text{e}^- \rightleftharpoons \text{Co}(s) + 2\text{OH}^-$	-0.746	$\text{FeO}_4^{2-} + 3\text{H}_2\text{O} + 3\text{e}^- \rightleftharpoons \text{FeOOH}(s) + 5\text{OH}^-$	0.80
Copper		$\text{Fe}^{3+} + \text{e}^- \rightleftharpoons \text{Fe}^{2+}$	$\begin{cases} 0.771 & 1 \text{ F HCl} \\ 0.732 & 1 \text{ F HClO}_4 \\ 0.767 & 1 \text{ F HNO}_3 \\ 0.746 & 1 \text{ F H}_2\text{SO}_4 \\ 0.68 & \end{cases}$
$\text{Cu}^+ + \text{e}^- \rightleftharpoons \text{Cu}(s)$	0.518	$\text{FeOOH}(s) + 3\text{H}^+ + \text{e}^- \rightleftharpoons \text{Fe}^{2+} + 2\text{H}_2\text{O}$	0.74
$\text{Cu}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cu}(s)$	0.339	$\text{ferricinium}^+ + \text{e}^- \rightleftharpoons \text{ferrocene}$	0.400
$\text{Cu}^{2+} + \text{e}^- \rightleftharpoons \text{Cu}^+$	0.161	$\text{Fe}(\text{CN})_6^{4-} + \text{e}^- \rightleftharpoons \text{Fe}(\text{CN})_6^{3-}$	0.356
$\text{CuCl}(s) + \text{e}^- \rightleftharpoons \text{Cu}(s) + \text{Cl}^-$	0.137	$\text{FeOH}^+ + \text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Fe}(s) + \text{H}_2\text{O}$	-0.16
$\text{Cu}(\text{IO}_3)_2(s) + 2\text{e}^- \rightleftharpoons \text{Cu}(s) + 2\text{IO}_3^-$	-0.079	$\text{Fe}^{2+} + 2\text{e}^- \rightleftharpoons \text{Fe}(s)$	-0.44
$\text{Cu}(\text{ethylenediamine})_2^+ + \text{e}^- \rightleftharpoons \text{Cu}(s) + 2 \text{ ethylenediamine}$	-0.119	$\text{FeCO}_3(s) + 2\text{e}^- \rightleftharpoons \text{Fe}(s) + \text{CO}_3^{2-}$	-0.756
$\text{CuI}(s) + \text{e}^- \rightleftharpoons \text{Cu}(s) + \text{I}^-$	-0.185	Lanthanum	
$\text{Cu}(\text{EDTA})^{2-} + 2\text{e}^- \rightleftharpoons \text{Cu}(s) + \text{EDTA}^{4-}$	-0.216	$\text{La}^{3+} + 3\text{e}^- \rightleftharpoons \text{La}(s)$	-2.379
$\text{Cu}(\text{OH})_2(s) + 2\text{e}^- \rightleftharpoons \text{Cu}(s) + 2\text{OH}^-$	-0.222	Lead	
$\text{Cu}(\text{CN})_2^- + \text{e}^- \rightleftharpoons \text{Cu}(s) + 2\text{CN}^-$	-0.429	$\text{Pb}^{4+} + 2\text{e}^- \rightleftharpoons \text{Pb}^{2+}$	1.69
$\text{CuCN}(s) + \text{e}^- \rightleftharpoons \text{Cu}(s) + \text{CN}^-$	-0.639	$\text{PbO}_2(s) + 4\text{H}^+ + \text{SO}_4^{2-} + 2\text{e}^- \rightleftharpoons \text{PbSO}_4(s) + 2\text{H}_2\text{O}$	1.685
Fluorine		$\text{PbO}_2(s) + 4\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Pb}^{2+} + 2\text{H}_2\text{O}$	1.458
$\text{F}_2(g) + 2\text{e}^- \rightleftharpoons 2\text{F}^-$	2.890	$3\text{PbO}_2(s) + 2\text{H}_2\text{O} + 4\text{e}^- \rightleftharpoons \text{Pb}_3\text{O}_4(s) + 4\text{OH}^-$	0.269
$\text{F}_2\text{O}(g) + 2\text{H}^+ + 4\text{e}^- \rightleftharpoons 2\text{F}^- + \text{H}_2\text{O}$	2.168	$\text{Pb}_3\text{O}_4(s) + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons 3\text{PbO}(s, \text{red}) + 2\text{OH}^-$	0.224
Gallium		$\text{Pb}_3\text{O}_4(s) + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons 3\text{PbO}(s, \text{yellow}) + 2\text{OH}^-$	0.207
$\text{Ga}^{3+} + 3\text{e}^- \rightleftharpoons \text{Ga}(s)$	-0.549	$\text{Pb}^{2+} + 2\text{e}^- \rightleftharpoons \text{Pb}(s)$	-0.126
$\text{GaOOH}(s) + \text{H}_2\text{O} + 3\text{e}^- \rightleftharpoons \text{Ga}(s) + 3\text{OH}^-$	-1.320	$\text{PbF}_2(s) + 2\text{e}^- \rightleftharpoons \text{Pb}(s) + 2\text{F}^-$	-0.350
Germanium		$\text{PbSO}_4(s) + 2\text{e}^- \rightleftharpoons \text{Pb}(s) + \text{SO}_4^{2-}$	-0.355
$\text{Ge}^{2+} + 2\text{e}^- \rightleftharpoons \text{Ge}(s)$	0.1	Lithium	
$\text{H}_4\text{GeO}_4 + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons \text{Ge}(s) + 4\text{H}_2\text{O}$	-0.039	$\text{Li}^+ + \text{e}^- \rightleftharpoons \text{Li}(s)$	-3.040
Gold		Magnesium	
$\text{Au}^+ + \text{e}^- \rightleftharpoons \text{Au}(s)$	1.69	$\text{Mg}(\text{OH})^+ + \text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Mg}(s) + \text{H}_2\text{O}$	-2.022
$\text{Au}^{3+} + 2\text{e}^- \rightleftharpoons \text{Au}^+$	1.41	$\text{Mg}^{2+} + 2\text{e}^- \rightleftharpoons \text{Mg}(s)$	-2.360
$\text{AuCl}_2^- + \text{e}^- \rightleftharpoons \text{Au}(s) + 2\text{Cl}^-$	1.154	$\text{Mg}(\text{C}_2\text{O}_4)(s) + 2\text{e}^- \rightleftharpoons \text{Mg}(s) + \text{C}_2\text{O}_4^{2-}$	-2.493
$\text{AuCl}_4^- + 2\text{e}^- \rightleftharpoons \text{AuCl}_2^- + 2\text{Cl}^-$	0.926	$\text{Mg}(\text{OH})_2(s) + 2\text{e}^- \rightleftharpoons \text{Mg}(s) + 2\text{OH}^-$	-2.690
Hydrogen		Manganese	
$2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2(g)$	0.000 0	$\text{MnO}_4^- + 4\text{H}^+ + 3\text{e}^- \rightleftharpoons \text{MnO}_2(s) + 2\text{H}_2\text{O}$	1.692
$\text{H}_2\text{O} + \text{e}^- \rightleftharpoons \frac{1}{2}\text{H}_2(g) + \text{OH}^-$	-0.828 0	$\text{Mn}^{3+} + \text{e}^- \rightleftharpoons \text{Mn}^{2+}$	1.56
Indium		$\text{MnO}_4^- + 8\text{H}^+ + 5\text{e}^- \rightleftharpoons \text{Mn}^{2+} + 4\text{H}_2\text{O}$	1.507
$\text{In}^{3+} + 3\text{e}^- \rightleftharpoons \text{In}(s)$	-0.338	$\text{Mn}_2\text{O}_3(s) + 6\text{H}^+ + 2\text{e}^- \rightleftharpoons 2\text{Mn}^{2+} + 3\text{H}_2\text{O}$	1.485
$\text{In}^{3+} + 2\text{e}^- \rightleftharpoons \text{In}^+$	-0.444	$\text{MnO}_2(s) + 4\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Mn}^{2+} + 2\text{H}_2\text{O}$	1.230
$\text{In}(\text{OH})_3(s) + 3\text{e}^- \rightleftharpoons \text{In}(s) + 3\text{OH}^-$	-0.99	$\text{Mn}(\text{EDTA})^- + \text{e}^- \rightleftharpoons \text{Mn}(\text{EDTA})^{2-}$	0.825
Iodine		$\text{MnO}_4^- + \text{e}^- \rightleftharpoons \text{MnO}_4^{2-}$	0.56
$\text{IO}_4^- + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{IO}_3^- + \text{H}_2\text{O}$	1.589	$3\text{Mn}_2\text{O}_3(s) + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons 2\text{Mn}_3\text{O}_4(s) + 2\text{OH}^-$	0.002
$\text{H}_5\text{IO}_6 + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{HIO}_3 + 3\text{H}_2\text{O}$	1.567	$\text{Mn}_3\text{O}_4(s) + 4\text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons 3\text{Mn}(\text{OH})_2(s) + 2\text{OH}^-$	-0.352
$\text{HOI} + \text{H}^+ + \text{e}^- \rightleftharpoons \frac{1}{2}\text{I}_2(s) + \text{H}_2\text{O}$	1.430	$\text{Mn}^{2+} + 2\text{e}^- \rightleftharpoons \text{Mn}(s)$	-1.182
$\text{ICl}_3(s) + 3\text{e}^- \rightleftharpoons \frac{1}{2}\text{I}_2(s) + 3\text{Cl}^-$	1.28	$\text{Mn}(\text{OH})_2(s) + 2\text{e}^- \rightleftharpoons \text{Mn}(s) + 2\text{OH}^-$	-1.565
$\text{ICl}(s) + \text{e}^- \rightleftharpoons \frac{1}{2}\text{I}_2(s) + \text{Cl}^-$	1.22		(continued)
$\text{IO}_3^- + 6\text{H}^+ + 5\text{e}^- \rightleftharpoons \frac{1}{2}\text{I}_2(s) + 3\text{H}_2\text{O}$	1.210		
$\text{IO}_3^- + 5\text{H}^+ + 4\text{e}^- \rightleftharpoons \text{HOI} + 2\text{H}_2\text{O}$	1.154		
$\text{I}_2(aq) + 2\text{e}^- \rightleftharpoons 2\text{I}^-$	0.620		
$\text{I}_2(s) + 2\text{e}^- \rightleftharpoons 2\text{I}^-$	0.535		
$\text{I}_3^- + 2\text{e}^- \rightleftharpoons 3\text{I}^-$	0.535		
$\text{IO}_3^- + 3\text{H}_2\text{O} + 6\text{e}^- \rightleftharpoons \text{I}^- + 6\text{OH}^-$	0.269		
Iron			
$\text{Fe}(\text{phenanthroline})_3^{3+} + \text{e}^- \rightleftharpoons \text{Fe}(\text{phenanthroline})_3^{2+}$	1.147		
$\text{Fe}(\text{bipyridyl})_3^{3+} + \text{e}^- \rightleftharpoons \text{Fe}(\text{bipyridyl})_3^{2+}$	1.120		

Reaction ^a	<i>E</i> ^o (volts)	Reaction ^a	<i>E</i> ^o (volts)
Mercury			
$2\text{Hg}^{2+} + 2e^- \rightleftharpoons \text{Hg}_2^{2+}$	0.908	$\frac{1}{2}\text{O}_2(g) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{H}_2\text{O}$	1.229 1
$\text{Hg}^{2+} + 2e^- \rightleftharpoons \text{Hg}(l)$	0.852	$\text{O}_2(g) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{H}_2\text{O}_2$	0.695
$\text{Hg}_2^{2+} + 2e^- \rightleftharpoons 2\text{Hg}(l)$	0.796	$\text{O}_2(g) + \text{H}^+ + e^- \rightleftharpoons \text{HO}_2$	-0.05
$\text{Hg}_2\text{SO}_4(s) + 2e^- \rightleftharpoons 2\text{Hg}(l) + \text{SO}_4^{2-}$	0.614		
$\text{Hg}_2\text{Cl}_2(s) + 2e^- \rightleftharpoons 2\text{Hg}(l) + 2\text{Cl}^-$	{ 0.268	Palladium	
	{ 0.241 (saturated calomel electrode)	$\text{Pd}^{2+} + 2e^- \rightleftharpoons \text{Pd}(s)$	0.915
$\text{Hg}(\text{OH})_3^- + 2e^- \rightleftharpoons \text{Hg}(l) + 3\text{OH}^-$	0.231	$\text{PdO}(s) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{Pd}(s) + \text{H}_2\text{O}$	0.79
$\text{Hg}(\text{OH})_2 + 2e^- \rightleftharpoons \text{Hg}(l) + 2\text{OH}^-$	0.206	$\text{PdCl}_6^{4-} + 2e^- \rightleftharpoons \text{Pd}(s) + 6\text{Cl}^-$	0.615
$\text{Hg}_2\text{Br}_2(s) + 2e^- \rightleftharpoons 2\text{Hg}(l) + 2\text{Br}^-$	0.140	$\text{PdO}_2(s) + \text{H}_2\text{O} + 2e^- \rightleftharpoons \text{PdO}(s) + 2\text{OH}^-$	0.64
$\text{HgO}(s, \text{yellow}) + \text{H}_2\text{O} + 2e^- \rightleftharpoons \text{Hg}(l) + 2\text{OH}^-$	0.098 3		
$\text{HgO}(s, \text{red}) + \text{H}_2\text{O} + 2e^- \rightleftharpoons \text{Hg}(l) + 2\text{OH}^-$	0.097 7		
Molybdenum			
$\text{MoO}_4^{2-} + 2\text{H}_2\text{O} + 2e^- \rightleftharpoons \text{MoO}_2(s) + 4\text{OH}^-$	-0.818		
$\text{MoO}_4^{2-} + 4\text{H}_2\text{O} + 6e^- \rightleftharpoons \text{Mo}(s) + 8\text{OH}^-$	-0.926		
$\text{MoO}_2(s) + 2\text{H}_2\text{O} + 4e^- \rightleftharpoons \text{Mo}(s) + 4\text{OH}^-$	-0.980		
Nickel			
$\text{NiOOH}(s) + 3\text{H}^+ + e^- \rightleftharpoons \text{Ni}^{2+} + 2\text{H}_2\text{O}$	2.05		
$\text{Ni}^{2+} + 2e^- \rightleftharpoons \text{Ni}(s)$	-0.236		
$\text{Ni}(\text{CN})_4^{2-} + e^- \rightleftharpoons \text{Ni}(\text{CN})_3^{2-} + \text{CN}^-$	-0.401		
$\text{Ni}(\text{OH})_2(s) + 2e^- \rightleftharpoons \text{Ni}(s) + 2\text{OH}^-$	-0.714		
Nitrogen			
$\text{HN}_3 + 3\text{H}^+ + 2e^- \rightleftharpoons \text{N}_2(g) + \text{NH}_4^+$	2.079		
$\text{N}_2\text{O}(g) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{N}_2(g) + \text{H}_2\text{O}$	1.769		
$2\text{NO}(g) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{N}_2\text{O}(g) + \text{H}_2\text{O}$	1.587		
$\text{NO}^+ + e^- \rightleftharpoons \text{NO}(g)$	1.46		
$2\text{NH}_3\text{OH}^+ + \text{H}^+ + 2e^- \rightleftharpoons \text{N}_2\text{H}_5^+ + 2\text{H}_2\text{O}$	1.40		
$\text{NH}_3\text{OH}^+ + 2\text{H}^+ + 2e^- \rightleftharpoons \text{NH}_4^+ + \text{H}_2\text{O}$	1.33		
$\text{N}_2\text{H}_5^+ + 3\text{H}^+ + 2e^- \rightleftharpoons 2\text{NH}_4^+$	1.250		
$\text{HNO}_2 + \text{H}^+ + e^- \rightleftharpoons \text{NO}(g) + \text{H}_2\text{O}$	0.984		
$\text{NO}_3^- + 4\text{H}^+ + 3e^- \rightleftharpoons \text{NO}(g) + 2\text{H}_2\text{O}$	0.955		
$\text{NO}_3^- + 3\text{H}^+ + 2e^- \rightleftharpoons \text{HNO}_2 + \text{H}_2\text{O}$	0.940		
$\text{NO}_3^- + 2\text{H}^+ + e^- \rightleftharpoons \frac{1}{2}\text{N}_2\text{O}_4(g) + \text{H}_2\text{O}$	0.798		
$\text{N}_2(g) + 8\text{H}^+ + 6e^- \rightleftharpoons 2\text{NH}_4^+$	0.274		
$\text{N}_2(g) + 5\text{H}^+ + 4e^- \rightleftharpoons \text{N}_2\text{H}_5^+$	-0.214		
$\text{N}_2(g) + 2\text{H}_2\text{O} + 4\text{H}^+ + 2e^- \rightleftharpoons 2\text{NH}_3\text{OH}^+$	-1.83		
$\frac{3}{2}\text{N}_2(g) + \text{H}^+ + e^- \rightleftharpoons \text{HN}_3$	-3.334		
Oxygen			
$\text{OH} + \text{H}^+ + e^- \rightleftharpoons \text{H}_2\text{O}$	2.56		
$\text{O}(g) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{H}_2\text{O}$	2.430 1		
$\text{O}_3(g) + 2\text{H}^+ + 2e^- \rightleftharpoons \text{O}_2(g) + \text{H}_2\text{O}$	2.075		
$\text{H}_2\text{O}_2 + 2\text{H}^+ + 2e^- \rightleftharpoons 2\text{H}_2\text{O}$	1.763		
$\text{HO}_2 + \text{H}^+ + e^- \rightleftharpoons \text{H}_2\text{O}_2$	1.44		
		$\text{Ag}^{2+} + e^- \rightleftharpoons \text{Ag}^+$	1.989
		$\text{Ag}^{3+} + 2e^- \rightleftharpoons \text{Ag}^+$	1.9
		$\text{AgO}(s) + \text{H}^+ + e^- \rightleftharpoons \frac{1}{2}\text{Ag}_2\text{O}(s) + \frac{1}{2}\text{H}_2\text{O}$	1.40
		$\text{Ag}^+ + e^- \rightleftharpoons \text{Ag}(s)$	0.799 3
		$\text{Ag}_2\text{C}_2\text{O}_4(s) + 2e^- \rightleftharpoons 2\text{Ag}(s) + \text{C}_2\text{O}_4^{2-}$	0.465
		$\text{AgN}_3(s) + e^- \rightleftharpoons \text{Ag}(s) + \text{N}_3^-$	0.293
		$\text{AgCl}(s) + e^- \rightleftharpoons \text{Ag}(s) + \text{Cl}^-$	{ 0.222 (saturated KCl)
		$\text{AgBr}(s) + e^- \rightleftharpoons \text{Ag}(s) + \text{Br}^-$	0.071
		$\text{Ag}(\text{S}_2\text{O}_3)^{3-} + e^- \rightleftharpoons \text{Ag}(s) + 2\text{S}_2\text{O}_3^{2-}$	0.017
		$\text{AgI}(s) + e^- \rightleftharpoons \text{Ag}(s) + \text{I}^-$	-0.152
		$\text{Ag}_2\text{S}(s) + \text{H}^+ + 2e^- \rightleftharpoons 2\text{Ag}(s) + \text{SH}^-$	-0.272

Reaction ^a	E° (volts)	Reaction ^a	E° (volts)
Sodium		Titanium	
$\text{Na}^+ + \frac{1}{2}\text{H}_2(g) + \text{e}^- \rightleftharpoons \text{NaH}(s)$	-2.367	$\text{TiO}^{2+} + 2\text{H}^+ + \text{e}^- \rightleftharpoons \text{Ti}^{3+} + \text{H}_2\text{O}$	0.1
$\text{Na}^+ + \text{e}^- \rightleftharpoons \text{Na}(s)$	-2.714	$\text{Ti}^{3+} + \text{e}^- \rightleftharpoons \text{Ti}^{2+}$	-0.9
Strontium		$\text{TiO}_2(s) + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons \text{Ti}(s) + 2\text{H}_2\text{O}$	-1.076
$\text{Sr}^{2+} + 2\text{e}^- \rightleftharpoons \text{Sr}(s)$	-2.889	$\text{TiF}_6^{2-} + 4\text{e}^- \rightleftharpoons \text{Ti}(s) + 6\text{F}^-$	-1.191
Sulfur		$\text{Ti}^{2+} + 2\text{e}^- \rightleftharpoons \text{Ti}(s)$	-1.60
$\text{S}_2\text{O}_8^{2-} + 2\text{e}^- \rightleftharpoons 2\text{SO}_4^{2-}$	2.01	Tungsten	
$\text{S}_2\text{O}_6^{2-} + 4\text{H}^+ + 2\text{e}^- \rightleftharpoons 2\text{H}_2\text{SO}_3$	0.57	$\text{W}(\text{CN})_8^{4-} + \text{e}^- \rightleftharpoons \text{W}(\text{CN})_8^{3-}$	0.457
$4\text{SO}_2 + 4\text{H}^+ + 6\text{e}^- \rightleftharpoons \text{S}_4\text{O}_6^{2-} + 2\text{H}_2\text{O}$	0.539	$\text{W}^6+ + \text{e}^- \rightleftharpoons \text{W}^5+$	0.26
$\text{SO}_2 + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons \text{S}(s) + 2\text{H}_2\text{O}$	0.450	$\text{WO}_3(s) + 6\text{H}^+ + 6\text{e}^- \rightleftharpoons \text{W}(s) + 3\text{H}_2\text{O}$	-0.091
$2\text{H}_2\text{SO}_3 + 2\text{H}^+ + 4\text{e}^- \rightleftharpoons \text{S}_2\text{O}_3^{2-} + 3\text{H}_2\text{O}$	0.40	$\text{W}^5+ + \text{e}^- \rightleftharpoons \text{W}^4+$	-0.3
$\text{S}(s) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2\text{S}(g)$	0.174	$\text{WO}_2(s) + 2\text{H}_2\text{O} + 4\text{e}^- \rightleftharpoons \text{W}(s) + 4\text{OH}^-$	-0.982
$\text{S}(s) + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2\text{S}(aq)$	0.144	$\text{WO}_4^{2-} + 4\text{H}_2\text{O} + 6\text{e}^- \rightleftharpoons \text{W}(s) + 8\text{OH}^-$	-1.060
$\text{S}_4\text{O}_6^{2-} + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons 2\text{HS}_2\text{O}_3^-$	0.10	Uranium	
$5\text{S}(s) + 2\text{e}^- \rightleftharpoons \text{S}_5^{2-}$	-0.340	$\text{UO}_2^+ + 4\text{H}^+ + \text{e}^- \rightleftharpoons \text{U}^{4+} + 2\text{H}_2\text{O}$	0.39
$2\text{S}(s) + 2\text{e}^- \rightleftharpoons \text{S}_2^{2-}$	-0.50	$\text{UO}_2^2+ + 4\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{U}^{4+} + 2\text{H}_2\text{O}$	0.273
$2\text{SO}_3^{2-} + 3\text{H}_2\text{O} + 4\text{e}^- \rightleftharpoons \text{S}_2\text{O}_3^{2-} + 6\text{OH}^-$	-0.566	$\text{UO}_2^2+ + \text{e}^- \rightleftharpoons \text{UO}_2^+$	0.16
$\text{SO}_3^{2-} + 3\text{H}_2\text{O} + 4\text{e}^- \rightleftharpoons \text{S}(s) + 6\text{OH}^-$	-0.659	$\text{U}^{4+} + \text{e}^- \rightleftharpoons \text{U}^{3+}$	-0.577
$\text{SO}_4^{2-} + 4\text{H}_2\text{O} + 6\text{e}^- \rightleftharpoons \text{S}(s) + 8\text{OH}^-$	-0.751	$\text{U}^{3+} + 3\text{e}^- \rightleftharpoons \text{U}(s)$	-1.642
$\text{SO}_4^{2-} + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{SO}_3^{2-} + 2\text{OH}^-$	-0.936	Vanadium	
$2\text{SO}_3^{2-} + 2\text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{S}_2\text{O}_4^{2-} + 4\text{OH}^-$	-1.130	$\text{VO}_2^+ + 2\text{H}^+ + \text{e}^- \rightleftharpoons \text{VO}^{2+} + \text{H}_2\text{O}$	1.001
$2\text{SO}_4^{2-} + 2\text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{S}_2\text{O}_6^{2-} + 4\text{OH}^-$	-1.71	$\text{VO}^{2+} + 2\text{H}^+ + \text{e}^- \rightleftharpoons \text{V}^{3+} + \text{H}_2\text{O}$	0.337
Thallium		$\text{V}^{3+} + \text{e}^- \rightleftharpoons \text{V}^{2+}$	-0.255
$\text{Ti}^3+ + 2\text{e}^- \rightleftharpoons \text{Ti}^+$	{ 1.280 0.77 1 F HCl 1.22 1 F H_2SO_4 1.23 1 F HNO_3 1.26 1 F HClO_4	$\text{V}^{2+} + 2\text{e}^- \rightleftharpoons \text{V}(s)$	-1.125
$\text{Ti}^+ + \text{e}^- \rightleftharpoons \text{Ti}(s)$	-0.336	Xenon	
Tin		$\text{H}_4\text{XeO}_6 + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{XeO}_3 + 3\text{H}_2\text{O}$	2.38
$\text{Sn}(\text{OH})_3^+ + 3\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Sn}^{2+} + 3\text{H}_2\text{O}$	0.142	$\text{XeF}_2 + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Xe}(g) + 2\text{HF}$	2.2
$\text{Sn}^{4+} + 2\text{e}^- \rightleftharpoons \text{Sn}^{2+}$	0.139	$\text{XeO}_3 + 6\text{H}^+ + 6\text{e}^- \rightleftharpoons \text{Xe}(g) + 3\text{H}_2\text{O}$	2.1
$\text{SnO}_2(s) + 4\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Sn}^{2+} + 2\text{H}_2\text{O}$	-0.094	Yttrium	
$\text{Sn}^{2+} + 2\text{e}^- \rightleftharpoons \text{Sn}(s)$	-0.141	$\text{Y}^{3+} + 3\text{e}^- \rightleftharpoons \text{Y}(s)$	-2.38
$\text{SnF}_6^{2-} + 4\text{e}^- \rightleftharpoons \text{Sn}(s) + 6\text{F}^-$	-0.25	Zinc	
$\text{Sn}(\text{OH})_6^{2-} + 2\text{e}^- \rightleftharpoons \text{Sn}(\text{OH})_3^+ + 3\text{OH}^-$	-0.93	$\text{ZnOH}^+ + \text{H}^+ + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + \text{H}_2\text{O}$	-0.497
$\text{Sn}(s) + 4\text{H}_2\text{O} + 4\text{e}^- \rightleftharpoons \text{SnH}_4(g) + 4\text{OH}^-$	-1.316	$\text{Zn}^{2+} + 2\text{e}^- \rightleftharpoons \text{Zn}(s)$	-0.762
$\text{SnO}_2(s) + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{SnO}(s) + 2\text{OH}^-$	-0.961	$\text{Zn}(\text{NH}_3)_4^{2+} + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + 4\text{NH}_3$	-1.04
		$\text{ZnCO}_3(s) + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + \text{CO}_3^{2-}$	-1.06
		$\text{Zn}(\text{OH})_3^- + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + 3\text{OH}^-$	-1.183
		$\text{Zn}(\text{OH})_4^{2-} + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + 4\text{OH}^-$	-1.199
		$\text{Zn}(\text{OH})_2(s) + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + 2\text{OH}^-$	-1.249
		$\text{ZnO}(s) + \text{H}_2\text{O} + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + 2\text{OH}^-$	-1.260
		$\text{ZnS}(s) + 2\text{e}^- \rightleftharpoons \text{Zn}(s) + \text{S}^{2-}$	-1.405

Appendix D

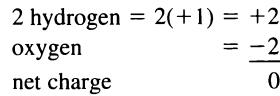
Oxidation Numbers and

Balancing Redox Equations

The *oxidation number*, or *oxidation state*, is a bookkeeping device used to keep track of the number of electrons formally associated with a particular element. The oxidation number is meant to tell how many electrons have been lost or gained by a neutral atom when it forms a compound. Because oxidation numbers have no real physical meaning, they are somewhat arbitrary, and not all chemists will assign the same oxidation number to a given element in an unusual compound. However, there are some ground rules that provide a useful start.

1. The oxidation number of an element by itself—e.g., Cu(s) or Cl₂(g)—is 0.
 2. The oxidation number of H is almost always +1, except in metal hydrides—e.g., NaH, in which H is -1.
 3. The oxidation number of oxygen is almost always -2. The only common exceptions are peroxides, in which two oxygen atoms are connected and each has an oxidation number of -1. Two examples are hydrogen peroxide (H—O—O—H) and its anion (H—O—O⁻). The oxidation number of oxygen in gaseous O₂ is 0.
 4. The alkali metals (Li, Na, K, Rb, Cs, Fr) almost always have an oxidation number of +1. The alkaline earth metals (Be, Mg, Ca, Sr, Ba, Ra) are almost always in the +2 oxidation state.
 5. The halogens (F, Cl, Br, I) are usually in the -1 oxidation state. Exceptions are when two different halogens are bound to each other or when a halogen is bound to more than one atom. When different halogens are bound to each other, we assign the oxidation number -1 to the more electronegative halogen.

The sum of the oxidation numbers of each atom in a molecule must equal the charge of the molecule. In H₂O, for example we have



Problems

Answers are given at the end of this appendix.

1. Write the oxidation state of the boldface atom in each of the following species.

In SO_4^{2-} , sulfur must have an oxidation number of +6 so that the sum of the oxidation numbers will be -2:

$$\begin{array}{rcl}
 \text{oxygen} & = 4(-2) & = -8 \\
 \text{sulfur} & & = +6 \\
 \text{net charge} & & = -2
 \end{array}$$

In benzene (C_6H_6), the oxidation number of each carbon must be -1 if hydrogen is assigned the number $+1$. In cyclohexane (C_6H_{12}), the oxidation number of each carbon must be -2 for the same reason. The carbons in benzene are in a higher oxidation state than those in cyclohexane.

The oxidation number of iodine in ICl_2^- is +1. This is unusual, because halogens are usually -1. However, because chlorine is more electronegative than iodine, we assign Cl as -1 thereby forcing I to be +1.

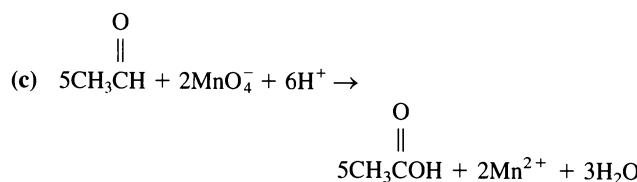
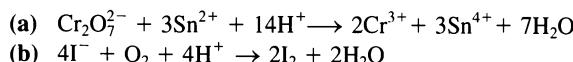
The oxidation number of As in As_2S_3 is +3, and the value for S is -2. This is arbitrary but reasonable. Because S is more electronegative than As, we make S negative and As positive and, because S is in the same family as oxygen, which is usually -2, we assign S as -2, thus leaving As as +3.

The oxidation number of S in $\text{S}_4\text{O}_6^{2-}$ (tetrathionate) is +2.5. The *fractional oxidation state* comes about because six O atoms contribute -12. Because the charge is -2, the four S atoms must contribute +10. The average oxidation number of S must be $+\frac{10}{4} = 2.5$.

The oxidation number of Fe in $K_3Fe(CN)_6$ is +3. To make this assignment, we first recognize cyanide (CN^-) as a common ion that carries a charge of -1. Six cyanide ions give -6, and three potassium ions (K^+) give +3. Therefore Fe should have an oxidation number of +3 for the whole formula to be neutral. In this approach, it is not necessary to assign individual oxidation numbers to carbon and nitrogen, as long as we recognize that the charge of CN is -1.

- | | | | | | |
|-----|------------------------------------|-----|-------------------------------|-----|--------------------------------|
| (g) | Cr^{3+} | (m) | ClO_2 | (s) | HAsO_3^{2-} |
| (h) | MnO_2 | (n) | ClO_2^- | (t) | $(\text{CH}_3)_4\text{Li}$ |
| (i) | Pb(OH)_3^- | (o) | $\text{Mn}(\text{CN})_6^{4-}$ | (u) | P_4O_{10} |
| (j) | Fe(OH)_3 | (p) | N_2 | (v) | $\text{C}_2\text{H}_6\text{O}$ |
| (k) | ClO^- | (q) | NH_4^+ | (w) | $\text{VO}(\text{SO}_4)_2$ |
| (l) | $\text{K}_4\text{Fe}(\text{CN})_6$ | (r) | N_2H_5^+ | (x) | Fe_3O_4 |

2. Identify the oxidizing agent and the reducing agent on the left side of each of the following reactions.



Balancing Redox Reactions

To balance a reaction involving oxidation and reduction, we must first identify which element is oxidized and which is reduced. We then break the net reaction into two imaginary *half-reactions*, one of which involves only oxidation and the other only reduction. Although free electrons never appear in a balanced net reaction, they do appear in balanced half-reactions. If we are dealing with aqueous solutions, we proceed to balance each half-reaction by using H_2O and either H^+ or OH^- , as necessary. A reaction is balanced when the number of atoms of each element is the same on both sides and the net charge is the same on both sides.

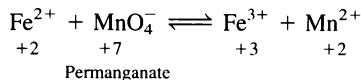
Acidic Solutions

Here are the steps that we will follow:

1. Assign oxidation numbers to the elements that are oxidized or reduced.
2. Break the reaction into two half-reactions, one involving oxidation and the other reduction.
3. For each half-reaction, balance the number of atoms that are oxidized or reduced.
4. Balance the electrons to account for the change in oxidation number by adding electrons to one side of each half-reaction.
5. Balance oxygen atoms by adding H_2O to one side of each half-reaction.
6. Balance the hydrogen atoms by adding H^+ to one side of each half-reaction.
7. Multiply each half-reaction by the number of electrons in the other half-reaction so that the number of electrons on each side of the total reaction will cancel. Then add the two half-reactions and simplify to the smallest integral coefficients.

Example Balancing a Redox Equation

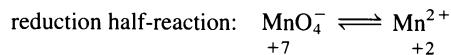
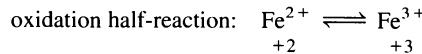
Balance the following equation by using H^+ but not OH^- :



SOLUTION

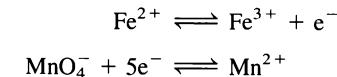
1. Assign oxidation numbers. These are assigned for Fe and Mn in each species in the preceding reaction.

2. Break the reaction into two half-reactions.



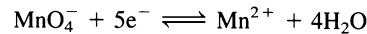
3. Balance the atoms that are oxidized or reduced. Because there is only one Fe or Mn in each species on each side of the equation, the atoms of Fe and Mn are already balanced.

4. Balance electrons. Electrons are added to account for the change in each oxidation state.

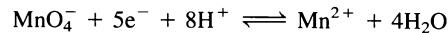


In the second case, we need 5e^- on the left side to take Mn from +7 to +2.

5. Balance oxygen atoms. There are no oxygen atoms in the Fe half-reactions. There are four oxygen atoms on the left side of the Mn reaction, so we add four molecules of H_2O to the right side:

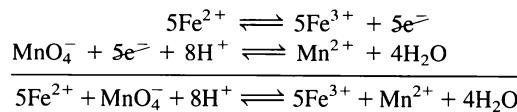


6. Balance hydrogen atoms. The Fe equation is already balanced. The Mn equation needs 8H^+ on the left.



At this point, each half-reaction must be completely balanced (the same number of atoms and charge on each side) or you have made a mistake.

7. Multiply and add the reactions. We multiply the Fe equation by 5 and the Mn equation by 1 and add:

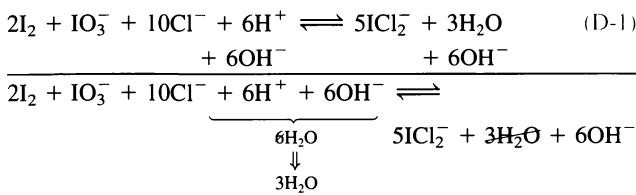


The total charge on each side is +17, and we find the same number of atoms of each element on each side. The equation is balanced.

Basic Solutions

The method preferred by many people for basic solutions is to balance the equation first with H^+ . The answer can then be converted into one in which OH^- is used instead. This is done by adding to each side of the equation a number of hydroxide ions equal to the number of H^+ ions appearing in the equation. For

example, to balance Equation D-1 with OH^- instead of H^+ , proceed as follows:



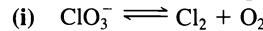
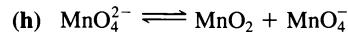
Realizing that $6\text{H}^+ + 6\text{OH}^- = 6\text{H}_2\text{O}$, and canceling $3\text{H}_2\text{O}$ on each side, gives the final result:



Problems

3. Balance the following reactions by using H^+ but not OH^- .

- (a) $\text{Fe}^{3+} + \text{Hg}_2^{2+} \rightleftharpoons \text{Fe}^{2+} + \text{Hg}^{2+}$
- (b) $\text{Ag} + \text{NO}_3^- \rightleftharpoons \text{Ag}^+ + \text{NO}$
- (c) $\text{VO}^{2+} + \text{Sn}^{2+} \rightleftharpoons \text{V}^{3+} + \text{Sn}^{4+}$
- (d) $\text{SeO}_4^{2-} + \text{Hg} + \text{Cl}^- \rightleftharpoons \text{SeO}_3^{2-} + \text{Hg}_2\text{Cl}_2$
- (e) $\text{CuS} + \text{NO}_3^- \rightleftharpoons \text{Cu}^{2+} + \text{SO}_4^{2-} + \text{NO}$
- (f) $\text{S}_2\text{O}_3^{2-} + \text{I}_2 \rightleftharpoons \text{I}^- + \text{S}_4\text{O}_6^{2-}$
- (g) $\text{Cr}_2\text{O}_7^{2-} + \text{CH}_3\text{CH} \rightleftharpoons \text{CH}_3\text{COH} + \text{Cr}^{3+}$



4. Balance the following equations by using OH^- but not H^+ .

- (a) $\text{PbO}_2 + \text{Cl}^- \rightleftharpoons \text{ClO}^- + \text{Pb}(\text{OH})_3^-$
- (b) $\text{HNO}_2 + \text{SbO}^+ \rightleftharpoons \text{NO} + \text{Sb}_2\text{O}_5$
- (c) $\text{Ag}_2\text{S} + \text{CN}^- + \text{O}_2 \rightleftharpoons \text{S} + \text{Ag}(\text{CN})_2 + \text{OH}^-$
- (d) $\text{HO}_2^- + \text{Cr}(\text{OH})_3 \rightleftharpoons \text{CrO}_4^{2-} + \text{OH}^-$
- (e) $\text{ClO}_2 + \text{OH}^- \rightleftharpoons \text{ClO}_2^- + \text{ClO}_3^-$
- (f) $\text{WO}_3^- + \text{O}_2 \rightleftharpoons \text{HW}_6\text{O}_{21}^{5-} + \text{OH}^-$

Answers to Problems

- | | | | |
|-----|--------|--------|----------|
| 1. | (a) +1 | (i) +2 | (q) -3 |
| (b) | +2 | (j) +3 | (r) -2 |
| (c) | +6 | (k) +1 | (s) +3 |
| (d) | +2 | (l) +2 | (t) -4 |
| (e) | -1/2 | (m) +4 | (u) +5 |
| (f) | +2 | (n) +3 | (v) -2 |
| (g) | +3 | (o) +2 | (w) +4 |
| (h) | +4 | (p) 0 | (x) +8/3 |

2. Oxidizing agent Reducing agent

- | | | |
|-----|------------------------------|-------------------------|
| (a) | $\text{Cr}_2\text{O}_7^{2-}$ | Sn^{2+} |
| (b) | O_2 | I^- |
| (c) | MnO_4^- | CH_3CHO |

3. (a) $2\text{Fe}^{3+} + \text{Hg}_2^{2+} \rightleftharpoons 2\text{Fe}^{2+} + 2\text{Hg}^{2+}$
 (b) $3\text{Ag} + \text{NO}_3^- + 4\text{H}^+ \rightleftharpoons 3\text{Ag}^+ + \text{NO} + 2\text{H}_2\text{O}$
 (c) $4\text{H}^+ + 2\text{VO}^{2+} + \text{Sn}^{2+} \rightleftharpoons 2\text{V}^{3+} + \text{Sn}^{4+} + 2\text{H}_2\text{O}$

- | | |
|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (d) | $2\text{Hg} + 2\text{Cl}^- + \text{SeO}_4^{2-} + 2\text{H}^+ \rightleftharpoons$
$\text{Hg}_2\text{Cl}_2 + \text{SeO}_3^{2-} + \text{H}_2\text{O}$ |
| (e) | $3\text{CuS} + 8\text{NO}_3^- + 8\text{H}^+ \rightleftharpoons$
$3\text{Cu}^{2+} + 3\text{SO}_4^{2-} + 8\text{NO} + 4\text{H}_2\text{O}$ |
| (f) | $2\text{S}_2\text{O}_3^{2-} + \text{I}_2 \rightleftharpoons \text{S}_4\text{O}_6^{2-} + 2\text{I}^-$ |
| (g) | $\text{Cr}_2\text{O}_7^{2-} + 3\text{CH}_3\text{CHO} + 8\text{H}^+ \rightleftharpoons$
$2\text{Cr}^{3+} + 3\text{CH}_3\text{CO}_2\text{H} + 4\text{H}_2\text{O}$ |
| (h) | $4\text{H}^+ + 3\text{MnO}_4^{2-} \rightleftharpoons \text{MnO}_2 + 2\text{MnO}_4^- + 2\text{H}_2\text{O}$ |
| (i) | $2\text{H}^+ + 2\text{ClO}_3^- \rightleftharpoons \text{Cl}_2 + \frac{5}{2}\text{O}_2 + \text{H}_2\text{O}$ |
4. (a) $\text{H}_2\text{O} + \text{OH}^- + \text{PbO}_2 + \text{Cl}^- \rightleftharpoons \text{Pb}(\text{OH})_3^- + \text{ClO}^-$
 (b) $4\text{HNO}_2 + 2\text{SbO}^+ + 2\text{OH}^- \rightleftharpoons 4\text{NO} + \text{Sb}_2\text{O}_5 + 3\text{H}_2\text{O}$
 (c) $\text{Ag}_2\text{S} + 4\text{CN}^- + \frac{1}{2}\text{O}_2 + \text{H}_2\text{O} \rightleftharpoons$
 $\text{S} + 2\text{Ag}(\text{CN})_2^- + 2\text{OH}^-$
 (d) $2\text{HO}_2^- + \text{Cr}(\text{OH})_3 \rightleftharpoons \text{CrO}_4^{2-} + \text{OH}^- + 2\text{H}_2\text{O}$
 (e) $2\text{ClO}_2 + 2\text{OH}^- \rightleftharpoons \text{ClO}_2^- + \text{ClO}_3^- + \text{H}_2\text{O}$
 (f) $12\text{WO}_3^- + 3\text{O}_2 + 2\text{H}_2\text{O} \rightleftharpoons 2\text{HW}_6\text{O}_{21}^{5-} + 2\text{OH}^-$

Glossary

abscissa The horizontal (x) axis of a graph.

absolute uncertainty The margin of uncertainty associated with a measurement. Absolute error could also refer to the difference between a measured value and the “true” value.

absorbance, A Defined as $A = \log(P_0/P)$, where P_0 is the radiant power of light (power per unit area) striking the sample on one side and P is the radiant power emerging from the other side. Also called *optical density*.

absorption Occurs when a substance is taken up *inside* another. See also **adsorption**.

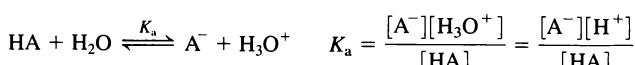
absorption spectrum A graph of absorbance or transmittance of light versus wavelength, frequency, or wavenumber.

accuracy A measure of how close a measured value is to the “true” value.

acid A substance that increases the concentration of H^+ when added to water.

acid-base titration One in which the reaction between analyte and titrant is an acid-base reaction.

acid dissociation constant, K_a The equilibrium constant for the reaction of an acid, HA, with H_2O :



acid error An error in glass pH electrodes that occurs in strongly acidic solutions, where electrodes tend to indicate a value of pH that is too high.

acid wash Procedure in which glassware is soaked in 3–6 M HCl for >1 h (followed by rinsing well with distilled water and soaking in distilled water) to remove traces of cations adsorbed on the surface of the glass and to replace them with H^+ .

acidic solution One in which the concentration of H^+ is greater than the concentration of OH^- .

activity, α The value that replaces concentration in a thermodynamically correct equilibrium expression. The activity of X is given by $\alpha_X = [X]\gamma_X$, where γ_X is the activity coefficient and $[X]$ is the concentration.

activity coefficient, γ The number by which the concentration must be multiplied to give activity.

adduct The product formed when a Lewis base combines with a Lewis acid.

adjusted retention time, t'_r In chromatography, this parameter is $t'_r = t_r - t_m$, where t_r is the retention time of a solute and t_m is the time needed for the mobile phase to travel the length of the column.

adsorption Occurs when a substance becomes attached to the *surface* of another substance. See also **absorption**.

adsorption chromatography A technique in which solute equilibrates between the mobile phase and adsorption sites on the stationary phase.

adsorption indicator Used for precipitation titrations, it becomes attached to a precipitate and changes color when the surface charge of the precipitate changes sign at the equivalence point.

aerosol A suspension of very small liquid or solid particles in air or gas. Examples include fog and smoke.

affinity chromatography A technique in which a particular solute is retained on a column by virtue of a specific interaction with a molecule covalently bound to the stationary phase.

aliquot Portion.

alkali flame detector Modified flame ionization detector that responds to N and P, which produce ions when they contact a Rb_2SO_4 -containing glass bead in the flame. Also called *nitrogen-phosphorus detector*.

alkaline error See **sodium error**.

amalgam A solution of anything in mercury.

ambient temperature The temperature of the surroundings (such as room temperature).

amine A compound with the general formula RNH_2 , R_2NH , or R_3N , where R is any group of atoms.

amino acid One of 20 building blocks of proteins, having the general structure



where R is a different substituent for each acid.

animeter Device for measuring electric current.

ammonium ion The ammonium ion is NH_4^+ . An ammonium ion is any ion of the type RNH_3^+ , $R_2NH_2^+$, R_3NH^+ , or R_4N^+ , where R is an organic substituent.

ampere, A SI unit of electric current. One ampere will produce a force of exactly 2×10^{-7} N/m when that current flows through two “infinitely” long, parallel conductors of negligible cross section, with a spacing of 1 m, in a vacuum. One ampere is a flow of one coulomb per second.

amperometry The measurement of electric current for analytical purposes.

amphiprotic molecule One that can act as both a proton donor and a proton acceptor. The intermediate species of a polyprotic acid is amphiprotic.

analyte The substance being measured or detected.

analytical chromatography Chromatography of small quantities of material conducted for the purpose of qualitative or quantitative analysis or both.

analytical concentration See **formal concentration**.

anhydrous Describes a substance from which all water has been removed.

anion A negatively charged ion.

anion exchanger An ion exchanger with positively charged groups covalently attached to the support. It can reversibly bind anions.

anode The electrode at which oxidation takes place. In electrophoresis, it is the positive electrode.

antibody A protein manufactured by an organism to sequester foreign molecules and mark them for destruction.

antigen A molecule that is foreign to an organism and causes the organism to make antibodies.

antilogarithm The antilogarithm of a is b if $10^a = b$.

- aprotic solvent** One that cannot donate protons (hydrogen ions) in an acid-base reaction.
- aqua regia** A 3:1 (vol/vol) mixture of concentrated (37 wt%) HCl and concentrated (70 wt%) HNO₃.
- aqueous** In water (as an *aqueous* solution).
- archiving** Storing for future reference.
- argentometric titration** One using Ag⁺ ion.
- ashless filter paper** Specially treated paper that leaves a negligible residue after ignition. It is used for gravimetric analysis.
- assessment** In quality assurance, the process of (1) collecting data to show that analytical procedures are operating within specified limits and (2) verifying that final results meet use objectives.
- asymmetry potential** When the activity of analyte is the same on the inside and outside of an ion-selective electrode, there should be no voltage across the membrane. In fact, the two surfaces are never identical, and some voltage (called the asymmetry potential) is usually observed.
- atmosphere, atm** One atmosphere is defined as a pressure of 101 325 Pa. It is equal to the pressure exerted by a column of Hg 760 mm in height at the Earth's surface.
- atmospheric pressure chemical ionization** A method for interfacing liquid chromatography to mass spectrometry. Liquid is nebulized into a fine aerosol by a coaxial gas flow and the application of heat. Electrons from a high-voltage corona discharge create cations and anions from analyte exiting the chromatography column. The most common species observed with this interface is MH⁺, the protonated analyte, with little fragmentation.
- atomic absorption spectroscopy** A technique in which the absorption of light by free gaseous atoms or ions in a flame, furnace, or plasma is used to measure concentration.
- atomic emission spectroscopy** A technique in which the emission of light by thermally excited atoms or ions in a flame, furnace, or plasma is used to measure concentration.
- atomic mass** The number of grams of an element containing Avogadro's number of atoms.
- atomization** The process in which a compound is decomposed into atoms at high temperature.
- autoprolysis** The reaction in which two molecules of the same species transfer a proton from one to the other; e.g., CH₃OH + CH₃OH \rightleftharpoons CH₃OH₂⁺ + CH₃O⁻. Also called *self-ionization*.
- autoprolysis constant** The equilibrium constant for an autoprolysis reaction.
- autotitrator** A device that dispenses measured amounts of titrant into a solution and monitors a property such as pH or electrode potential after each addition. The instrument performs the titration automatically and can determine the end point automatically. Data from the titration can be transferred to a spreadsheet for further interpretation.
- auxiliary complexing agent** A species, such as ammonia, used to stabilize another species and keep that other species in solution. It binds loosely enough to be displaced by a titrant.
- auxiliary electrode** The current-carrying partner of the working electrode in an electrolysis. Also called *counter electrode*.
- average** The sum of several values divided by the number of values. Also called *mean*.
- Avogadro's number** The number of atoms in exactly 0.012 kg of ¹²C, approximately 6.022×10^{23} .
- background correction** In atomic spectroscopy, a means of distinguishing signal due to analyte from signal due to absorption, emission, or scattering by the flame, furnace, plasma, or sample matrix.
- background electrolyte** In capillary electrophoresis, the buffer in which separation is carried out. Also called *run buffer*.
- back titration** One in which an excess of standard reagent is added to react with analyte. Then the excess reagent is titrated with a second reagent or with a standard solution of analyte.
- base** A substance that decreases the concentration of H⁺ when added to water.
- base "dissociation" constant** A misnomer for *base hydrolysis constant, K_b*.
- base hydrolysis constant, K_b** The equilibrium constant for the reaction of a base, B, with H₂O:
- $$B + H_2O \rightleftharpoons BH^+ + OH^- \quad K_b = \frac{[BH^+][OH^-]}{[B]}$$
- base peak** The most intense peak in a mass spectrum.
- basic solution** One in which the concentration of OH⁻ is greater than the concentration of H⁺.
- beam chopper** A rotating mirror that directs light alternately through the sample and reference cells of a double-beam spectrophotometer.
- Beer's law** Relates absorbance (A) of a sample to concentration (c), pathlength (b), and molar absorptivity (ε): $A = \epsilon bc$. This equation is more correctly called the *Beer-Lambert-Bouguer law*.
- biological oxygen demand (BOD)** In a water sample, the quantity of dissolved oxygen consumed by microorganisms during a 5-day incubation in a sealed vessel at 20°C. Oxygen consumption is limited by organic nutrients, so BOD is a measure of pollutant concentration.
- biosensor** Device that uses biological components such as enzymes, antibodies, or DNA, in combination with electrical, optical, or other signals, to achieve selective response to one analyte.
- blank** A sample not intended to contain analyte. See also **field blank, method blank, reagent blank**.
- blank titration** One in which a solution containing all reagents except analyte is titrated. The volume of titrant needed in the blank titration is subtracted from the volume needed to titrate unknown.
- bleed** In gas chromatography, slow loss of stationary phase from evaporation, thermal decomposition, or oxidation.
- blind sample** See **performance test sample**.
- blocking** Occurs when a metal ion binds tightly to a metal ion indicator. A blocked indicator is unsuitable for a titration because no color change is observed at the end point.
- Boltzmann distribution** The relative population of two states at thermal equilibrium:
- $$\frac{N_2}{N_1} = \frac{g_2}{g_1} e^{-(E_2 - E_1)/kT}$$
- where N_i is the population of the state, g_i is the degeneracy of the state, E_i is the energy of the state, k is Boltzmann's constant, and T is temperature in kelvins. Degeneracy refers to the number of states with the same energy.
- bomb** Sealed vessel for conducting high-temperature, high-pressure reactions.
- bonded stationary phase** In HPLC, a stationary liquid phase covalently attached to the solid support.
- Brønsted-Lowry acid** A proton (hydrogen ion) donor.
- Brønsted-Lowry base** A proton (hydrogen ion) acceptor.
- buffer** A mixture of a weak acid and its conjugate base. A buffered solution is one that resists changes in pH when acids or bases are added.
- buffer capacity, β** A measure of the ability of a buffer to resist changes in pH. The larger the buffer capacity, the greater the resistance to pH change.

The definition of buffer capacity is $\beta = dC_b/dpH = -dC_a/dpH$, where C_a and C_b are the number of moles of strong acid or base per liter needed to produce a unit change in pH. Also called *buffer intensity*.

bulk sample Material taken from lot being analyzed—usually chosen to be representative of the entire lot. Also called *gross sample*.

buoyancy Upward force exerted on an object in a liquid or gaseous fluid. An object weighed in air appears lighter than its actual mass by an amount equal to the mass of air that it displaces.

buret A calibrated glass tube with a stopcock at the bottom. Used to deliver known volumes of liquid.

calibration Process of relating the actual quantity (such as mass, volume, force, or electric current) to the quantity indicated on the scale of an instrument.

calibration check In a series of analytical measurements, a calibration check is an analysis of a solution formulated by the analyst to contain a known concentration of analyte. It is the analyst's own check that procedures and instruments are functioning correctly.

calibration curve A graph showing the value of some property versus concentration of analyte. When the corresponding property of an unknown is measured, its concentration can be determined from the graph. Also called *standard curve*.

calomel electrode A common reference electrode based on the half-reaction $Hg_2Cl_2(s) + 2e^- \rightleftharpoons 2Hg(l) + 2Cl^-$. See also **saturated calomel electrode**.

capacitance The electric capacitance of two parallel, charged surfaces is the charge on each surface divided by the electric potential difference (volts) between the two surfaces.

capillary electrophoresis Separation of a mixture into its components by a strong electric field imposed between the two ends of a narrow capillary tube filled with electrolyte solution.

capillary gel electrophoresis A form of capillary electrophoresis in which the tube is filled with a polymer gel that serves as a sieve for macromolecules. The largest molecules migrate slowest through the gel.

capillary zone electrophoresis A form of capillary electrophoresis in which ionic solutes are separated because of differences in their electrophoretic mobility.

carboxylate anion The conjugate base (RCO_2^-) of a carboxylic acid.

carboxylic acid A molecule with the general structure RCO_2H , where R is any group of atoms.

carcinogen A cancer-causing agent.

carrier gas The mobile phase gas in gas chromatography.

catalyst A substance that increases the rate of a selected chemical reaction.

cathode The electrode at which reduction takes place. In electrophoresis, it is the negative electrode.

cation A positively charged ion.

cation exchanger An ion exchanger with negatively charged groups covalently attached to the support. It can reversibly bind cations.

certified reference material Samples sold by national measurement institutes containing known quantities of analytes to test accuracy of analytical procedures. The U.S. National Institute of Standards and Technology calls its certified materials *Standard Reference Materials*.

characteristic The part of a logarithm to the left of the decimal point.

charge balance The sum of all positive charge in solution equals the magnitude of the sum of all negative charge in solution.

charged aerosol detector Sensitive, nearly universal detector for liquid chromatography in which solvent is evaporated from eluate to leave an aerosol of fine particles of nonvolatile solute. Aerosol particles are

charged by adsorption of N_2^+ ions and flow to a collector that measures total charge reaching the detector versus time.

charging current Electric current arising from migration of ions and electrons to or from an electrode when the potential of the electrode is changed. There is no redox reaction of the ions at the electrode. Also called *capacitor current* or *condenser current*.

charring In a gravimetric analysis, the precipitate and filter paper are first *dried* gently. Then the filter paper is *charred* at intermediate temperature to destroy the paper without letting it inflame. Finally, precipitate is *ignited* at high temperature to convert it to its analytical form.

chelating ligand A ligand that binds to a metal through more than one atom.

chemical interference In atomic spectroscopy, any chemical reaction that decreases the efficiency of atomization.

chemical ionization A gentle method of producing ions for a mass spectrometer without extensive fragmentation of the analyte molecule, M. A reagent gas such as CH_4 is bombarded with electrons to make CH_5^+ , which transfers H^+ to M, giving MH^+ .

chemical oxygen demand (COD) In a natural water or industrial effluent sample, the quantity of O_2 equivalent to the quantity of $K_2Cr_2O_7$ consumed by refluxing the sample with a standard dichromate-sulfuric acid solution containing Ag^+ catalyst. Because 1 mol of $K_2Cr_2O_7$ consumes $6e^-$ ($Cr^{6+} \rightarrow Cr^{3+}$), it is equivalent to 1.5 mol of O_2 ($O \rightarrow O^{2-}$).

chemiluminescence Emission of light by an excited-state product of a chemical reaction.

chiral molecule One that is not superimposable on its mirror image in any accessible conformation. Also called an *optically active molecule*, a chiral molecule rotates the plane of polarization of light.

chromatogram A graph showing chromatography detector response as a function of elution time or volume.

chromatograph A machine used to perform chromatography.

chromatography A technique in which molecules in a mobile phase are separated because of their different affinities for a stationary phase. The greater the affinity for the stationary phase, the longer the molecule is retained.

Clark electrode One that measures dissolved oxygen by amperometry.

co-chromatography In chromatography, addition of a known compound to an unknown to see if it is eluted at the same time as a component of the unknown. See **spike**.

coefficient of variation The standard deviation (s) expressed as a percentage of the mean value \bar{x} : coefficient of variation = $100 \times s/\bar{x}$.

Also called *relative standard deviation*.

cold trapping Splitless gas chromatography injection technique in which solute is condensed far below its boiling point in a narrow band at the start of the column.

collimated light Light in which all rays travel in parallel paths.

collimation The process of making light rays travel parallel to one another.

collisionally activated dissociation Fragmentation of an ion in a mass spectrometer by high-energy collisions with gas molecules. In atmospheric pressure chemical ionization or electrospray interfaces, collisionally activated dissociation at the inlet to the mass filter can be promoted by varying the cone voltage. In tandem mass spectrometry, dissociation occurs in a collision cell between the two mass separators.

collision cell The middle stage of a tandem mass spectrometer in which the precursor ion selected by the first stage is fragmented by collisions with gas molecules.

colloid A dissolved particle with a diameter in the approximate range 1–500 nm. It is too large to be considered one molecule but too small to simply precipitate.

combination electrode Consists of a glass electrode with a concentric reference electrode built on the same body.

combustion analysis A technique in which a sample is heated in an atmosphere of O₂ to oxidize it to CO₂ and H₂O, which are collected and weighed or measured by gas chromatography. Modifications permit the simultaneous analysis of N, S, and halogens.

common ion effect Occurs when a salt is dissolved in a solution already containing one of the ions of the salt. The salt is less soluble than it would be in a solution without that surplus ion. An application of Le Châtelier's principle.

complex ion Historical name for any ion containing two or more ions or molecules that are each stable by themselves; e.g., CuCl₃⁻ contains Cu⁺ + 3Cl⁻.

complexometric titration One in which the reaction between analyte and titrant involves complex formation.

composite sample A representative sample prepared from a heterogeneous material. If the material consists of distinct regions, the composite is made of portions of each region, with relative amounts proportional to the size of each region.

compound electrode An ion-selective electrode consisting of a conventional electrode surrounded by a barrier that is selectively permeable to the analyte of interest. Alternatively, the barrier region might contain an enzyme that converts external analyte into a species to which the inner electrode is sensitive.

concentration An expression of the quantity per unit volume or unit mass of a substance. Common measures of concentration are molarity (mol/L) and molality (mol/kg of solvent).

conditional formation constant The equilibrium constant for formation of a complex under a particular, stated set of conditions, such as pH, ionic strength, and concentration of auxiliary complexing species. Also called *effective formation constant*.

confidence interval The range of values within which there is a specified probability that the “true” value will be found.

conjugate acid-base pair An acid and a base that differ only through the gain or loss of a single proton.

constant mass In gravimetric analysis, the product is heated and cooled to room temperature in a desiccator until successive weighings are “constant.” There is no standard definition of “constant mass”; but, for ordinary work, it is usually taken to be about ±0.3 mg. Constancy is usually limited by the irreproducible regain of moisture during cooling and weighing.

control chart A graph in which successive observations of a process are recorded to determine whether the process is within specified control limits.

coprecipitation Occurs when a substance whose solubility is not exceeded precipitates along with one whose solubility is exceeded.

correlation coefficient The square of the correlation coefficient, R², is a measure of goodness of fit of data points to a straight line. The closer R² is to 1, the better the fit.

coulomb, C The amount of charge per second that flows past any point in a circuit when the current is 1 ampere. There are approximately 96 485 coulombs in a mole of electrons.

coulometry A technique in which the quantity of analyte is determined by measuring the number of coulombs needed for complete electrolysis.

counter electrode The current-carrying partner of the working electrode. Same as *auxiliary electrode*.

counterion An ion with a charge opposite that of the ion of interest. **coupled equilibria** Reversible chemical reactions in which the product of one reaction is a reactant in another reaction.

cross-linking Covalent linkage between different strands of a polymer. **cumulative formation constant, β_n** The equilibrium constant for a reaction of the type M + nX ⇌ MX_n. Also called as *overall formation constant*.

current, I The amount of charge flowing through a circuit per unit time (C/s).

cuvet A cell with transparent walls used to hold samples for spectrophotometric measurements.

dalton, Da Unit of atomic mass defined as 1/12 of the mass of ¹²C. **Debye-Hückel equation** Gives the activity coefficient (γ) as a function of ionic strength (μ). The *extended Debye-Hückel equation*, applicable to ionic strengths up to about 0.1 M, is $\log \gamma = [-0.51z^2\sqrt{\mu}]/[1 + (\alpha\sqrt{\mu}/305)]$, where z is the ionic charge and α is the effective hydrated diameter in picometers.

decant To pour liquid off a solid or, perhaps, a denser liquid. The denser phase is left behind.

degrees of freedom In statistics, the number of observations minus the number of parameters estimated from the observations.

deionized water Water that has been passed through a cation exchanger (in the H⁺ form) and an anion exchanger (in the OH⁻ form) to remove ions from the solution.

density Mass per unit volume.

derivatization Chemical alteration to attach a group to a molecule so that it can be detected conveniently. Alternatively, treatment can alter volatility or solubility to allow easier separation.

desiccant A drying agent.

desiccator A sealed chamber in which samples can be dried in the presence of a desiccant or by vacuum pumping or both.

detection limit The smallest quantity of analyte that is “significantly different” from a blank. The detection limit is often taken as the mean signal for blanks plus three times the standard deviation of a low-concentration sample. Also called *lower limit of detection*.

determinate error See *systematic error*.

dialysis A technique in which solutions are placed on either side of a semipermeable membrane that allows small molecules, but not large molecules, to cross. Small molecules in the two solutions diffuse across and equilibrate between the two sides. Large molecules are retained on their original side.

diastereoisomers Dissymmetric molecules that are not mirror images.

diffraction The bending of light rays by a grating. This process occurs when electromagnetic radiation passes through or is reflected from slits with a spacing comparable to the wavelength. Interference of waves from adjacent slits produces a spectrum of radiation, with each wavelength emerging at a different angle.

diffuse part of the double layer Region of solution near a charged surface in which excess counterions are attracted to the charge. The thickness of this layer is 0.3–10 nm.

diffusion Net transport of a solute from a region of high concentration to a region of low concentration caused by the random movement of molecules in a liquid or gas (or, very slowly, in a solid).

diffusion current In polarography, the current observed when the rate of reaction is limited by the rate of diffusion of analyte to the electrode.

digestion (1) The process in which a precipitate is left (usually warm) in the presence of mother liquor to promote particle recrystallization and growth. Purer, more easily filterable crystals result. (2) Any

chemical treatment in which a substance is decomposed to transform the analyte into a form suitable for analysis.

dilution factor The factor (initial volume of reagent)/(total volume of solution) used to multiply the initial concentration of reagent to find the diluted concentration.

dimer A molecule made from two identical units.

diprotic acids and bases Compounds that can donate or accept two protons.

direct titration One in which the analyte is treated with titrant, and the volume of titrant required for complete reaction is measured.

displacement titration An EDTA titration procedure in which analyte is treated with excess MgEDTA^{2-} to displace Mg^{2+} : $\text{M}^{n+} + \text{MgEDTA}^{2-} \rightleftharpoons \text{MEDTA}^{n-4} + \text{Mg}^{2+}$. The liberated Mg^{2+} is then titrated with EDTA. This procedure is useful if there is no suitable indicator for direct titration of M^{n+} .

disproportionation A reaction in which an element in one oxidation state gives products containing that element in both higher and lower oxidation states; e.g., $2\text{Cu}^+ \rightleftharpoons \text{Cu}^{2+} + \text{Cu}(s)$.

doping Deliberate addition of an impurity to a substance for the purpose of modifying a property of the substance.

double-junction electrode An electrode with inner and outer compartments designed to minimize contact between analyte solution and the contents of the inner electrode. The outer compartment serves as a salt bridge with ions that are chemically compatible with the analyte.

dynamic flash combustion Procedure for elemental analysis in which a sample in a tin capsule is dropped into a preheated furnace for rapid oxidation of the sample.

dynamic range The range of analyte concentration over which a change in concentration gives a change in detector response.

dynode A metal surface that easily emits several electrons each time it is struck by one accelerated electron in a photomultiplier tube or an electron multiplier.

E° The standard reduction potential.

E°' The effective standard reduction potential at pH 7 (or at some other specified conditions).

EDTA (ethylenediaminetetraacetic acid) ($\text{HO}_2\text{CCH}_2)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$, the most widely used reagent for complexometric titrations. It forms 1:1 complexes with virtually all cations with a charge of 2 or more.

effective formation constant See **conditional formation constant**.

electric charge, q Quantity of electricity, measured in coulombs.

electric double layer Region comprising the charged surface of an electrode or a particle plus the oppositely charged region of solution adjacent to the surface. Also called *double layer*.

electric potential The electric potential (in volts) at a point is the energy (in joules) needed to bring one coulomb of positive charge from infinity to that point. The *potential difference* between two points is the energy needed to transport one coulomb of positive charge from the negative point to the positive point.

electroactive species Any species that can be oxidized or reduced at an electrode.

electrochemical detector Liquid chromatography detector that measures current when an electroactive solute emerges from the column and passes over a working electrode held at a fixed potential with respect to a reference electrode. Also called *amperometric detector*.

electrochemistry Use of electrical measurements on a chemical system for analytical purposes. Also refers to use of electricity to drive a chemical reaction or use of a chemical reaction to produce electricity.

electrode A device through which electrons flow into or out of chemical species involved in a redox reaction.

electrogravimetric analysis A technique in which the mass of an electrolytic deposit is used to quantify the analyte.

electrolysis A chemical reaction driven by passage of electric current.

electrolyte A substance that produces ions when dissolved.

electromagnetic spectrum The whole range of electromagnetic radiation, including visible light, radio waves, X-rays, etc.

electron capture detector Gas chromatography detector that is particularly sensitive to compounds with halogen atoms, nitro groups, and other groups with high electron affinity. Makeup gas (N_2 or 5% CH_4 in Ar) is ionized by β -rays from ^{63}Ni to liberate electrons that produce a small, steady current. High-electron-affinity analytes capture some of the electrons and reduce the detector current.

electronic balance A weighing device that uses an electromagnetic servomotor to balance the load on the pan. The mass of the load is proportional to the current needed to balance it.

electronic transition One in which an electron goes from one energy level to another.

electron ionization Method used to create ions from gaseous molecules in the inlet of a mass spectrometer by bombardment of the gas with high-energy electrons.

electron multiplier An ion detector that works like a photomultiplier tube. Cations striking a cathode liberate electrons. A series of *dynodes* multiplies the number of electrons by $\sim 10^5$ before they reach the anode.

electroosmosis Bulk flow of fluid in a capillary tube induced by an electric field. Mobile ions in the diffuse part of the double layer at the wall of the capillary serve as the “pump.” Also called *electroendosmosis*.

electropherogram A graph of detector response versus time for electrophoresis.

electrophoresis Migration of ions in solution in an electric field. Cations move toward the cathode and anions move toward the anode. Ions can be separated from one another by their differing rates of migration in a strong electric field.

electrospray A method for interfacing liquid chromatography to mass spectrometry. A high potential applied to the liquid at the column exit creates charged droplets in a fine aerosol. Gaseous ions are derived from ions that were already in the mobile phase on the column. It is common to observe protonated bases (BH^+), ionized acids (A^-), and complexes formed between analyte, M (which could be neutral or charged), and stable ions such as NH_4^+ , Na^+ , HCO_3^- , or CH_3CO_2^- that were already in solution.

eluate What comes out of a chromatography column. Also called *effluent*.

eluent The solvent applied to the beginning of a chromatography column.

eluent strength, ϵ° A measure of the ability of a solvent to elute solutes from a chromatography column. Eluent strength is a measure of the adsorption energy of a solvent on the stationary phase in chromatography. Also called *solvent strength*.

eluotropic series Ranks solvents according to their ability to displace solutes from the stationary phase in adsorption chromatography.

elution The process of passing a liquid or a gas through a chromatography column.

emission spectrum A graph of luminescence intensity versus luminescence wavelength (or frequency or wavenumber), obtained with a fixed excitation wavelength.

emulsion A stable dispersion of immiscible liquids, which might be made by vigorous shaking. Milk is an emulsion of cream in an aqueous phase. Emulsions usually require an emulsifying agent (a surfactant) for stability. The emulsifying agent stabilizes the interface between the two phases by its affinity for both phases.

enantiomers Mirror image isomers that cannot be superimposed on each other. Also called *optical isomers*.

end point The point in a titration at which there is a sudden change in a physical property, such as indicator color, pH, conductivity, or absorbance. Used as a measure of the equivalence point.

enthalpy change, ΔH The heat absorbed or released when a reaction occurs at constant pressure.

entropy A measure of the “disorder” of a substance.

enzyme A protein that catalyzes a chemical reaction.

enzyme-linked immunosorbent assay (ELISA) A biochemical assay incorporating an enzyme attached to an antibody. After antibody binds to the intended analyte and excess antibody is washed away, a reagent is added that is converted to a detectable product (such as a colored or fluorescent substance) by the enzyme. The quantity of detectable product is proportional to the amount of analyte.

equilibrium The state in which the forward and reverse rates of all reactions are equal, so that the concentrations of all species remain constant.

equilibrium constant, K For the reaction $aA + bB \rightleftharpoons cC + dD$, $K = \mathcal{A}_C^c \mathcal{A}_D^d / \mathcal{A}_A^a \mathcal{A}_B^b$, where \mathcal{A}_i is the activity of the i th species. If we ignore activity coefficients, which we usually do in this book, the equilibrium constant is written in terms of concentrations: $K = [C]^c [D]^d / [A]^a [B]^b$.

equivalence point The point in a titration at which the quantity of titrant is exactly sufficient for stoichiometric reaction with the analyte.

equivalent For a redox reaction, the amount of reagent that can donate or accept one mole of electrons. For an acid-base reaction, the amount of reagent that can donate or accept one mole of protons.

equivalent weight The mass of substance containing one equivalent.

error bar Graphical depiction of the uncertainty in a measurement.

evaporative light-scattering detector A liquid chromatography detector that makes a fine mist of eluate and evaporates solvent from the mist in a heated zone. The remaining particles of liquid or solid solute flow past a laser beam and are detected by their ability to scatter the light.

excited state Any state of an atom or a molecule having more than the minimum possible energy.

extended Debye-Hückel equation See **Debye-Hückel equation**.

extraction The process in which a solute is transferred from one phase to another. Analyte is sometimes removed from a sample by extraction into a solvent that dissolves the analyte.

extrapolation Estimation of a value that lies beyond the range of measured data.

F test For two variances, s_1^2 and s_2^2 (with s_1 chosen to be the larger of the two), the statistic F is defined as $F = s_1^2/s_2^2$. To decide whether s_1 is significantly greater than s_2 , we compare F with the critical values in a table based on a certain confidence level. If the calculated value of F is greater than the value in the table, the difference is significant.

Fajans titration A precipitation titration in which the end point is signaled by adsorption of a colored indicator on the precipitate.

false negative A conclusion that the concentration of analyte is below a certain limit when, in fact, the concentration is above the limit.

false positive A conclusion that the concentration of analyte exceeds a certain limit when, in fact, the concentration is below the limit.

faradaic current That component of current in an electrochemical cell due to oxidation and reduction reactions.

Faraday constant, F The number of coulombs in a mole of elementary charges, approximately 9.648×10^4 C/mol of charge.

field blank A blank sample exposed to the environment at the sample collection site and transported in the same manner as other samples between the lab and the field.

filtrate Portion of a sample (usually the liquid) that passes through a filter.

first derivative The slope of a curve ($\Delta y / \Delta x$) measured at each point along the curve. The first derivative reaches a maximum value at the steepest point on the curve.

flame ionization detector Gas chromatography detector in which solute is burned in a H_2 -air flame to produce CHO^+ ions. The current carried through the flame by these ions is proportional to the concentration of susceptible species in the eluate.

flame photometer A device that uses flame atomic emission and a filter photometer to quantify Li, Na, K, and Ca in liquid samples.

flame photometric detector Gas chromatography detector that measures optical emission from S, P, Pb, Sn, or other elements in a H_2 - O_2 flame.

flocculate To cause particles of a dispersion, such as a colloid, to aggregate into larger particles.

fluorescence The process in which a molecule emits a photon 10^{-8} to 10^{-4} s after absorbing a photon. It results from a transition between states of the same spin multiplicity (e.g., singlet \rightarrow singlet).

fluorescence detector Liquid chromatography detector that uses a strong light or laser to irradiate eluate emerging from a column and detects radiant emission from fluorescent solutes.

flux In sample preparation, flux is a solid such as NaOH that is melted to dissolve a sample.

formal concentration, F The molarity of a substance if it did not change its chemical form on being dissolved. It represents the total number of moles of substance dissolved in a liter of solution, regardless of any reactions that take place when the solute is dissolved. Also called *analytical concentration* or *formality*.

formality, F See **formal concentration**.

formal potential The potential of a half-reaction (relative to a standard hydrogen electrode) when the formal concentrations of reactants and products are unity. Any other conditions (such as pH, ionic strength, and concentrations of ligands) also must be specified.

formation constant, K_f The equilibrium constant for the reaction of a metal with its ligands to form a metal-ligand complex. Same as *stability constant*.

formula mass, FM The mass containing one mole of the indicated chemical formula of a substance. For example, the formula mass of $CuSO_4 \cdot 5H_2O$ is the sum of the masses of copper, sulfate, and five water molecules.

fortification See **spike**.

fraction of association, α For the reaction of a base (B) with H_2O , the fraction of base in the form BH^+ . $\alpha = [BH^+]/([B] + [BH^+])$.

fraction of dissociation, α For the dissociation of an acid (HA), the fraction of acid in the form A^- . $\alpha = [A^-]/([HA] + [A^-])$.

frequency The number of cycles per unit time for a repetitive event. For a light wave, it is the number of complete oscillations of the electromagnetic field per second.

fugacity The activity of a gas. The activity coefficient for the gas is called the fugacity coefficient.

fused-core particle See **superficially porous particle**.

fusion The process in which an otherwise insoluble substance is dissolved in a molten salt such as Na_2CO_3 , Na_2O_2 , or KOH . Once the substance has dissolved, the melt is cooled, dissolved in aqueous solution, and analyzed.

galvanic cell One that produces electricity by means of a spontaneous chemical reaction. Also called a *voltaic cell*.

gas chromatography A form of chromatography in which the mobile phase is a gas.

gathering A process in which a trace constituent of a solution is intentionally coprecipitated with a major constituent.

Gaussian distribution Theoretical bell-shaped distribution of measurements when all error is random. The center of the curve is the mean (μ) and the width is characterized by the standard deviation (σ). A *normalized Gaussian distribution*, also called the *normal error curve*, has an area of unity and is given by

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/2\sigma^2}$$

gel Chromatographic stationary phase particles, such as Sephadex or polyacrylamide, which are soft and pliable.

gel filtration chromatography See **molecular exclusion chromatography**.

gel permeation chromatography See **molecular exclusion chromatography**.

glass electrode One that has a thin glass membrane across which a pH-dependent voltage develops. The voltage (and hence pH) is measured by a pair of reference electrodes on either side of the membrane.

gradient elution Chromatography in which the composition of the mobile phase is progressively changed to increase the eluent strength of the solvent.

graduated cylinder A tube with volume calibrations along its length.

gram-atom The amount of an element containing Avogadro's number of atoms; it is the same as a mole of the element.

graphite furnace A graphite tube that can be heated electrically to about 2 500 K to atomize a sample for atomic spectroscopy.

grating Either a reflective or a transmitting surface etched with closely spaced lines; used to disperse light into its component wavelengths.

gravimetric analysis Any analytical method that relies on measuring the mass of a substance (such as a precipitate) to complete the analysis.

gravimetric titration A titration in which the mass of titrant is measured, instead of the volume. Titrant concentration is conveniently expressed as mol reagent/kg titrant solution. Gravimetric titrations can be more accurate and precise than volumetric titrations.

green chemistry Principles intended to change our behavior in a manner that will help sustain the habitability of the Earth. Green chemistry seeks to design chemical products and processes to reduce the use of resources and energy and the generation of hazardous waste.

greenhouse gas A component of Earth's atmosphere that absorbs infrared radiation from the ground and reradiates some of it back to the ground, thereby keeping Earth warmer than it would be in the absence of the greenhouse gas.

gross sample See **bulk sample**.

ground state The state of an atom or a molecule with the minimum possible energy.

Grubbs test Statistical test used to decide whether to discard a datum that appears discrepant.

guard column In HPLC, a short, disposable column packed with the same material as the main column and placed between the injec-

tor and the main column. The guard column removes impurities that might irreversibly bind to the main column and degrade it. Also called *precolumn*. In gas chromatography, the guard column is a length of empty tubing with chemically deactivated walls to minimize retention. The guard column collects nonvolatile components of the sample that would remain on and degrade the analytical column.

half-cell Part of an electrochemical cell in which half of an electrochemical reaction (either the oxidation or the reduction reaction) occurs.

half-reaction Any redox reaction can be conceptually broken into two half-reactions, one involving only oxidation and one involving only reduction.

half-wave potential Potential at the midpoint of the rise in the current of a polarographic wave.

half-width Width of a signal at half of its maximum height.

hardness Total concentration of alkaline earth ions in natural water expressed as mg CaCO_3 per liter of water as if all of the alkaline earths present were CaCO_3 .

Henderson-Hasselbalch equation A logarithmic rearranged form of the acid dissociation equilibrium equation:

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

Henry's law The partial pressure of a gas in equilibrium with gas dissolved in a solution is proportional to the concentration of dissolved gas: $P = k[\text{dissolved gas}]$. The constant k is called the *Henry's law constant*. It is a function of the gas, the liquid, and the temperature.

hertz, Hz SI unit of frequency, s^{-1} ; also called *reciprocal seconds*.

heterogeneous Having a composition that is not uniform throughout.

HETP (height equivalent to a theoretical plate) The length of a chromatography column divided by the number of theoretical plates in the column.

hexadentate ligand One that binds to a metal atom through six ligand atoms.

high-performance liquid chromatography (HPLC) A chromatographic technique using small stationary phase particles and high pressure to force solvent through the column.

HILIC See **hydrophilic interaction chromatography**.

hollow-cathode lamp One that emits sharp atomic lines characteristic of the cathode material.

homogeneous Having the same composition everywhere.

homogeneous precipitation A technique in which a precipitating agent is generated slowly by a reaction in homogeneous solution, effecting a slow crystallization instead of a rapid precipitation of product.

HPLC See **high-performance liquid chromatography**.

hydrated radius The effective size of an ion or a molecule plus its associated water molecules in solution.

hydrolysis "Reaction with water." The reaction $\text{B} + \text{H}_2\text{O} \rightleftharpoons \text{BH}^+ + \text{OH}^-$ is called hydrolysis of a base.

hydronium ion, H_3O^+ What we really mean when we write $\text{H}^+(\text{aq})$.

hydrophilic interaction chromatography (HILIC) Liquid chromatographic separation of polar solutes with a hydrophilic stationary phase and mixed organic-aqueous eluent. Eluent strength increases with *decreasing* organic solvent.

hydrophilic substance One that is soluble in water or attracts water to its surface.

hydrophobic substance One that is insoluble in water or repels water from its surface.

hygroscopic substance One that readily picks up water from the atmosphere.

hypothesis test Statistical test to evaluate the probability that a statement is false.

ignition Heating a gravimetric precipitate to high temperature to convert it into a known, constant composition that can be weighed.

immiscible liquids Two liquids that do not form a single phase when mixed together.

immunoassay An analytical measurement using antibodies.

inclusion An impurity that occupies lattice sites in a crystal.

indeterminate error See **random error**.

indicator A compound having a physical property (usually color) that changes abruptly near the equivalence point of a chemical reaction.

indicator electrode One that develops a potential whose magnitude depends on the activity of one or more species in contact with the electrode.

indicator error The difference between the indicator end point of a titration and the true equivalence point.

indirect detection Chromatographic detection based on the *absence* of signal from a background species. For example, in ion chromatography, a light-absorbing ionic species can be added to the eluent. Non-absorbing analyte replaces an equivalent amount of light-absorbing eluent when analyte emerges from the column, thereby decreasing the absorbance of eluate.

indirect titration One that is used when the analyte cannot be directly titrated. For example, analyte A may be precipitated with excess reagent R. The product is filtered, and the excess R washed away. Then AR is dissolved in a new solution, and R can be titrated.

inductively coupled plasma A high-temperature plasma that derives its energy from an oscillating radio-frequency field. It is used to atomize a sample for atomic emission spectroscopy.

inflection point One at which the derivative of the slope is 0: $d^2y/dx^2 = 0$. That is, the slope reaches a maximum or minimum value.

inorganic carbon In a natural water or industrial effluent sample, the quantity of dissolved carbonate and bicarbonate.

intensity Power per unit area of a beam of electromagnetic radiation (W/m^2). Also called *radiant power* or *irradiance*.

intercept For a straight line whose equation is $y = mx + b$, b is the intercept. It is the value of y when $x = 0$.

interference A phenomenon in which the presence of one substance changes the signal in the analysis of another substance.

internal conversion A radiationless electronic transition between states of the same energy and electron-spin multiplicity.

internal standard A known quantity of a compound other than analyte added to a solution containing an unknown quantity of analyte. The concentration of analyte is then measured relative to that of the internal standard.

interpolation The estimation of the value of a quantity that lies between two known values.

intersystem crossing A radiationless electronic transition between states of different electron-spin multiplicity.

iodometry The use of triiodide (or iodine) as a titrant.

iodometry A technique in which an oxidant is treated with I^- to produce I_3^- , which is then titrated (usually with thiosulfate).

ion chromatography HPLC ion-exchange separation of ions.

ion-exchange chromatography A technique in which solute ions are retained by oppositely charged sites in the stationary phase.

ionic atmosphere The region of solution around an ion or a charged particle. It contains an excess of oppositely charged ions.

ionic liquid A salt that melts near or below room temperature and has a large temperature range over which it remains liquid.

ionic radius The effective size of an ion in a crystal.

ionic strength, μ A measure of the total concentration of ions in solution, given by $\mu = \frac{1}{2} \sum_i c_i z_i^2$, where c_i is the concentration of the i th ion in solution and z_i is the charge on that ion. The sum extends over all ions in solution, including the ions whose activity coefficients are being calculated.

ionization interference In atomic spectroscopy, loss of signal as a result of ionization of analyte atoms.

ionization suppressor An element used in atomic spectroscopy to decrease the extent of ionization of the analyte.

ionophore A molecule with a hydrophobic outside and a polar inside that can engulf an ion and carry the ion through a hydrophobic phase (such as a cell membrane).

ion pair A closely associated anion and cation, held together by electrostatic attraction. In solvents less polar than water, ions are usually found as ion pairs.

ion-selective electrode One whose potential is selectively dependent on the concentration of one particular ion in solution.

irradiance Power per unit area of a beam of electromagnetic radiation (W/m^2). Also called *radiant power* or *intensity*.

isobaric interference In mass spectrometry, overlap of two peaks with nearly the same mass. For example, $^{41}\text{K}^+$ and $^{40}\text{ArH}^+$ differ by 0.01 atomic mass unit and appear as a single peak unless the spectrometer resolution is great enough to separate them.

isocratic elution Chromatography using a single solvent (or single solvent mixture) for the mobile phase.

isoelectric focusing A technique in which a sample containing polyprotic molecules is subjected to a strong electric field in a medium with a pH gradient. Each species migrates until it reaches the region of its isoelectric pH. In that region, the molecule has no net charge, ceases to migrate, and remains focused in a narrow band.

isoelectric point That pH at which the average charge of a polyprotic species is 0. Same as *isoelectric pH*.

isosbestic point A wavelength at which the absorbance spectra of two species cross each other. The appearance of isosbestic points in a solution in which a chemical reaction is occurring is evidence that there are only two components present, with a constant total concentration.

isotope ratio mass spectrometry A mass spectrometric technique designed to provide accurate measurements of the ratio of different ions of a selected element. The instrument has one detector dedicated to each isotope.

joule, J SI unit of energy. One joule is expended when a force of 1 N acts over a distance of 1 m. This energy is equivalent to that required to raise 102 g (about $\frac{1}{4}$ pound) by 1 m at sea level.

junction potential An electric potential that exists at the junction between two different electrolyte solutions or substances. It arises in solutions as a result of unequal rates of diffusion of different ions.

kelvin, K SI unit of temperature defined such that the temperature of water at its triple point (where water, ice, and water vapor are at equilibrium) is 273.16 K and the absolute zero of temperature is 0 K. Also called *absolute temperature*.

kilogram, kg SI unit of mass equal to the mass of a particular Pt-Ir cylinder kept at the International Bureau of Weights and Measures, Sèvres, France.

Kjeldahl nitrogen analysis Procedure for the analysis of nitrogen in organic compounds. The compound is digested with boiling H₂SO₄ to convert nitrogen into NH₄⁺, which is treated with base and distilled as NH₃ into a standard acid solution. The moles of acid consumed equal the moles of NH₃ liberated from the compound.

lab-on-a-chip See **microfluidic chip**.

laboratory sample Portion of bulk sample taken to the lab for analysis. Must have the same composition as the bulk sample.

law of mass action States that for the chemical reaction $aA + bB \rightleftharpoons cC + dD$, the condition at equilibrium is $K = \mathcal{A}_C^c \mathcal{A}_D^d / \mathcal{A}_A^a \mathcal{A}_B^b$, where \mathcal{A}_i is the activity of the i th species. The law is usually used in approximate form, in which activities are replaced by concentrations.

least squares Process of fitting a mathematical function to a set of measured points by minimizing the sum of the squares of the distances from the points to the curve.

Le Châtelier's principle If a system at equilibrium is disturbed, the direction in which it proceeds back to equilibrium is such that the disturbance is partly offset.

Lewis acid One that can form a chemical bond by sharing a pair of electrons donated by another species.

Lewis base One that can form a chemical bond by sharing a pair of its electrons with another species.

lifetime In spectroscopy, the lifetime for an excited state is the time required for the population to decrease to 1/e times its initial value, where e is the base of the natural logarithm.

ligand An atom or a group attached to a central atom in a molecule. The term is often used to mean any group attached to anything else of interest.

linear flow rate In chromatography, the distance per unit time traveled by the mobile phase.

linear interpolation A form of interpolation in which it is assumed that the variation in some quantity is linear. For example, to find the value of b when $a = 32.4$ in the following table,

<i>a:</i>	32	32.4	33
<i>b:</i>	12.85	<i>x</i>	17.96

you can set up the proportion

$$\frac{32.4 - 32}{33 - 32} = \frac{x - 12.85}{17.96 - 12.85}$$

which gives $x = 14.89$.

linearity A measure of how well data in a graph follow a straight line, showing that response is proportional to the quantity of analyte.

linear range The concentration range over which the change in detector response is proportional to the change in analyte concentration.

linear response The case in which the analytical signal is directly proportional to the concentration of analyte.

liquid-based ion-selective electrode One that has a hydrophobic membrane separating an inner reference electrode from the analyte solution.

The membrane contains an ion exchanger dissolved in nonpolar solvent. The ion-exchange equilibrium of analyte between the liquid ion exchanger and the aqueous solution gives rise to the electrode potential.

liquid chromatography A form of chromatography in which the mobile phase is a liquid.

liter, L Common unit of volume equal to exactly 1 000 cm³.

logarithm The base 10 logarithm of n is a if 10 ^{a} = n (which means log $n = a$). The natural logarithm of n is a if e ^{a} = n (which means ln $n = a$). The number e (= 2.718 28 . . .) is called the base of the natural logarithm.

longitudinal diffusion Diffusion of solute molecules parallel to the direction of travel through a chromatography column.

lot Entire material that is to be analyzed. Examples are a bottle of reagent, a lake, or a truckload of gravel.

lower limit of detection See **detection limit**.

lower limit of quantitation Smallest amount of analyte that can be measured with reasonable accuracy. Usually taken as 10 times the standard deviation of a low-concentration sample.

luminescence Any emission of light by a molecule.

L'vov platform Platform on which sample is placed in a graphite-tube furnace for atomic spectroscopy to prevent sample vaporization before the walls reach constant temperature.

makeup gas Gas added to the exit stream from a gas chromatography column for the purpose of changing flow rate or gas composition to optimize detection of analyte.

mantissa The part of a logarithm to the right of the decimal point.

masking The process of adding a chemical substance (*a masking agent*) to a sample to prevent one or more components from interfering in a chemical analysis.

masking agent A reagent that selectively reacts with one (or more) component(s) of a solution to prevent the component(s) from interfering in a chemical analysis.

mass balance The sum of the moles of any element in all of its forms in a solution must equal the moles of that element delivered to the solution. Also called *material balance*.

mass spectrometer An instrument that converts gaseous molecules into ions, accelerates them in an electric field, separates them according to their mass-to-charge ratio, and detects the amount of each species.

mass spectrometry A technique in which gaseous molecules are ionized, accelerated by an electric field, and then separated according to their mass-to-charge ratio.

mass spectrum In mass spectrometry, a graph showing the relative abundance of each ion as a function of its mass-to-charge ratio.

mass titration One in which the mass of titrant, instead of the volume, is measured.

mass-to-charge ratio, m/z The mass of an ion in daltons divided by the charge of the ion measured in multiples of the elementary charge. For ²³Na⁺, for example, $m/z = 23/1 = 23$.

matrix The medium containing analyte (that is, everything in a sample other than the analyte). For many analyses, it is important that standards be prepared in the same matrix as the unknown.

matrix effect A change in analytical signal caused by anything in the sample other than analyte.

matrix modifier Substance added to sample for atomic spectroscopy to make the matrix more volatile or the analyte less volatile so that the matrix evaporates before analyte does.

mean The sum of a set of results divided by the number of values in the set. Also called *average*.

mechanical balance A balance having a beam that pivots on a fulcrum. Standard masses are used to measure the mass of an unknown.

median For a set of data, that value above and below which there are equal numbers of data.

mediator In electrolysis, a molecule that carries electrons between the electrode and the intended analyte. Used when the analyte cannot react directly at the electrode or when analyte concentration is so low that other reagents react instead. Mediator is recycled indefinitely by oxidation or reduction at the counter electrode.

meniscus The curved surface of a liquid.

metal ion buffer Consists of a metal-ligand complex plus excess free ligand. The two serve to fix the concentration of free metal ion through the reaction $M + nL \rightleftharpoons ML_n$.

metal ion indicator A compound whose color changes when it binds to a metal ion.

meter, m SI unit of length defined as the distance that light travels in a vacuum during $\frac{1}{299792458}$ of a second.

method blank A sample without deliberately added analyte. The method blank is taken through all steps of a chemical analysis, including sample preparation. See also **reagent blank**.

method of least squares Process of fitting a mathematical function to a set of measured points by minimizing the sum of the squares of the distances from the points to the curve.

method validation The process of proving that an analytical method is acceptable for its intended purpose.

micellar electrokinetic capillary chromatography A form of capillary electrophoresis in which a micelle-forming surfactant is present. Migration times of solutes depend on the fraction of time spent in the micelles.

micelle An aggregate of molecules with ionic headgroups and long, nonpolar tails. The inside of the micelle resembles hydrocarbon solvent, whereas the outside interacts strongly with aqueous solution.

microfluidic chip Also called “lab-on-a-chip,” a glass or plastic or other “chip” with features such as channels, valves, pumps, and reaction chambers designed to carry out chemical separations, analysis, or manipulations.

microporous particles Chromatographic stationary phase consisting of highly porous particles 1.5–10 mm in diameter, with high efficiency and high capacity for solute.

migration Electrostatically induced motion of ions in a solution under the influence of an electric field.

migration time Time required for a solute to reach the detector in capillary electrophoresis.

miscible liquids Two liquids that form a single phase when mixed in any ratio.

mobile phase In chromatography, the phase that travels through the column.

mobility The terminal velocity that an ion reaches in a field of 1 V/m. Velocity = mobility \times field.

molality, m A measure of concentration equal to the number of moles of solute per kilogram of solvent.

molar absorptivity, ϵ Constant of proportionality in Beer's law: $A = \epsilon bc$, where A is absorbance, b is pathlength, and c is the molarity of the absorbing species. Molar absorptivity tells how much light is absorbed at a particular wavelength by a particular substance. Also called *extinction coefficient*.

molarity, M A measure of concentration equal to the number of moles of solute per liter of solution.

mole, mol SI unit for the amount of substance that contains as many molecules as there are atoms in 12 g of ^{12}C . There are approximately 6.022×10^{23} molecules per mole.

molecular exclusion chromatography A technique in which the stationary phase has a porous structure into which small molecules can enter but large molecules cannot. Molecules are separated by size, with larger molecules moving faster than smaller ones. Also called *size exclusion, gel filtration, or gel permeation chromatography*.

molecular ion A gas-phase ion that has not lost or gained any atoms during ionization.

molecularly imprinted polymer A polymer synthesized in the presence of a template molecule. After the template is removed, the polymer has a void with the right shape to hold the template or closely related molecules. Polymer functional groups are positioned correctly to bind to template functional groups.

molecular mass The number of grams of a substance that contains Avogadro's number of molecules.

molecular orbital Describes the distribution of an electron within a molecule.

molecular sieve A crystalline solid particle with pores the size of small molecules. Zeolites (sodium aluminosilicates) are a common type.

mole fraction The number of moles of a substance in a mixture divided by the total number of moles of all components present.

monochromatic light Light with a very narrow range of wavelengths (“one color”).

monochromator A device (usually a grating or prism) that disperses light into its component wavelengths and selects a narrow band of wavelengths to pass through the exit slit.

monoclonal antibody Identical antibody molecules produced by a single type of cell.

monodentate ligand One that binds to a metal ion through only one atom.

monoprotic acids and bases Compounds that can donate or accept one proton.

mortar and pestle A mortar is a hard ceramic or steel vessel in which a solid sample is ground with a hard tool called a pestle.

mother liquor The solution from which a substance has crystallized or precipitated.

multidentate ligand One that binds to a metal ion through more than one atom.

m/z See **mass-to-charge ratio**.

natural logarithm The natural logarithm (\ln) of a is b if $e^b = a$. See also **logarithm**.

nebulization The process of breaking a liquid into a mist of fine droplets.

nebulizer In atomic spectroscopy, this device breaks the liquid sample into a mist of fine droplets.

Nernst equation Relates the voltage of a cell (E) to the activities of reactants and products:

$$E = E^\circ - \frac{RT}{nF} \ln Q = E^\circ - \frac{0.05916 \text{ V}}{n} \log Q \text{ (at } 298.15 \text{ K)}$$

where R is the gas constant, T is temperature in kelvins, F is the Faraday constant, Q is the reaction quotient, and n is the number of electrons transferred in the balanced reaction. E° is the cell voltage when all activities are unity.

neutralization The process in which a stoichiometric equivalent of acid is added to a base (or *vice versa*).

newton, N SI unit of force. One newton will accelerate a mass of 1 kg by 1 m/s².

nitrogen-phosphorus detector See **alkali flame detector**.

nitrogen rule A compound with an odd number of nitrogen atoms—in addition to C, H, halogens, O, S, Si, and P—will have an odd molecular mass. A compound with an even number of nitrogen atoms (0, 2, 4, etc.) will have an even molecular mass.

noise Signals originating from sources other than those intended to be measured.

nominal mass The *integer* mass of the species with the most abundant isotope of each of the constituent atoms.

nonpolar compound A compound, such as a hydrocarbon, with little charge separation within the molecule and no net ionic charge. Nonpolar compounds interact with other substances by weak van der Waals forces and are generally not soluble in water.

normal error curve A Gaussian distribution whose area is unity.

normal hydrogen electrode (N.H.E.) See **standard hydrogen electrode**.

normality n times the molarity of a redox reagent, where n is the number of electrons donated or accepted by that species in a particular chemical reaction. For acids and bases, it is also n times the molarity, but n is the number of protons donated or accepted by the species.

normalized peak area In capillary electrophoresis, the measured peak area divided by the migration time. Used in electrophoresis because different solutes migrate at different rates and pass through the detector at different rates. In chromatography, all solutes pass through the detector at the same rate, so normalization is not necessary.

normal-phase chromatography A chromatographic separation utilizing a polar stationary phase and a less polar mobile phase.

nucleation The process whereby molecules in solution come together randomly to form small crystalline aggregates that can grow into larger crystals.

null hypothesis In statistics, the supposition that two quantities do not differ from each other or that two methods do not give different results.

occlusion An impurity that becomes trapped (sometimes with solvent) in a pocket within a growing crystal.

ohm, Ω SI unit of electric resistance.

on-column injection Used in gas chromatography to place a thermally unstable sample directly on the column without excessive heating in an injection port. Solute is condensed at the start of the column by low temperature, and then the temperature is raised to initiate chromatography.

open tubular column In chromatography, a hollow capillary column whose walls are coated with stationary phase.

optical isomers Molecules that are the mirror image of each other and cannot be superimposed on each other. Same as *enantiomers*.

optically active molecule See **chiral molecule**.

order of magnitude A power of 10.

ordinate The vertical (y) axis of a graph.

outlier A datum that is far from the other points in a data set.

overall formation constant, β_n See **cumulative formation constant**.

oxidant See **oxidizing agent**.

oxidation A loss of electrons or a raising of the oxidation state.

oxidation number See **oxidation state**.

oxidation state A bookkeeping device used to tell how many electrons have been gained or lost by a neutral atom when it forms a compound. Also called *oxidation number*.

oxidizing agent A substance that takes electrons in a chemical reaction. Also called *oxidant*.

p function The negative logarithm (base 10) of a quantity: $pX = -\log X$.
packed column A chromatography column filled with stationary phase particles.

parallax error The apparent displacement of an object when the observer changes position. Occurs when the scale of an instrument is viewed from a position that is not perpendicular to the scale. The apparent reading is not correct.

particle growth The stage of crystal growth in which solute crystallizes onto the surface of preexisting crystals.

partition chromatography A technique in which separation is achieved by equilibration of solute between two phases.

parts per billion (ppb) An expression of concentration denoting nanograms (10^{-9} g) of solute per gram of solution.

parts per million (ppm) An expression of concentration denoting micrograms (10^{-6} g) of solute per gram of solution.

pascal, Pa SI unit of pressure equal to 1 N/m^2 . There are 10^5 Pa in 1 bar and 101 325 Pa in 1 atm.

peptization Occurs when washing some ionic precipitates with distilled water causes the ions that neutralize the charges of individual particles to be washed away. The particles then repel one another, disintegrate, and pass through the filter with the wash liquid.

performance test sample In a series of analytical measurements, a performance test sample is inserted to see whether the procedure gives correct results when the analyst does not know the right answer. The performance test sample is formulated by someone other than the analyst to contain a known concentration of analyte. Also called a *quality control sample* or *blind sample*.

permanent hardness Component of water hardness not due to dissolved alkaline earth bicarbonates. This hardness remains in the water after boiling.

pH Defined as $\text{pH} = -\log A_{\text{H}^+}$ where A_{H^+} is the activity of H^+ . In most approximate applications, the pH is taken as $-\log[\text{H}^+]$.

phosphorescence Emission of light during a transition between states of different spin multiplicity (e.g., triplet \rightarrow singlet). Phosphorescence is slower than fluorescence, with emission occurring $\sim 10^{-4}$ to 10^2 s after absorption of a photon.

photochemistry Chemical reaction initiated by absorption of a photon.

photodiode array An array of semiconductor diodes used to detect light. The array is normally used to detect light that has been spread into its component wavelengths. One small band of wavelengths falls on each detector.

photomultiplier tube One in which the cathode emits electrons when struck by light. The electrons then strike a series of dynodes (plates that are positive with respect to the cathode), and more electrons are released each time a dynode is struck. As a result, more than 10^6 electrons may reach the anode for every photon striking the cathode.

photon A “particle” of light with energy $h\nu$, where h is Planck’s constant and ν is the frequency of the light.

pipet A glass tube calibrated to deliver a fixed or variable volume of liquid.

pK The negative logarithm (base 10) of an equilibrium constant: $pK = -\log K$.

plane-polarized light Light whose electric field oscillates in one plane.

plasma A gas that is hot enough to contain free ions and electrons, as well as neutral molecules.

plate height, H The length of a chromatography column divided by the number of theoretical plates in the column.

polar compound A compound, such as an alcohol, that has positive and negative regions that attract neighboring molecules by electrostatic forces. Polar compounds tend to be soluble in water and insoluble in nonpolar solvents such as hydrocarbons.

polarogram A graph showing the relation between current and potential during a polarographic experiment.

polarograph An instrument used to obtain and record a polarogram.

polarographic wave The flattened S-shaped increase in current during a redox reaction in polarography.

polarography A voltammetry experiment using a dropping-mercury electrode.

- polychromatic light** Light of many wavelengths (“many colors”).
- polychromator** A device that spreads light into its component wavelengths and directs each small band of wavelengths to a different region where it is detected by a photodiode array.
- polyprotic acids and bases** Compounds that can donate or accept more than one proton.
- population mean, μ** Mean value for an infinite population of data. Same as *true mean*.
- population standard deviation, σ** Standard deviation for an infinite population of data. Same as *true standard deviation*.
- postprecipitation** The adsorption of otherwise soluble impurities on the surface of a precipitate after the precipitation is over.
- potential** See **electric potential**.
- potential difference** See **electric potential**.
- potentiometer** A device that measures electric potential difference. A potentiometer measures the same quantity as that measured by a voltmeter, but the potentiometer is designed to draw much less current from the circuit being measured.
- potentiometry** An analytical method in which an electric potential difference (a voltage) of a cell is measured.
- potentiostat** An electronic device that maintains a chosen constant or time-varying voltage between a pair of electrodes.
- power** The amount of energy per unit time ($J/s = \text{watts}, W$) being expended.
- ppb** See **parts per billion**.
- ppm** See **parts per million**.
- precipitant** A substance that precipitates a species from solution.
- precipitation** Occurs when a substance leaves solution rapidly (to form either microcrystalline or amorphous solid).
- precipitation titration** One in which the analyte forms a precipitate with the titrant.
- precision** How well replicate measurements agree with one another.
- precolumn** See **guard column**.
- preconcentration** The process of concentrating trace components of a mixture prior to their analysis.
- precursor ion** In tandem mass spectrometry (selected reaction monitoring), the ion selected by the first mass separator for fragmentation in the collision cell.
- premix burner** In atomic spectroscopy, one in which the sample is nebulized and simultaneously mixed with fuel and oxidant before being fed into the flame.
- preoxidation** In some redox titrations, adjustment of the analyte oxidation state to a higher value so that it can be titrated with a reducing agent.
- preparative chromatography** Chromatography of large quantities of material conducted for the purpose of isolating pure material.
- prereduction** The process of reducing an analyte to a lower oxidation state prior to performing a titration with an oxidizing agent.
- pressure** Force per unit area, commonly measured in pascals (N/m^2) or bars.
- pressure broadening** In spectroscopy, line broadening due to collisions between molecules.
- primary standard** A reagent that is pure enough and stable enough to be used directly after weighing. The entire mass is considered to be pure reagent.
- prism** A transparent, triangular solid. Each wavelength of light passing through the prism is bent (refracted) at a different angle.
- product** The species created in a chemical reaction. Products appear on the right side of the chemical equation.
- product ion** In tandem mass spectrometry (selected reaction monitoring), the fragment ion from the collision cell selected by the final mass separator for passage through to the detector.
- protic solvent** One with an acidic hydrogen atom.
- protocol** In quality assurance, written directions stating what must be documented and how the documentation is to be done.
- proton** The ion H^+ .
- proton acceptor** A Brønsted-Lowry base: a molecule that combines with H^+ .
- protonated molecule** In mass spectrometry, the ion MH^+ resulting from addition of H^+ to analyte M.
- proton donor** A Brønsted-Lowry acid: a molecule that can provide H^+ to another molecule.
- purge** To force a fluid (usually gas) to flow through a substance or a chamber, usually to extract something from the substance being purged or to replace the fluid in the chamber with the purging fluid.
- purge and trap** A method for removing volatile analytes from liquids or solids, concentrating the analytes, and introducing them into a gas chromatograph. A carrier gas bubbled through a liquid or solid extracts volatile analytes, which are then trapped in a tube containing adsorbent. After analyte has been collected, the adsorbent tube is heated and purged in the reverse direction to desorb the analytes, which are collected by cold trapping in a gas chromatography column.
- pyrolysis** Thermal decomposition of a substance.
- quadratic equation** An equation that can be rearranged to the form $ax^2 + bx + c = 0$.
- qualitative analysis** The process of determining the identity of the constituents of a substance.
- quality assurance** Practices that demonstrate the reliability of analytical data.
- quality control** Active measures taken to ensure the required accuracy and precision of a chemical analysis.
- quality control sample** See **performance test sample**.
- quantitative analysis** Process of measuring how much of a constituent is present in a substance.
- quantitative transfer** Transferring the entire contents from one vessel to another, usually accomplished by rinsing the first vessel several times with fresh liquid and pouring each rinse into the receiving vessel.
- radiant power** Power per unit area (W/m^2) of a beam of electromagnetic radiation. Also called *intensity, irradiance, or radiant flux*.
- random error** A type of error, which can be either positive or negative and cannot be eliminated, based on the ultimate limitations on a physical measurement. Also called *indeterminate error*.
- random heterogeneous material** A material in which there are differences in composition with no pattern or predictability and on a fine scale. When you collect a portion of the material for analysis, you obtain some of each of the different compositions.
- random sample** Bulk sample constructed by taking portions of the entire lot at random.
- range** The difference between the highest and lowest values in a set of data. Also called *spread*. With respect to an analytical method, range is the concentration interval over which linearity, accuracy, and precision are all acceptable.
- raw data** Individual values of a measured quantity, such as peak areas from a chromatogram or volumes from a buret.
- reactant** The species that is consumed in a chemical reaction. It appears on the left side of a chemical equation.

reaction quotient, Q Expression having the same form as the equilibrium constant for a reaction. However, the reaction quotient is evaluated for a particular set of existing activities (concentrations), which are generally not the equilibrium values. At equilibrium, $Q = K$.
reagent blank A solution prepared from all of the reagents except analyte. The blank measures the response of the analytical method to impurities in the reagents or any other effects caused by any component other than the analyte. A reagent blank is similar to a method blank, but it has not been subjected to all sample preparation procedures.

reagent grade chemical A high-purity chemical generally suitable for use in quantitative analysis and meeting purity requirements set by organizations such as the American Chemical Society.

reconstructed total ion chromatogram In chromatography, a graph of the sum of intensities of all ions detected at all masses (above a selected cutoff) versus time.

redox couple A pair of reagents related by electron transfer; e.g., $\text{Fe}^{3+}/\text{Fe}^{2+}$ or $\text{MnO}_4^-/\text{Mn}^{2+}$.

redox indicator A compound used to find the end point of a redox titration because its various oxidation states have different colors. The standard potential of the indicator must be such that its color changes near the equivalence point of the titration.

redox reaction A chemical reaction in which electrons are transferred from one element to another.

redox titration One in which the reaction between analyte and titrant is an oxidation-reduction reaction.

reducing agent A substance that donates electrons in a chemical reaction. Also called *reductant*.

reductant See **reducing agent**.

reduction A gain of electrons or a lowering of the oxidation state.

reference electrode One that maintains a constant potential against which the potential of another half-cell may be measured.

refraction Bending of light when it passes between media with different refractive indexes.

refractive index, n The speed of light in any medium is c/n , where c is the speed of light in vacuum and n is the refractive index of the medium. The refractive index also measures the angle at which a light ray is bent when it passes from one medium into another. Snell's law states that $n_1 \sin \theta_1 = n_2 \sin \theta_2$, where n_i is the refractive index for each medium and θ_i is the angle of the ray with respect to the normal between the two media.

refractive index detector Liquid chromatography detector that measures the change in refractive index of eluate as solutes emerge from the column.

releasing agent In atomic spectroscopy, a substance that prevents chemical interference.

relative standard deviation See **coefficient of variation**.

relative uncertainty The uncertainty of a quantity divided by the value of the quantity. It is usually expressed as a percentage of the measured quantity.

replicate measurements Repeated measurements of the same quantity.

reporting limit Concentration below which regulations dictate that an analyte is reported as "not detected." The reporting limit is typically set 5 to 10 times higher than the detection limit.

reprecipitation Sometimes a gravimetric precipitate can be freed of impurities only by redissolving it in fresh solvent and reprecipitating it. The impurities are present at lower concentration during the second precipitation and are less likely to coprecipitate.

residual current Small current observed in the absence of analyte reaction in an electrolysis.

resin An ion exchanger, such as polystyrene with ionic substituents, which exists as small, hard particles.

resolution How close two bands in a spectrum or a chromatogram can be to each other and still be seen as two peaks. In chromatography, it is defined as the difference in retention times of adjacent peaks divided by their width.

response factor, F The relative response of a detector to analyte (X) and internal standard (S): $(\text{signal from X})/[\text{X}] = F(\text{signal from S})/[\text{S}]$. Once you have measured F with a standard mixture, you can use it to find $[\text{X}]$ in an unknown if you know $[\text{S}]$ and the quotient $(\text{signal from X})/(\text{signal from S})$.

results What we ultimately report after applying statistics to treated data.

retention time, t_r The time from injection for an individual solute to reach the detector of a chromatography column.

retention volume, V_r The volume of solvent needed to elute a solute from a chromatography column.

reversed-phase chromatography Liquid chromatography in which the stationary phase is less polar than the mobile phase.

robustness The ability of an analytical method to be unaffected by small changes in operating conditions.

rotational transition Occurs when a molecule changes its rotation energy.

rubber policeman A glass rod with a flattened piece of rubber on the tip. The rubber is used to scrape solid particles from glass surfaces in gravimetric analysis.

run buffer See **background electrolyte**.

salt An ionic solid.

salt bridge A conducting ionic medium in contact with two electrolyte solutions. It allows ions to flow without allowing immediate diffusion of one electrolyte solution into the other. It usually contains a salt that minimizes the junction potential at each end of the bridge.

sample cleanup Removal of portions of the sample that do not contain analyte and may interfere with analysis.

sample preparation Transforming a sample into a state that is suitable for analysis. This process can include concentrating a dilute analyte and removing or masking interfering species.

sampling The process of collecting a representative sample for analysis.

saturated calomel electrode (S.C.E.) A calomel electrode saturated with KCl. The electrode half-reaction is $\text{Hg}_2\text{Cl}_2(s) + 2e^- \rightleftharpoons 2\text{Hg}(l) + 2\text{Cl}^-$. The compound Hg_2Cl_2 is called calomel.

saturated solution One that contains the maximum amount of a compound that can dissolve at equilibrium.

S.C.E. See **saturated calomel electrode**.

schlieren Streaks in a liquid mixture observed before the two phases have mixed. Streaks arise from regions that refract light differently.

second, s SI unit of time equal to the duration of 9 192 631 770 periods of the radiation corresponding to the transition between two hyperfine levels of the ground state of ^{133}Cs .

second derivative The slope of the slope, $\Delta(\text{slope})/\Delta x$, of a curve, measured at each point along the curve. When the slope reaches a maximum or minimum, the second derivative is 0.

segregated heterogeneous material A material in which differences in composition are on a large scale. Different regions have obviously different composition.

selected ion chromatogram A graph of detector response versus time when a mass spectrometer monitors just one or a few species of selected mass-to-charge ratio emerging from a chromatograph.

selected ion monitoring Use of a mass spectrometer to monitor species with just one or a few mass-to-charge ratios (m/z).

selected reaction monitoring A technique in which a precursor ion selected by one mass separator passes through a collision cell in which the precursor breaks into several product ions. A second mass separator then selects one (or a few) of these ions for detection. Selected reaction monitoring improves chromatographic signal-to-noise ratio because it is insensitive to almost everything other than the intended analyte. Also called *mass spectrometry-mass spectrometry (MS-MS)* or *tandem mass spectrometry*.

selectivity The capability of an analytical method to distinguish analyte from other species in the sample. Also called *specificity*.

selectivity coefficient With respect to an ion-selective electrode, a measure of the relative response of the electrode to two different ions. In ion-exchange chromatography, the selectivity coefficient is the equilibrium constant for displacement of one ion by another from the resin.

self-absorption In a luminescence measurement, a high concentration of analyte molecules can absorb excitation energy from excited analyte. If the absorbed energy is dissipated as heat instead of light, fluorescence does not increase in proportion to analyte concentration. Analyte concentration can be so high that fluorescence *decreases* with increasing concentration. In flame emission atomic spectroscopy, there is a lower concentration of excited-state atoms in the cool, outer part of the flame than in the hot, inner flame. The cool atoms can absorb emission from the hot ones and thereby decrease the observed signal.

self-ionization See *autoprotolysis*.

semipermeable membrane A thin layer of material that allows some substances, but not others, to pass across the material. A dialysis membrane allows small molecules to pass, but not large molecules.

sensitivity The magnitude of instrument response per unit change in concentration of analyte.

separator column Ion-exchange column used to separate analyte species in ion chromatography.

septum A disk, usually made of silicone rubber, covering the injection port of a gas chromatograph. The sample is injected by syringe through the septum.

SI units The international system of units based on the meter, kilogram, second, ampere, kelvin, candela, mole, radian, and steradian.

sieving In electrophoresis, the separation of macromolecules by migration through a polymer gel. The smallest molecules move fastest and the largest move slowest.

signal averaging Improvement of a signal by averaging successive scans. The signal increases in proportion to the number of scans accumulated. The noise increases in proportion to the square root of the number of scans. Therefore the signal-to-noise ratio improves in proportion to the square root of the number of scans collected.

signal-to-noise ratio The height of a signal divided by the noise in the baseline around the signal. The higher the signal-to-noise ratio, the less uncertainty there is in the signal.

significant figure The number of significant digits in a quantity is the minimum number of digits needed to express the quantity in scientific notation without loss of precision. In experimental data, the first uncertain figure is the last significant figure.

silanization Treatment of a chromatographic solid support or glass column with hydrophobic silicon compounds that bind to the most reactive Si—OH groups. It reduces irreversible adsorption and tailing of polar solutes.

silver-silver chloride electrode A common reference electrode containing a silver wire coated with AgCl paste and dipped in a solution saturated with AgCl and (usually) KCl. The half-reaction is $\text{AgCl}(s) + \text{e}^- \rightleftharpoons \text{Ag}(s) + \text{Cl}^-$.

singlet state One in which all electron spins are paired.

size exclusion chromatography See **molecular exclusion chromatography**.

slope For a straight line whose equation is $y = mx + b$, the value of m is the slope. It is the ratio $\Delta y / \Delta x$ for any segment of the line.

slurry A suspension of a solid in a solvent.

sodium error Occurs when a glass pH electrode is placed in a strongly basic solution containing very little H^+ and a high concentration of Na^+ . The electrode begins to respond to Na^+ as if it were H^+ , so the pH reading is lower than the actual pH. Also called *alkaline error*.

solid-phase extraction Sample preparation procedure in which a solution is passed through a short column of chromatographic stationary phase. Unretained components of the sample are washed through the column. Components of interest can be eluted in batches with successively stronger solvents. Analyte can be partially purified and preconcentrated by this means.

solid-phase microextraction Extraction of compounds from liquids or gases into a coated fiber dispensed from a syringe needle. After extraction, the fiber is withdrawn into the needle and the needle is injected through the septum of a chromatograph. The fiber is extended inside the injection port and adsorbed solutes are desorbed by heating (for gas chromatography) or solvent (for liquid chromatography).

solid-state ion-selective electrode An ion-selective electrode that has a solid membrane made of an inorganic salt crystal.

Ion-exchange equilibria between the solution and the surface of the crystal account for the electrode potential.

solubility product, K_{sp} The equilibrium constant for the dissociation of a solid salt to give its ions in solution. For the reaction $\text{M}_m\text{N}_n(s) \rightleftharpoons m\text{M}^{n+} + n\text{N}^{m-}$, $K_{\text{sp}} = [\text{M}^{n+}]^m[\text{N}^{m-}]^n$.

solute A minor component of a solution.

solvation The interaction of solvent molecules with solute. Solvent molecules orient themselves around solute to minimize the energy through dipole and van der Waals forces.

solvent The major constituent of a solution.

solvent strength See *eluent strength*.

solvent trapping Splitless gas chromatography injection technique in which solvent is condensed below its boiling point at the start of the column. Solutes dissolve in a narrow band in the condensed solvent.

species Chemists refer to any element, compound, or ion of interest as a *species*. The word *species* is both singular and plural.

specifications In quality assurance, specifications are written statements describing how good analytical results need to be and what precautions are required in an analytical method.

specific gravity A dimensionless quantity equal to the mass of a substance divided by the mass of an equal volume of water at 4°C. Specific gravity is virtually identical with density in g/mL.

specificity Capability of an analytical method to distinguish analyte from other species in the sample. Also called *selectivity*.

spectral interference In atomic spectroscopy, any physical process that affects light intensity at the analytical wavelength. Created by substances that absorb, scatter, or emit light at the analytical wavelength.

spectrophotometer A device used to measure absorption of light. It includes a source of light, a wavelength selector (monochromator), and an electrical means of detecting light.

spectrophotometric analysis Any method in which light absorption, emission, reflection, or scattering is used to measure chemical concentrations.

spectrophotometric titration One in which absorption or emission of light is used to monitor the progress of the chemical reaction.

spectrophotometry Any method using light to measure chemical concentrations.

specular reflection Reflection of light at an angle equal to the angle of incidence.

spike Addition of a known compound (usually at a known concentration) to an unknown. In isotope dilution mass spectrometry, the spike is the added, unusual isotope. *Spike* is a noun and a verb. Also called a *fortification*.

spike recovery The fraction of a spike found by chemical analysis of the spiked sample.

split injection Used in capillary gas chromatography to inject a small fraction of sample onto the column; the rest of the sample is blown out to waste.

splitless injection Used in capillary gas chromatography for trace analysis and quantitative analysis. The entire sample in a low-boiling solvent is directed to the column, where the sample is concentrated by *solvent trapping* (condensing the solvent below its boiling point) or *cold trapping* (condensing solutes far below their boiling range). The column is then warmed to initiate separation.

spontaneous process One that is energetically favorable. It will eventually occur, but thermodynamics makes no prediction about how long it will take.

spread See **range**.

square wave voltammetry A form of *voltammetry* in which the potential waveform consists of a square wave superimposed on a voltage staircase. The technique is faster and more sensitive than voltammetry with other waveforms.

stability constant See **formation constant**.

stacking In electrophoresis, the process of concentrating a dilute electrolyte into a narrow band by an electric field. Stacking occurs because the electric field in the dilute electrolyte is stronger than the field in more concentrated surrounding electrolyte. Ions in the low-conductivity region migrate rapidly until they reach the interface, where the electric field is much smaller.

standard addition A technique in which an analytical signal due to an unknown is first measured. Then a known quantity of analyte is added, and the increase in signal is recorded. From the response, it is possible to calculate what quantity of analyte was in the unknown.

standard curve A graph showing the response of an analytical technique to known quantities of analyte. Also called *calibration curve*.

standard deviation A statistic measuring how closely data are clustered about the mean value. For a finite set of data, the standard deviation, s , is computed from the formula

$$s = \sqrt{\frac{\sum_i(x_i - \bar{x})^2}{n - 1}} = \sqrt{\frac{\sum_i(x_i^2)}{n - 1} - \frac{(\sum_i x_i)^2}{n(n - 1)}}$$

where n is the number of results, x_i is an individual result, and \bar{x} is the mean result. For a large number of measurements, s approaches σ , the true standard deviation of the population, and \bar{x} approaches μ , the true population mean.

standard deviation of the mean The standard deviation of a set of measurements (s) divided by the square root of the number of measurements (n) in the set.

standard hydrogen electrode (S.H.E.) One that contains $H_2(g)$ bubbling over a catalytic Pt surface immersed in aqueous H^+ . The activities of H_2 and H^+ are both unity in the hypothetical standard electrode. The reaction is $H^+ + e^- \rightleftharpoons \frac{1}{2}H_2(g)$. Also called *normal hydrogen electrode (N.H.E.)*.

standardization Process of determining the concentration of a reagent by reaction with a known quantity of a second reagent.

standard reduction potential, E° The voltage that would be measured when a hypothetical cell containing the desired half-reaction (with all species present at unit activity) is connected to a standard hydrogen electrode anode.

Standard Reference Material See **certified reference material**.

standard solution A solution whose composition is known by virtue of the way that it was made from a reagent of known purity or by virtue of its reaction with a known quantity of a standard reagent.

standard state The standard state of a solute is 1 M and the standard state of a gas is 1 bar. Pure solids and liquids are considered to be in their standard states. In equilibrium constants, dimensionless concentrations are expressed as a ratio of the concentration of each species to its concentration in its standard state.

stationary phase In chromatography, the phase that does not move through the column.

stepwise formation constant, K_n The equilibrium constant for a reaction of the type $ML_{n-1} + L \rightleftharpoons ML_n$

steradian, sr SI unit of solid angle. There are 4π steradians in a complete sphere.

stir-bar sorptive extraction Sample preparation method similar to solid-phase microextraction, except the sorptive layer is coated on the outside of a stirring bar. Coating volume is greater than the fiber volume in solid-phase microextraction, so it provides $\sim 10^2$ times greater sensitivity for traces of analyte. Analyte is removed from the coating by thermal desorption for chromatography.

stoichiometry Ratios of substances participating in a chemical reaction.

stray light In spectrophotometry, light that reaches the detector but is not part of the narrow set of wavelengths expected from the monochromator.

stripping analysis A sensitive polarographic technique in which analyte is concentrated from dilute solution by reduction into a drop (or a film) of Hg. It is then analyzed polarographically during an anodic redissolution process. Some analytes can be oxidatively concentrated onto an electrode other than Hg and stripped in a reductive process.

strong acids and bases Those that are completely dissociated (to H^+ or OH^-) in water.

strong electrolyte One that mostly dissociates into ions in solution.

Student's t A statistical tool used to express confidence intervals and to compare results from different experiments.

sulfur chemiluminescence detector Gas chromatography detector for the element sulfur. Exhaust from a flame ionization detector is mixed with O_3 to form an excited state of SO_2 that emits light, which is detected.

superficially porous particle Chromatographic stationary phase particle consisting of porous outside layer (e.g., 0.25 μm thick) on a nonporous core. Solutes only need to diffuse through the depth of the porous layer, so mass transfer between the stationary and mobile phases is rapid and separations are efficient. The overall diameter of the particle is large enough that extreme pressure is not required for chromatography. Also called *fused-core particle*.

supernatant liquid Liquid remaining above the solid after a precipitation. Also called *supernate*.

supersaturated solution One that contains more dissolved solute than would be present at equilibrium.

supporting electrolyte An unreactive salt added in high concentration to solutions for voltammetric measurements. Supporting electrolyte carries most of the ion-migration current and therefore decreases the coulombic migration of electroactive species to a negligible level. The electrolyte also decreases the resistance of the solution.

suppressed-ion chromatography Separation of ions by using an ion-exchange column followed by a suppressor (membrane or column) to remove ionic eluent.

suppressor In ion chromatography, a device that transforms ionic eluent into a nonionic form.

surfactant A molecule with an ionic or polar headgroup and a long, nonpolar tail. Surfactants aggregate in aqueous solution to form micelles. Surfactants derive their name from the fact that they accumulate at boundaries between polar and nonpolar phases and modify the surface tension, which is the free energy of formation of the surface. Soaps are surfactants.

syringe A device having a calibrated barrel into which liquid is sucked by a plunger. The liquid is expelled through a needle by pushing on the plunger.

systematic error Error due to procedural or instrumental factors that cause a measurement to be consistently too large or too small. The error can, in principle, be discovered and corrected. Also called *determinate error*.

systematic treatment of equilibrium A method that uses the charge balance, mass balance(s), and equilibria to completely specify the system's composition.

***t* test** Statistical test used to decide whether the results of two experiments are within experimental uncertainty of each other. The uncertainty must be specified to within a certain probability.

tailing Asymmetric chromatographic band in which the later part elutes very slowly. It often results from adsorption of a solute onto a few active sites on the stationary phase.

tandem mass spectrometry See **selected reaction monitoring**.

tare As a noun, *tare* is the mass of an empty vessel used to receive a substance to be weighed. As a verb, *tare* means setting the balance reading to 0 when an empty vessel or weighing paper is placed on the pan.

temperature programming Raising the temperature of a gas chromatography column during a separation to reduce the retention time of late-eluting components.

temporary hardness Component of water hardness due to dissolved alkaline earth bicarbonates. It is temporary because boiling causes precipitation of the carbonates.

test portion Part of the laboratory sample used for one analysis. Also called *aliquot*.

theoretical plate An imaginary construct in chromatography denoting a segment of a column in which one equilibration of solute occurs between the stationary and mobile phases. The number of theoretical plates on a column with Gaussian bandshapes is defined as $N = t_r^2/\sigma^2$, where t_r is the retention time of a peak and σ is the standard deviation of the band.

thermal conductivity, κ Rate at which a substance transports heat (energy per unit time per unit area) through a temperature gradient (degrees per unit distance). Energy flow [$J/(s \cdot m^2)$] = $-\kappa(dT/dx)$, where κ is the thermal conductivity [$W/(m \cdot K)$] and dT/dx is the temperature gradient (K/m).

thermal conductivity detector A device that detects substances eluted from a gas chromatography column by measuring changes in the thermal conductivity of the gas stream.

thermogravimetric analysis A technique in which the mass of a substance is measured as the substance is heated. Changes in mass indicate decomposition of the substance, often to well-defined products.

thin-layer chromatography Liquid chromatography in which the stationary phase is coated on a flat glass or plastic plate. Solute is spotted near the bottom of the plate. The bottom edge of the plate is placed in contact with solvent, which creeps up the plate by capillary action. **titer** A measure of concentration, usually defined as how many milligrams of reagent B will react with 1 mL of reagent A. One milliliter of $AgNO_3$ solution with a titer of 1.28 mg NaCl/mL will be consumed by 1.28 mg NaCl in the reaction $Ag^+ + Cl^- \rightarrow AgCl(s)$. The same solution of $AgNO_3$ has a titer of 0.993 mg of KH_2PO_4 /mL, because 1 mL of $AgNO$ solution will be consumed by 0.993 mg KH_2PO_4 to precipitate Ag_2PO_4 .

titrant The substance added to the analyte in a titration.

titration A procedure in which one substance (titrant) is carefully added to another (analyte) until complete reaction has occurred. The quantity of titrant required for complete reaction tells how much analyte is present.

titration curve A graph showing how the concentration of a reactant or a physical property of the solution varies as one reactant (the titrant) is added to another (the analyte).

titration error The difference between the observed end point and the true equivalence point in a titration.

tolerance Manufacturer's stated uncertainty in the accuracy of a device such as a buret or volumetric flask. A 100-mL flask with a tolerance of ± 0.08 mL may contain 99.92 to 100.08 mL and be within tolerance.

total carbon In a natural water or industrial effluent sample, the quantity of CO_2 produced when the sample is completely oxidized by oxygen at 900°C in the presence of a catalyst.

total ion chromatogram A graph of detector response versus time when a mass spectrometer monitors all ions above a selected m/z ratio emerging from a chromatograph.

total organic carbon In a natural water or industrial effluent sample, the quantity of CO_2 produced when the sample is first acidified and purged to remove carbonate and bicarbonate and then completely oxidized by oxygen at 900°C in the presence of a catalyst.

total oxygen demand In a natural water or industrial effluent sample, the quantity of O_2 required for complete oxidation of species in the water at 900°C in the presence of a catalyst.

trace Tiny amount of a substance.

trace analysis Chemical analysis of very low levels of analyte, typically ppm and lower.

transition range For an acid-base indicator, the pH range over which the color change occurs. For a redox indicator, the potential range over which the color change occurs.

transmission quadrupole mass spectrometer A mass spectrometer that separates ions by passing them between four metallic cylinders to which are applied direct current and oscillating electric fields. Resonant ions with the right mass-to-charge ratio pass through the chamber to the detector, while nonresonant ions are deflected into the cylinders and are lost.

transmittance, T Defined as $T = P/P_0$, where P_0 is radiant power of light striking the sample on one side and P is radiant power emerging from the other side of the sample.

treated data Concentrations or amounts of analyte found from raw data with a calibration curve or some other calibration method.

triplet state An electronic state in which there are two unpaired electrons.

ultra-performance liquid chromatography (UPLC) Liquid chromatography with stationary phase particles in the 1.5- to 2- μm range. Higher pressure is required to force liquid through a UPLC column than through columns with larger particle size.

ultraviolet detector Liquid chromatography detector that measures ultraviolet absorbance of solutes emerging from the column.

uncertainty Variability within a set of measurements of the same quantity.

UPLC See **ultra-performance liquid chromatography**.

use objectives In quality assurance, use objectives are a written statement of how results will be used. Use objectives are required before specifications can be written for the method.

validation See **method validation**.

van Deemter equation Describes the dependence of chromatographic plate height (H) on linear flow rate (u , in units such as m/s): $H = A + B/u + Cu$. The constant A depends on band broadening processes such as multiple flow paths that are independent of flow rate. B depends on the rate of diffusion of solute in the mobile phase. C depends on the rate of mass transfer (that is, the equilibration time) between the stationary and mobile phases.

variance The square of the standard deviation.

vibrational transition Occurs when a molecule changes its vibrational energy.

viscosity Resistance to flow in a fluid.

void volume, V_0 The volume of the mobile phase *outside* of the stationary phase in a molecular exclusion chromatography column. The total volume of mobile phase is the void volume plus the volume of solvent inside the stationary phase particles.

volatile Easily vaporized.

Volhard titration Titration of Ag^+ with SCN^- in the presence of Fe^{3+} . Formation of red $\text{Fe}(\text{SCN})^{2+}$ marks the end point.

volt, V SI unit of electric potential. If the potential difference between two points is one volt, then one joule of energy is required to move one coulomb of charge between the two points.

voltammetry An analytical method in which the relationship between current and voltage is observed during an electrochemical reaction.

voltammogram A graph of current versus electrode potential in an electrochemical cell.

voltmeter Device for measuring electric potential difference. See also **potentiometer**.

volume flow rate In chromatography, the volume of mobile phase per unit time eluted from the column.

volume percent, vol % (Volume of solute/volume of solution) $\times 100$.

volumetric analysis A technique in which the volume of material needed to react with the analyte is measured.

volumetric flask One having a tall, thin neck with a calibration mark. When the liquid level is at the calibration mark, the flask contains its specified volume of liquid.

watt, W SI unit of power equal to an energy flow of one joule per second. When an electric current of one ampere flows through a potential difference of one volt, the power is one watt.

wavelength, λ Distance between consecutive crests of a wave.

wavenumber, $\tilde{\nu}$ Reciprocal of the wavelength, $1/\lambda$.

weak acids and bases Those whose dissociation constants are not large.

weak electrolyte One that only partly dissociates into ions when it dissolves.

weighing paper Paper on which to place a solid reagent on a balance. Weighing paper has a very smooth surface, from which solids fall easily for transfer to a vessel.

weight percent, wt % (Mass of solute/mass of solution) $\times 100$.

weight/volume percent (Mass of solute/volume of solution) $\times 100$.

width at half-height, $w_{1/2}$ Width of a chromatographic peak or spectroscopic signal at half of the maximum height of the peak.

work Energy required or released when an object is moved from one point to another. Unit of work is the joule (J).

working electrode One at which the reaction of interest occurs.

y-intercept The value of y at which a line crosses the y -axis.

Zeeman background correction Technique used in atomic spectroscopy in which analyte signals are shifted outside the detector monochromator range by applying a strong magnetic field to the sample. Signal that remains is the background.

zwitterion A neutral molecule with a positive charge localized at one position and a negative charge localized at another position.

Solutions to “Ask Yourself” Questions

Chapter 0

- 0-A.** (a) A heterogeneous material has different compositions in different regions. A homogeneous material is the same everywhere.
 (b) The composition of a random heterogeneous material varies from place to place with no pattern or predictability to the variation. A segregated heterogeneous material has large regions of distinctly different compositions.
 (c) A random sample is selected by taking material at random from the lot. That is, the locations from which material is selected should follow no pattern. You could do this by dividing the lot into many imaginary regions and assigning a number to each. Then use your computer or calculator to generate random numbers and take a sample from each region whose number is selected by the computer. A composite sample is selected deliberately by taking predetermined portions of the lot from selected regions. Random sampling is appropriate for a random heterogeneous lot. Composite sampling is appropriate when the lot is segregated into regions of different composition.

Chapter 1

- 1-A.** (a) See Table 1-3

$$\begin{aligned} \text{(b)} \quad & 8 \times 10^{-7} \text{ L O}_3 \times \left(\frac{1 \text{ nL}}{10^{-9} \text{ L}} \right) \\ & = 800 \text{ nL O}_3 \text{ per liter of air (800 nL O}_3/\text{L}) \\ & 8 \times 10^{-7} \text{ L O}_3 \times \left(\frac{1 \mu\text{L}}{10^{-6} \text{ L}} \right) \\ & = 0.8 \mu\text{L O}_3 \text{ per liter of air (0.8 } \mu\text{L O}_3/\text{L}) \end{aligned}$$

$$\begin{aligned} \text{1-B. (a)} \quad & \text{Office worker: } 2.2 \times 10^6 \frac{\text{eat}}{\text{day}} \times 4.184 \frac{\text{J}}{\text{eat}} \\ & = 9.2 \times 10^6 \text{ J/day} \end{aligned}$$

$$\begin{aligned} & \text{Mountain climber: } 3.4 \times 10^6 \frac{\text{eat}}{\text{day}} \times 4.184 \frac{\text{J}}{\text{eat}} \\ & = 14.2 \times 10^6 \text{ J/day} \end{aligned}$$

$$\text{(b)} \quad 60 \frac{\text{s}}{\text{min}} \times 60 \frac{\text{min}}{\text{h}} \times 24 \frac{\text{h}}{\text{day}} = 8.64 \times 10^4 \text{ s/day}$$

$$\text{(c)} \quad \frac{9.2 \times 10^6 \frac{\text{J}}{\text{day}}}{8.64 \times 10^4 \frac{\text{s}}{\text{day}}} = 1.1 \times 10^2 \text{ W;}$$

$$\frac{14.2 \times 10^6 \frac{\text{J}}{\text{day}}}{8.64 \times 10^4 \frac{\text{s}}{\text{day}}} = 1.6 \times 10^2 \text{ W}$$

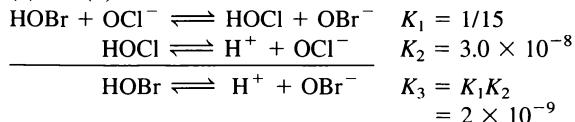
570

- (d) The office worker consumes more power than does the light bulb.

$$\begin{aligned} \text{1-C. (a)} \quad & \left(1.67 \frac{\text{g solution}}{\text{mL}} \right) \left(1000 \frac{\text{mL}}{\text{L}} \right) = 1.670 \frac{\text{g solution}}{\text{L}} \\ \text{(b)} \quad & \left(0.705 \frac{\text{g HClO}_4}{\text{g solution}} \right) \left(1.670 \frac{\text{g solution}}{\text{L}} \right) \\ & = 1.18 \times 10^3 \frac{\text{g HClO}_4}{\text{L}} \\ \text{(c)} \quad & \left(1.18 \times 10^3 \frac{\text{g}}{\text{L}} \right) / \left(100.458 \frac{\text{g}}{\text{mol}} \right) = 11.7 \frac{\text{mol}}{\text{L}} \\ \text{1-D. (a)} \quad & \left(1.50 \frac{\text{g solution}}{\text{mL solution}} \right) \left(1000 \frac{\text{mL solution}}{\text{L solution}} \right) \\ & = 1.50 \times 10^3 \frac{\text{g solution}}{\text{L solution}} \\ \text{(b)} \quad & \left(0.480 \frac{\text{g HBr}}{\text{g solution}} \right) \left(1.50 \times 10^3 \frac{\text{g solution}}{\text{L solution}} \right) \\ & = 7.20 \times 10^2 \frac{\text{g HBr}}{\text{L solution}} \\ \text{(c)} \quad & \left(7.20 \times 10^2 \frac{\text{g HBr}}{\text{L solution}} \right) / \left(\frac{80.912 \text{ g HBr}}{\text{mol}} \right) \\ & = 8.90 \text{ M} \end{aligned}$$

$$\text{(d)} \quad M_{\text{conc}} \cdot V_{\text{conc}} = M_{\text{dil}} \cdot V_{\text{dil}} \\ (8.90 \text{ M})(x \text{ mL}) = (0.160 \text{ M})(250 \text{ mL}) \Rightarrow x = 4.49 \text{ mL}$$

- 1-E.** (a) and (b)



- (c) Consumption of a product drives the reaction forward (to the right).

Chapter 2

- 2-A.** The lab notebook must (1) state what was done; (2) state what was observed; and (3) be understandable to a stranger.

$$\text{2-B. (a)} \quad m = \frac{(24.913 \text{ g}) \left(1 - \frac{0.0012 \text{ g/mL}}{8.0 \text{ g/mL}} \right)}{\left(1 - \frac{0.0012 \text{ g/mL}}{1.00 \text{ g/mL}} \right)} = 24.939 \text{ g}$$

$$\begin{aligned} \text{(b)} \quad & \text{density} = \frac{\text{mass}}{\text{volume}} \Rightarrow \text{volume} = \frac{\text{mass}}{\text{density}} \\ & \text{volume} = (24.939 \text{ g}) / (0.99800 \text{ g/mL}) \\ & = 24.989 \text{ mL} \end{aligned}$$

2-C. Dissolve (0.250 0 L)(0.150 0 mol/L) = 0.037 50 mol of K₂SO₄ in less than 250 mL of water in a 250-mL volumetric flask. Add more water and mix. Dilute to the 250.0-mL mark and invert the flask many times for complete mixing.

2-D. Transfer pipet. The adjustable 100-μL micropipet has a tolerance of ±1.8% at 10 μL and ±0.6% at 100 μL. The uncertainty at 10 μL is ±1.8% of 10 μL = (0.018)(10 μL) = ±0.18 μL. The uncertainty at 100 μL is ±0.6% of 100 μL = (0.006)(100 μL) = ±0.6 μL.

2-E. mass delivered × conversion factor
 $= (10.000\ 0\ \text{g})(1.002\ 0\ \text{mL/g}) = 10.020\ \text{mL}$

2-F. S²⁻ + 2H⁺ → H₂S. H₂S(g) is lost when the solution is boiled to dryness.

Chapter 3

3-A. (a) 5 (b) 4 (c) 3

3-B. (a) 3.71 (b) 10.7
 (c) 4.0×10^1 (d) 2.85×10^{-6}
 (e) 12.625 1 (f) 6.0×10^{-4}
 (g) 242

3-C. (a) Carmen (b) Cynthia (c) Chastity (d) Cheryl

3-D. (a) percent relative uncertainty in mass
 $= (0.002/4.635) \times 100 = 0.04_3\%$
 percent relative uncertainty in volume
 $= (0.05/1.13) \times 100 = 4.4\%$

$$\begin{aligned} \text{(b) density} &= \frac{4.635 \pm 0.002\ \text{g}}{1.13 \pm 0.05\ \text{mL}} \\ &= \frac{4.635(\pm 0.04_3\%) \text{ g}}{1.3(\pm 4.4\%) \text{ mL}} = 4.10 \pm ?\ \text{g/mL} \end{aligned}$$

$$\text{uncertainty} = \sqrt{(0.04_3)^2 + (4.4)^2} = 4.4\%$$

4.4% of 4.10 = 0.18. The answer can be written
 $4.1_0 \pm 0.1_8$ or $4.1 \pm 0.2\ \text{g/mL}$

$$\begin{aligned} \text{(c) For pH} &= 8.82 \pm 0.02, [\text{H}^+] = 10^{-8.82} = 1.514 \times 10^{-9}\ \text{M} \\ \text{uncertainty in } [\text{H}^+] &= 2.303[\text{H}^+](\text{uncertainty in pH}) \\ &= 2.303[1.514 \times 10^{-9}\ \text{M}](0.02) \\ &= 6.97 \times 10^{-11}\ \text{M} = 0.069\ 7 \times 10^{-9}\ \text{M} \\ [\text{H}^+] &= 1.51 (\pm 0.07) \times 10^{-9}\ \text{M} \end{aligned}$$

3-E. $-196^\circ\text{C} = 77\ \text{K} = -321^\circ\text{F}$

Chapter 4

4-A. $\bar{x} = \frac{821 + 783 + 834 + 855}{4} = 823.2$
 $s = \sqrt{\frac{(821 - 823.2)^2 + (783 - 823.2)^2 + (834 - 823.2)^2 + (855 - 823.2)^2}{4 - 1}}$
 $= 30.3$

relative standard deviation = $(30.3/823.2) \times 100 = 3.68\%$
 median = $(821 + 834)/2 = 827.5$; range = $855 - 783 = 72$

$$\begin{aligned} \text{4-B. (a)} \quad \bar{x}_1 &= \frac{31.40 + 31.24 + 31.18 + 31.43}{4} \\ &= 31.31_2\ \text{mM} \\ s_1 &= \sqrt{\frac{(31.40 - 31.31_2)^2 + (31.24 - 31.31_2)^2 + \dots + (31.43 - 31.31_2)^2}{4 - 1}} \\ &= 0.12_1\ \text{mM} \\ \bar{x}_2 &= \frac{30.70 + 29.49 + 30.01 + 30.15}{4} \\ &= 30.08_8\ \text{mM} \\ s_2 &= \sqrt{\frac{(30.70 - 30.08_8)^2 + (29.49 - 30.08_8)^2 + \dots + (30.15 - 30.08_8)^2}{4 - 1}} \\ &= 0.49_7\ \text{mM} \\ \text{(b)} \quad F_{\text{calculated}} &= \frac{s_2^2}{s_1^2} = \frac{(0.49_7)^2}{(0.12_1)^2} = 16.8 \end{aligned}$$

For 3 degrees of freedom in both standard deviations, $F_{\text{table}} = 9.28$. $F_{\text{calculated}} > F_{\text{table}}$, so standard deviations are significantly different.

$$\begin{aligned} \text{4-C. (a)} \quad \bar{x} &= \frac{117 + 119 + 111 + 115 + 120}{5} \\ &= 116.4\ \mu\text{mol}/100\ \text{mL} \\ s &= \sqrt{\frac{(117 - 116.4)^2 + (119 - 116.4)^2 + \dots + (120 - 116.4)^2}{5 - 1}} \\ &= 3.58\ \mu\text{mol}/100\ \text{mL} \end{aligned}$$

$$\text{(b)} \quad F_{\text{calculated}} = \frac{s_2^2}{s_1^2} = \frac{(3.58)^2}{(2.8)^2} = 1.63$$

For 4 degrees of freedom in the numerator and 3 degrees of freedom in the denominator, $F_{\text{table}} = 9.12$. $F_{\text{calculated}} < F_{\text{table}}$, so standard deviations are not significantly different.

(c) Because the standard deviations are not significantly different, we use Equations 4-6 and 4-5:

$$\begin{aligned} s_{\text{pooled}} &= \sqrt{\frac{2.8^2(4 - 1) + 3.58^2(5 - 1)}{4 + 5 - 2}} = 3.27 \\ t_{\text{calculated}} &= \frac{|111.0 - 116.4|}{3.27} \sqrt{\frac{4 \cdot 5}{4 + 5}} = 2.46 \end{aligned}$$

$t_{\text{table}} = 2.365$ for 95% confidence and $4 + 5 - 2 = 7$ degrees of freedom.

$t_{\text{calculated}} > t_{\text{table}}$, so the difference is significant at the 95% confidence level.

$$\begin{aligned} \text{4-E.} \quad \bar{x} &= 201.8; s = 9.34 \\ G_{\text{calculated}} &= |216 - 201.8| / 9.34 = 1.52 \\ G_{\text{table}} &= 1.672 \text{ for five measurements} \\ \text{Because } G_{\text{calculated}} &< G_{\text{table}}, \text{ we should not reject 216.} \end{aligned}$$

4-F.

x_i	y_i	$x_i y_i$	x_i^2	$d_i (= y_i - mx_i - b)$	d_i^2
1	3	3	1	-0.167	0.02789
3	2	6	9	0.333	0.11089
5	0	0	25	-0.167	0.02789
$\Sigma x_i = 9$	$\Sigma y_i = 5$	$\Sigma x_i y_i = 9$	$\Sigma (x_i^2) = 35$		$\Sigma (d_i^2) = 0.16667$

$$D = n\Sigma(x_i^2) - (\Sigma x_i)^2 = (3 \cdot 35) - 9^2 = 24$$

$$m = [n\Sigma x_i y_i - \Sigma x_i \Sigma y_i]/D \\ = [(3 \cdot 9) - (9 \cdot 5)]/24 = -0.750$$

$$b = [\Sigma(x_i^2)\Sigma y_i - \Sigma(x_i y_i)\Sigma x_i]/D \\ = [(35 \cdot 5) - (9 \cdot 9)]/24 = 3.917$$

$$s_y = \sqrt{\frac{\Sigma(d_i^2)}{3-2}} = \sqrt{\frac{0.16667}{1}} = 0.4082$$

$$s_m = s_y \sqrt{\frac{n}{D}} = 0.4082 \sqrt{\frac{3}{24}} = 0.144$$

$$s_b = s_y \sqrt{\frac{\Sigma(x_i^2)}{D}} = 0.4082 \sqrt{\frac{35}{24}} = 0.493$$

$$y(\pm 0.4_{08}) = -0.75_0(\pm 0.14_4)x + 3.9_{17}(\pm 0.4_{93})$$

$$4-G. \quad x = \frac{y - b}{m} = \frac{1.00 - 3.9_{17}}{-0.750} = 3.89$$

$$\bar{x} = (1 + 3 + 5)/3 = 3; \bar{y} = (3 + 2 + 0)/3 = 1.667$$

$$\text{uncertainty in } x = \frac{s_y}{|m|} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \Sigma(x_i - \bar{x})^2}}$$

$$= \frac{0.4082}{0.750} \times$$

$$\sqrt{\frac{1}{5} + \frac{1}{3} + \frac{(1.00 - 1.667)^2}{(-0.750)^2[(1 - 3)^2 + (3 - 3)^2 + (5 - 3)^2]}}$$

$$= 0.43$$

Final answer: $x = 3.9 \pm 0.4$

Actions: Compare data and results with specifications. Document procedures and keep records suitable for meeting use objectives. Verify that the use objectives were met.

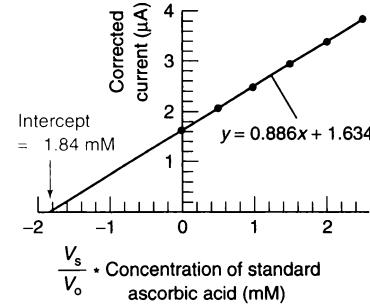
5-B. Precision is demonstrated by the repeatability of analyses of replicate samples and replicate portions of the same sample. Accuracy is demonstrated by fortification recovery, calibration checks, blanks, and quality control samples (blind samples).

5-C. The equation of the least-squares line through the data points is $y = 0.886x + 1.634$. To find the x -intercept, set $y = 0$.

$$0 = 0.886x + 1.634 \Rightarrow x = -1.84 \text{ mM}$$

concentration of ascorbic acid in orange juice = 1.84 mM

	A	B	C	D	E
1	Vitamin C standard addition experiment				
2	Add 25.0 mM ascorbic acid to 50.0 mL of orange juice				
3					
4	Vs =				
5	Vo (mL) =	mL ascorbic acid added	x-axis function	I(s+x) =	y-axis function
6		50	Si*Vs/Vo	signal (μA)	$I(s+x)*V/Vo$
7	[Sji] (mM) =	0.000	0.000	1.66	1.660
8	25	1.000	0.500	2.03	2.071
9		2.000	1.000	2.39	2.486
10		3.000	1.500	2.79	2.957
11		4.000	2.000	3.16	3.413
12		5.000	2.500	3.51	3.861
13					
14	C7 = \$A\$8*B7/\$A\$6	E7 = D7*(\\$A\$6+B7)/\\$A\$6			



5-D. First evaluate the response factor from the known mixture:

$$\frac{A_x}{[X]} = F \left(\frac{A_s}{[S]} \right)$$

$$\frac{0.644}{52.4 \text{ nM}} = F \left(\frac{1.000}{38.9 \text{ nM}} \right) \Rightarrow F = 0.478_1$$

For the unknown mixture, we can write

$$\frac{A_X}{[X]} = F\left(\frac{A_S}{[S]}\right)$$

$$\frac{1.093}{[X]} = 0.478_1 \left(\frac{1.000}{742 \text{ nM}}\right) \Rightarrow$$

$$[X] = 1.70 \times 10^3 \text{ nM} = 1.70 \mu\text{M}$$

Chapter 6

- 6-A.** (a) The concentrations of reagents used in an analysis are determined either by weighing out pure primary standards or by reaction with such standards. If the standards are not pure, none of the concentrations will be correct.
 (b) In a blank titration, the quantity of titrant required to reach the end point in the absence of analyte is measured. This quantity is subtracted from the quantity of titrant needed in the presence of analyte, thereby reducing the error caused by over-titration.
 (c) In a direct titration, titrant reacts directly with analyte. In a back titration, a known excess of reagent that reacts with analyte is used. The excess is then titrated.
 (d) The uncertainty in the equivalence point is constant at $\pm 0.04 \text{ mL}$. The relative uncertainty in delivering 20 mL is $0.04/20 = 0.2\%$. The relative uncertainty in delivering 40 mL is only half as great: $0.04/40 = 0.1\%$.
- 6-B.** (a) 0.197 g of ascorbic acid = $1.118 \text{ g} \times 10^{-3} \text{ mol}$, which requires $1.118 \text{ g} \times 10^{-3} \text{ mol I}_3^-$. $[\text{I}_3^-] = 1.118 \text{ g} \times 10^{-3} \text{ mol} / 0.02941 \text{ L} = 0.03803 \text{ M}$.
 (b) mol I_3^- required to react with 0.4242 g of powder from vitamin C tablet = $(0.03163 \text{ L I}_3^-)(0.03803 \text{ M}) = 1.203 \times 10^{-3} \text{ mol I}_3^-$. Because 1 mol I_3^- reacts with 1 mol ascorbic acid, there must have been $1.203 \times 10^{-3} \text{ mol}$ ascorbic acid in the sample that was titrated.
 (c) mass of ascorbic acid titrated
 $= (1.203 \times 10^{-3} \text{ mol ascorbic acid})(176.12 \text{ g/mol})$
 $= 0.2119 \text{ g ascorbic acid}$
 wt% ascorbic acid in tablet
 $= \frac{0.2119 \text{ g ascorbic acid}}{0.4242 \text{ g tablet}} \times 100 = 49.95 \text{ wt\%}$
- 6-C.** (a) moles of $\text{Na}_2\text{C}_2\text{O}_4$ in 1 L = $(3.514 \text{ g}) / (134.00 \text{ g/mol})$
 $= 0.026224 \text{ mol}$
 $\text{C}_2\text{O}_4^{2-}$ in 25.00 mL = $(0.026224 \text{ M})(0.02500 \text{ L})$
 $= 6.556_0 \times 10^{-4} \text{ mol}$
 $\text{mol MnO}_4^- = (\text{mol C}_2\text{O}_4^{2-}) \left(\frac{2 \text{ mol MnO}_4^-}{5 \text{ mol C}_2\text{O}_4^{2-}} \right)$
 $= (6.556_0 \times 10^{-4} \text{ mol}) \left(\frac{2}{5} \right)$
 $= 2.622_4 \times 10^{-4} \text{ mol}$
 equivalence volume of KMnO_4
 $= 24.44 - 0.03 = 24.41 \text{ mL}$
 $[\text{MnO}_4^-] = \frac{2.622_4 \times 10^{-4} \text{ mol}}{0.02441 \text{ L}} = 0.01074_3 \text{ M}$

(b) moles of KMnO_4 consumed

$$= (0.02500 \text{ L})(0.01074_3 \text{ M}) = 2.685_8 \times 10^{-4} \text{ mol}$$

$$\text{mol NaNO}_2 \text{ reacting with KMnO}_4$$

$$= (2.685_8 \times 10^{-4} \text{ mol KMnO}_4) \left(\frac{5 \text{ mol NaNO}_2}{2 \text{ mol KMnO}_4} \right)$$

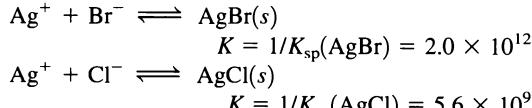
$$= 6.714_4 \times 10^{-4} \text{ mol}$$

$$[\text{NaNO}_2] = \frac{6.714_4 \times 10^{-4} \text{ mol}}{0.03811 \text{ L}} = 0.01762 \text{ M}$$

- 6-D.** (a) $\text{PbBr}_2(s) \xrightleftharpoons{K_{sp}} \text{Pb}^{2+} + 2\text{Br}^-$
 $x(2x)^2 = 2.1 \times 10^{-6} \Rightarrow$
 $x = [\text{Pb}^{2+}] = 8.0_7 \times 10^{-3} \text{ M}$

(b) $\text{PbBr}_2(s) \xrightleftharpoons{K_{sp}} \text{Pb}^{2+} + 2\text{Br}^-$
 $x \quad 0.10 \text{ M}$
 $[\text{Pb}^{2+}](0.10)^2 = 2.1 \times 10^{-6} \Rightarrow$
 $[\text{Pb}^{2+}] = 2.1 \times 10^{-4} \text{ M}$

- 6-E.** (a) For salts with identical stoichiometry of cations and anions, the one with smaller K_{sp} (AgBr) precipitates first. You can also see this by writing the reverse reaction and looking for the larger equilibrium constant:



(b) mol Br^- = mol Ag^+ at first end point
 $= (0.01555 \text{ L})(0.03333 \text{ M})$
 $= 5.183 \times 10^{-4} \text{ mol}$

The original concentration of Br^- was therefore

$$[\text{Br}^-] = (5.183 \times 10^{-4} \text{ mol}) / (0.02500 \text{ L})$$

$$= 0.02073 \text{ M}$$

(c) mol Cl^- = mol Ag^+ at second end point
 $- \text{mol Ag}^+$ at first end point
 $= (0.04223 \text{ L} - 0.01555 \text{ L})(0.03333 \text{ M})$
 $= 8.892 \times 10^{-4} \text{ mol}$

The original concentration of Cl^- was therefore
 $[\text{Cl}^-] = (8.892 \times 10^{-4} \text{ mol}) / (0.02500 \text{ L})$
 $= 0.03557 \text{ M}$

- 6-F.** (a) AgCl is more soluble than AgSCN and will slowly dissolve in the presence of excess SCN^- . This reaction consumes the SCN^- and causes the red end-point color to fade. If the AgCl is removed by filtration, it is no longer present when SCN^- is added in the back titration.
 (b) Consider the titration of C^+ (in a flask) by A^- (from a buret). Before the equivalence point, there is excess C^+ in solution. Selective adsorption of C^+ on the CA crystal surface gives the crystal a positive charge. After the equivalence point, there is excess A^- in solution. Selective adsorption of A^- on the CA crystal surface gives it a negative charge.
 (c) Beyond the equivalence point, there is excess $\text{Fe}(\text{CN})_6^{4-}$ in solution. Adsorption of this anion on the precipitate will make the particles *negative*.

Chapter 7

7-A. (a) $\frac{0.214\text{ g AgBr}}{187.772\text{ g AgBr/mol}} = 1.142\text{ g} \times 10^{-3}\text{ mol AgBr}$

(b) $[\text{NaBr}] = \frac{1.142\text{ g} \times 10^{-3}\text{ mol}}{50.00\text{ g} \times 10^{-3}\text{ L}} = 0.022\text{ M}$

- 7-B. (a) Absorbed impurities are taken into a substance. Adsorbed impurities are found on the surface of a substance.
- (b) Included impurities occupy lattice sites of the host crystal. Occluded impurities are trapped in a pocket inside the host.
- (c) An ideal gravimetric precipitate should be insoluble, be easily filtered, possess a known, constant composition, and be stable to heat.
- (d) High supersaturation often leads to formation of colloidal product with a large amount of impurities.
- (e) Supersaturation can be decreased by increasing temperature (for most solutions), mixing well during addition of precipitant, and using dilute reagents. Homogeneous precipitation also reduces supersaturation.
- (f) Washing with electrolyte preserves the electric double layer and prevents peptization.
- (g) The volatile HNO_3 evaporates during drying. NaNO_3 is nonvolatile and will lead to a high mass for the precipitate.
- (h) During the first precipitation, the concentration of impurities in the solution is high, giving a relatively high concentration of impurities in the precipitate. In the reprecipitation, the level of solution impurities is reduced, thus giving a purer precipitate.
- (i) In thermogravimetric analysis, the mass of a sample is measured as the sample is heated. The mass lost during decomposition provides information about the composition of the sample.

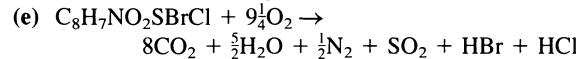
7-C. (a) $\frac{0.104\text{ g CeO}_2}{172.115\text{ g CeO}_2/\text{mol}} = 6.043 \times 10^{-4}\text{ mol CeO}_2$
 $6.043 \times 10^{-4}\text{ mol CeO}_2 \times \frac{1\text{ mol Ce}}{1\text{ mol CeO}_2} = 6.043 \times 10^{-4}\text{ mol Ce}$
 $(6.043 \times 10^{-4}\text{ mol Ce})(140.116\text{ g Ce/mol Ce}) = 0.084\text{ g Ce}$

(b) $\text{wt\% Ce} = \frac{0.084\text{ g Ce}}{4.37\text{ g unknown}} \times 100 = 1.94\text{ wt\%}$

- 7-D. (a) In *combustion*, a substance is heated in the presence of excess O_2 to convert carbon into CO_2 and hydrogen into H_2O . In *pyrolysis*, the substance is decomposed by heating in the absence of added O_2 . All oxygen in the sample is converted into CO by passage through a suitable catalyst.
- (b) WO_3 catalyzes the complete combustion of C to CO_2 in the presence of excess O_2 . Cu reduces SO_3 to SO_2 and removes excess O_2 from the gas stream.
- (c) The tin capsule melts and is oxidized to SnO_2 to liberate heat and crack the sample. Tin uses the available oxygen

immediately, ensures that sample oxidation occurs in the gas phase, and acts as an oxidation catalyst.

- (d) By dropping the sample in before very much O_2 is present, pyrolysis of the sample to give gaseous products occurs prior to oxidation. This practice minimizes the formation of nitrogen oxides.



Chapter 8

- 8-A. hydronium ion . . . proton donor . . . proton acceptor . . . neutralize . . . conjugate

- 8-B. (a) Our strategy is to write the solubility product for $\text{Mg}(\text{OH})_2$ and substitute the known value of $[\text{Mg}^{2+}] = 0.050\text{ M}$. Then we can solve for $[\text{OH}^-]$.

$$[\text{Mg}^{2+}][\text{OH}^-]^2 = K_{\text{sp}} = 7.1 \times 10^{-12}$$

$$\frac{0.050\text{ M}}{?} = (0.050)[\text{OH}^-]^2$$

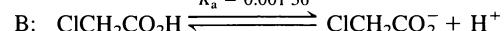
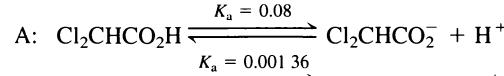
$$\Rightarrow [\text{OH}^-] = 1.19 \times 10^{-5}\text{ M}$$

- (b) Knowing the concentration of OH^- , we can compute $[\text{H}^+]$ and pH.

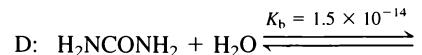
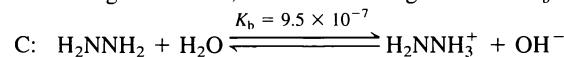
$$[\text{H}^+] = K_w/[\text{OH}^-] = 8.39 \times 10^{-10}\text{ M}$$

$$\Rightarrow \text{pH} = -\log[\text{H}^+] = 9.08$$

- 8-C. The stronger acid is A, which has the larger value of K_a .



The stronger base is C, which has the larger value of K_b .



- 8-D. (a) (i) $\text{pH} = -\log[\text{H}^+] = -\log(1.0 \times 10^{-3}) = 3.00$

$$\text{(ii) pH} = -\log[\text{H}^+] = -\log(K_w/[\text{OH}^-]) = -\log(1.0 \times 10^{-14}/1.0 \times 10^{-2}) = 12.00$$

- (b) (i) $\text{pH} = -\log(3.2 \times 10^{-5}) = 4.49$

$$\text{(ii) pH} = -\log(1.0 \times 10^{-14}/0.0077) = 11.89$$

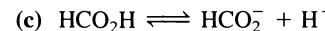
- (c) $[\text{H}^+] = 10^{-4.44} = 3.6 \times 10^{-5}\text{ M}$

- (d) $[\text{H}^+] = K_w/[\text{OH}^-] = 1.0 \times 10^{-14}/0.0077 = 1.3 \times 10^{-12}\text{ M}$
 H^+ is derived from $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$

- (e) The concentration of the strong base is too low to perturb the pH of pure water. The pH is very close to 7.00.

- 8-E. (a) $\text{p}K_a = 3$

- (b) $\text{p}K_b = 3$



- (d) HCO_2^-

$$\text{(e) } K_a = \frac{[\text{HCO}_2^-][\text{H}^+]}{[\text{HCO}_2\text{H}]} = 1.80 \times 10^{-4}$$

$$\text{(f) } K_b = \frac{[\text{HCO}_2\text{H}][\text{OH}^-]}{[\text{HCO}_2^-]}$$

$$\text{(g) } K_b = \frac{K_w}{K_a} = 5.56 \times 10^{-11}$$

- 8-F. (a) Let $x = [\text{H}^+] = [\text{A}^-]$ and $0.100 - x = [\text{HA}]$.

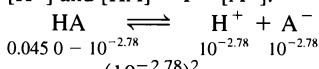
$$\frac{x^2}{0.100 - x} = 1.00 \times 10^{-5} \Rightarrow x = 9.95 \times 10^{-4}\text{ M}$$

$$\Rightarrow \text{pH} = -\log x = 3.00$$

fraction of dissociation

$$= \frac{[\text{A}^-]}{[\text{A}^-] + [\text{HA}]} = \frac{9.95 \times 10^{-4}}{0.100} = 9.95 \times 10^{-3}$$

- (b) Write the acid dissociation equation and substitute $[\text{H}^+] = 10^{-\text{pH}} = 10^{-2.78}$. But, we also know that $[\text{A}^-] = [\text{H}^+]$ and $[\text{HA}] = F - [\text{A}^-]$.

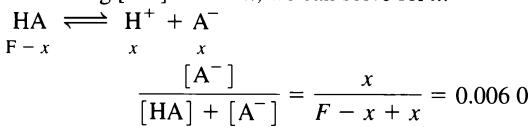


$$K_a = \frac{(10^{-2.78})^2}{0.0450 - 10^{-2.78}} = 6.4 \times 10^{-5} \Rightarrow \text{p}K_a = 4.19$$

- (c) We are told that the fraction of dissociation is 0.60%,

$$\text{which means that } \frac{[\text{A}^-]}{([\text{A}^-] + [\text{HA}])} = 0.0060.$$

Writing the weak-acid equilibrium and setting $[\text{H}^+] = [\text{A}^-] = x$ and setting $[\text{HA}] = F - x$, we can solve for x :



With $F = 0.0450 \text{ M}$, $x = 2.7 \times 10^{-4} \text{ M}$

$$\Rightarrow K_a = \frac{[\text{A}^-][\text{H}^+]}{[\text{HA}]} = \frac{x^2}{F - x} = 1.6 \times 10^{-6}$$

$$\Rightarrow \text{p}K_a = 5.79$$

- 8-G. (a) Let $x = [\text{OH}^-] = [\text{BH}^+]$ and $0.100 - x = [\text{B}]$.

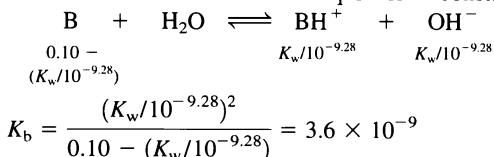
$$\frac{x^2}{0.100 - x} = 1.00 \times 10^{-5}$$

$$\Rightarrow x = 9.95 \times 10^{-4} \text{ M} \Rightarrow [\text{H}^+] = \frac{K_w}{x}$$

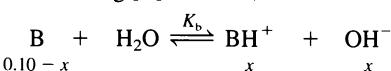
$$= 1.005 \times 10^{-11} \Rightarrow \text{pH} = 11.00$$

$$\frac{[\text{BH}^+]}{[\text{B}] + [\text{BH}^+]} = \frac{9.95 \times 10^{-4}}{0.100} = 9.95 \times 10^{-3}$$

- (b) We know that $[\text{H}^+] = 10^{-\text{pH}} = 10^{-9.28}$. We also know that $[\text{OH}^-] = K_w/[\text{H}^+]$. In the weak-base equilibrium, $[\text{BH}^+] = [\text{OH}^-]$ and $[\text{B}] = F - [\text{BH}^+]$. Inserting all these values lets us solve for the equilibrium constant.



- (c) We are told that the fraction of association is 2.0%, which means that $\frac{[\text{BH}^+]}{([\text{B}] + [\text{BH}^+])} = 0.020$. Writing the weak-base equilibrium and setting $[\text{BH}^+] = [\text{OH}^-] = x$ and setting $[\text{B}] = F - x$, we can solve for x :



$$\frac{x}{0.10 - x} = 0.020 \Rightarrow x = 0.0020$$

$$K_b = \frac{[\text{BH}^+][\text{OH}^-]}{[\text{B}]} = \frac{x^2}{0.10 - x}$$

$$= \frac{(0.0020)^2}{0.10 - 0.0020} = 4.1 \times 10^{-5}$$

Chapter 9

$$9-\text{A. (a)} \text{ pH} = \text{p}K_a + \log\left(\frac{[\text{A}^-]}{[\text{HA}]}\right)$$

$$= 5.00 + \log\left(\frac{0.050}{0.100}\right) = 4.70$$

$$(b) \text{ pH} = 3.744 + \log\left(\frac{[\text{HCO}_3^-]}{[\text{HCO}_2\text{H}]}\right) = 3.744 + \log R,$$

where we abbreviate the quotient $[\text{HCO}_3^-]/[\text{HCO}_2\text{H}]$ by R . At pH 3.00, we solve for R as follows:

$$3.00 = 3.744 + \log R$$

$$\log R = 3.00 - 3.744 = -0.744$$

$$10^{\log R} = 10^{-0.744}$$

$$R = 0.180$$

pH:	3.000	3.744	4.000
$[\text{HCO}_3^-]/[\text{HCO}_2\text{H}]$:	0.180	1.00	1.80

$$9-\text{B. (a)} \text{ pH} = \text{p}K_a + \log\left(\frac{[\text{tris}]}{[\text{trisH}^+]}\right)$$

$$= 8.07 + \log\left(\frac{(10.0 \text{ g})/(121.14 \text{ g/mol})}{(10.0 \text{ g})/(157.60 \text{ g/mol})}\right) = 8.18$$

- (b) The number of mmol of HClO_4 added is $(10.5 \text{ mL}) \times (0.500 \text{ M}) = 5.25 \text{ mmol}$

	tris	$+$	H^+	\rightarrow	tris H^+	$+$	H_2O
Initial mmol	82.55		5.25		63.45		
Final mmol	77.30		—		68.70		

$$\text{pH} = 8.07 + \log\left(\frac{77.30}{68.70}\right) = 8.12$$

- (c) The number of mmol of NaOH added is $(10.5 \text{ mL}) \times (0.500 \text{ M}) = 5.25 \text{ mmol}$.

	tris H^+	$+$	OH^-	\rightarrow	tris	$+$	H_2O
Initial mmol	63.45		5.25		82.55		
Final mmol	58.20		—		87.80		

$$\text{pH} = 8.07 + \log\left(\frac{87.80}{58.20}\right) = 8.25$$

- 9-C. (a) mol tris = $10.0 \text{ g}/(121.14 \text{ g/mol})$

$$= 0.0825_5 \text{ mol} = 82.5_5 \text{ mmol}$$

	tris	$+$	H^+	\rightarrow	tris H^+	$+$	H_2O
Initial mmol	82.5_5		x		—		
Final mmol	82.5_5 - x		—		x		

$$\text{pH} = \text{p}K_a + \log\left(\frac{[\text{tris}]}{[\text{trisH}^+]}\right)$$

$$7.60 = 8.07 + \log\left(\frac{82.5_5 - x}{x}\right)$$

$$-0.47 = \log\left(\frac{82.5_5 - x}{x}\right) \Rightarrow 10^{-0.47} = 10^{\log[(82.5_5 - x)/x]}$$

$$0.33_{88} = \frac{82.5_5 - x}{x} \Rightarrow x = 61.66 \text{ mmol}$$

volume of HCl required

$$= (0.061.66 \text{ mol})/(1.20 \text{ M}) = 51.4 \text{ mL}$$

- (b) I would weigh out 0.020 0 mol of acetic acid (= 1.201 g) and place it in a beaker with ~75 mL of water. While monitoring the pH with a pH electrode, I would add 3 M NaOH (~4 mL are required) until the pH is exactly 5.00. I would then pour the solution into a 100-mL volumetric flask and wash the beaker several times with a few milliliters of distilled water. Each washing would be added to the volumetric flask to ensure quantitative transfer from the beaker to the flask. After swirling the volumetric flask to mix the solution, I would carefully add water up to the 100-mL mark, insert the cap, and invert many times to ensure complete mixing.

9-D. (a) Compound	pK_a
Hydroxybenzene	9.98
Propanoic acid	4.87
Cyanoacetic acid	2.47 ← Most suitable, because pK_a is closest to pH
Sulfuric acid	1.99

- (b) Buffer capacity is based on the ability of buffer to react with added acid or base, without making a large change in the ratio of concentrations $[A^-]/[HA]$. The greater the concentration of each component, the less relative change is brought about by reaction with a small increment of added acid or base.

- (c) From the given values of K_b , compute $K_a = K_w/K_b$ and $pK_a = -\log K_a$.

Compound	pK_a (for conjugate acid)
Ammonia	9.26 ← Most suitable, because pK_a is closest to pH
Aniline	4.60
Hydrazine	7.98
Pyridine	5.20

- 9-E. (a) The quotient $[HIn]/[In^-]$ changes from 10:1 when $pH = pK_{HIn} - 1$ to 1:10 when $pH = pK_{HIn} + 1$. This change is generally sufficient to cause a complete color change.

- (b) red, orange, yellow

Chapter 10

- 10-A. The titration reaction is $H^+ + OH^- \rightarrow H_2O$ and $V_e = 5.00 \text{ mL}$ because

$$\underbrace{(V_e \text{ (mL)})(0.100 \text{ M})}_{\text{mmol HCl at } V_e} = \underbrace{(50.00 \text{ mL})(0.010 0 \text{ M})}_{\text{Initial mmol NaOH}} \Rightarrow V_e = 5.00 \text{ mL}$$

Representative calculations:

$$V_a = 1.00 \text{ mL}$$

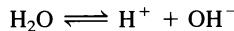
$$\begin{aligned} OH^- &= \underbrace{(50.00 \text{ mL})(0.010 0 \text{ M})}_{\text{Initial mmol NaOH}} - \underbrace{(1.00 \text{ mL})(0.100 \text{ M})}_{\text{Added mmol HCl}} \\ &= 0.400 \text{ mmol} \end{aligned}$$

$$[OH^-] = (0.400 \text{ mmol})/(51.00 \text{ mL}) = 0.007 84 \text{ M}$$

$$[H^+] = K_w/(0.007 84 \text{ M}) = 1.28 \times 10^{-12} \text{ M}$$

$$pH = -\log(1.28 \times 10^{-12}) = 11.89$$

$$V_a = V_e = 5.00 \text{ mL}$$



$$K_w = x^2 \Rightarrow x = 1.00 \times 10^{-7} \text{ M} \Rightarrow pH = 7.00$$

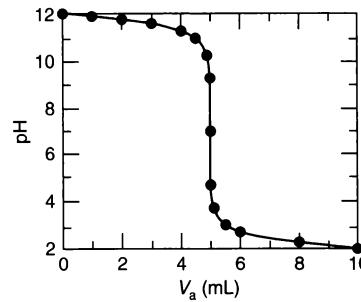
$$V_a = 5.01 \text{ mL}$$

$$\text{excess H}^+ = (0.01 \text{ mL})(0.100 \text{ M}) = 0.001 \text{ mmol}$$

$$[H^+] = (0.001 \text{ mmol})/(55.01 \text{ mL}) = 1.8 \times 10^{-5} \text{ M}$$

$$pH = -\log(1.8 \times 10^{-5}) = 4.74$$

V_a (mL)	pH	V_a (mL)	pH	V_a (mL)	pH
0.00	12.00	4.50	10.96	5.10	3.74
1.00	11.89	4.90	10.26	5.50	3.05
2.00	11.76	4.99	9.26	6.00	2.75
3.00	11.58	5.00	7.00	8.00	2.29
4.00	11.27	5.01	4.74	10.00	2.08



- 10-B. Titration reaction: $HCO_2H + OH^- \rightarrow HCO_2^- + H_2O$

$$\underbrace{(V_e \text{ (mL)})(0.050 0 \text{ M})}_{\text{mmol KOH at } V_e} = \underbrace{(50.0 \text{ mL})(0.050 0 \text{ M})}_{\text{Initial mmol HCO}_2\text{H}}$$

$$\Rightarrow V_e = 50.0 \text{ mL}$$

Representative calculations:



$$\begin{array}{ccccccc} 0.050 0 & - & x & & x & & \\ \hline K_a & = & 1.80 \times 10^{-4} & & pK_a & = 3.744 & \\ \frac{x^2}{0.050 0 - x} & = & K_a & \Rightarrow & x & = 2.91 \times 10^{-3} & \Rightarrow pH = 2.54 \end{array}$$

$$V_b = 48.0 \text{ mL}$$

	HA	+	OH^-	→	A^-	+	H_2O
Initial mmol	2.50		2.40		—		
Final mmol	0.10		—		2.40		

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right) = 3.744 + \log\left(\frac{2.40}{0.10}\right) = 5.12$$

$$V_b = 50.0 \text{ mL}: \text{formal conc. of } A^-$$

$$= \frac{(50.0 \text{ mL})(0.050 0 \text{ M})}{100.0 \text{ mL}} = 0.025 0 \text{ M}$$

$$A^- + H_2O \rightleftharpoons HA + OH^-$$

$$0.0250 - x \quad x \quad x$$

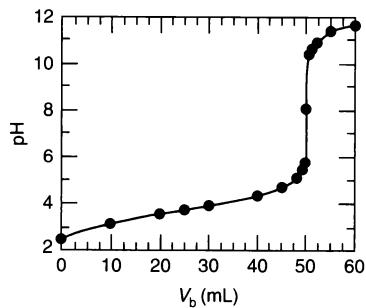
$$K_b = K_w/K_a = 5.56 \times 10^{-11}$$

$$\frac{x^2}{0.0250 - x} = K_b \Rightarrow x = 1.18 \times 10^{-6} \Rightarrow$$

$$pH = -\log\left(\frac{K_w}{x}\right) = 8.07$$

$$V_b = 60.0 \text{ mL: excess } [OH^-] = \frac{(10.0 \text{ mL})(0.0500 \text{ M})}{110.0 \text{ mL}} = 4.55 \times 10^{-3} \text{ M} \Rightarrow pH = 11.66$$

V_a (mL)	pH	V_a (mL)	pH	V_a (mL)	pH
0.0	2.54	45.0	4.70	50.5	10.40
10.0	3.14	48.0	5.12	51.0	10.69
20.0	3.57	49.0	5.44	52.0	10.99
25.0	3.74	49.5	5.74	55.0	11.38
30.0	3.92	50.0	8.07	60.0	11.66
40.0	4.35				

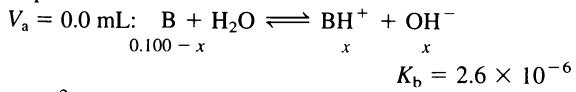


At $V_b = \frac{1}{2} V_e$, the pH should equal pK_a , which it does.

- 10-C. (a) Titration of a weak base, B, produces the conjugate acid, BH^+ , which is necessarily acidic.
 (b) The titration reaction is $B + H^+ \rightarrow BH^+$.

$$\text{Find } V_e: (V_e)(0.200 \text{ M}) = (100.0 \text{ mL})(0.100 \text{ M}) \Rightarrow V_e = 50.0 \text{ mL}$$

Representative calculations:



$$\frac{x^2}{0.100 - x} = K_b = 2.6 \times 10^{-6} \Rightarrow x = 5.09 \times 10^{-4}$$

$$pH = -\log\left(\frac{K_w}{x}\right) = 10.71$$

$V_a = 20.0 \text{ mL: }$

B	+ H ⁺	→ BH ⁺
Initial mmol	10.00	4.00
Final mmol	6.00	—

Initial mmol	10.00	4.00	—
Final mmol	6.00	—	4.00

$$pH = pK_a (\text{for } BH^+) + \log\left(\frac{[B]}{[BH^+]}\right)$$

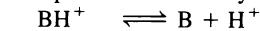
$$K_a = \frac{K_w}{K_b}$$

$$= 8.41 + \log\left(\frac{6.00}{4.00}\right) = 8.59$$

$V_a = V_e = 50 \text{ mL: All B has been converted into the conjugate acid, } BH^+. \text{ The formal concentration of } BH^+ \text{ is}$

$$F' = \frac{(100.0 \text{ mL})(0.100 \text{ M})}{150.0 \text{ mL}} = 0.0667 \text{ M}$$

The pH is determined by the reaction



$$\frac{x^2}{0.0667 - x} = K_a = \frac{K_w}{K_b} \Rightarrow x = 1.60 \times 10^{-5}$$

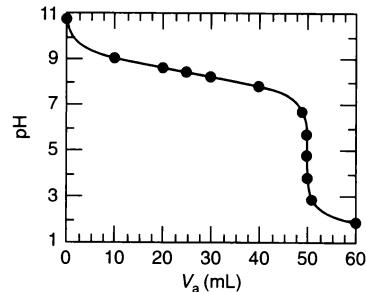
$$\Rightarrow pH = 4.80$$

$V_a = 51.0 \text{ mL: There is excess } [H^+].$

$$\text{excess } [H^+] = \frac{(1.0 \text{ mL})(0.200 \text{ M})}{(151.0 \text{ mL})} = 1.32 \times 10^{-3}$$

$$\Rightarrow pH = 2.88$$

V_a (mL)	pH	V_a (mL)	pH	V_a (mL)	pH
0.0	10.71	30.0	8.23	50.0	4.80
10.0	9.01	40.0	7.81	50.1	3.88
20.0	8.59	49.0	6.72	51.0	2.88
25.0	8.41	49.9	5.71	60.0	1.90



10-D. (a) Figure 10-1: bromothymol blue: blue → yellow

Figure 10-2: thymol blue: yellow → blue

Figure 10-11 ($pK_a = 8$): thymolphthalein: colorless → blue

- (b) The derivatives are shown in the following spreadsheet. In the first derivative graph, the maximum is near 119 μL. In Figure 10-6, the second derivative graph gives an end point of 118.9 μL.

A	B	C	D	E	F
1			First Derivative		Second Derivative
2	μL NaOH	pH	μL	Derivative	μL
3	107	6.921			
4	110	7.117	108.5	6.533E-02	
5	113	7.359	111.5	8.067E-02	110
6	114	7.457	113.5	9.800E-02	112.5
7	115	7.569	114.5	1.120E-01	114
8	116	7.705	115.5	1.360E-01	115
9	117	7.878	116.5	1.730E-01	116
10	118	8.090	117.5	2.120E-01	117
11	119	8.343	118.5	2.530E-01	118
12	120	8.591	119.5	2.480E-01	119
13	121	8.794	120.5	2.030E-01	120
14	122	8.952	121.5	1.580E-01	121
15					
16	C4 = (A4+A3)/2			E5 = (C5+C4)/2	
17	D4 = (B4-B3)/(A4-A3)			F5 = (D5-D4)/(C5-C4)	

10-E. (a) Tris(hydroxymethyl)aminomethane ($\text{H}_2\text{NC}(\text{CH}_2\text{OH})_3$), sodium carbonate (Na_2CO_3), or borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) can be used to standardize HCl. Potassium hydrogen phthalate ($\text{HO}_2\text{C}-\text{C}_6\text{H}_4-\text{CO}_2\text{K}^+$) or potassium hydrogen iodate ($\text{KH}(\text{IO}_3)_2$) can be used to standardize NaOH.

(b) $30 \text{ mL of } 0.05 \text{ M OH}^- = 1.5 \text{ mmol OH}^- = 1.5 \text{ mmol potassium hydrogen phthalate; } (1.5 \times 10^{-3} \text{ mol}) \times (204.22 \text{ g/mol}) = 0.30 \text{ g of potassium hydrogen phthalate}$

10-F. (a) $5.00 \text{ mL of } 0.0336 \text{ M HCl} = 0.168_0 \text{ mmol}$

$6.34 \text{ mL of } 0.0100 \text{ M NaOH} = 0.0634 \text{ mmol}$

$$\text{HCl consumed by NH}_3 = 0.168_0 - 0.0634 \\ = 0.104_6 \text{ mmol}$$

$$\text{mol NH}_3 = \text{mol HCl} = 0.104_6 \text{ mmol}$$

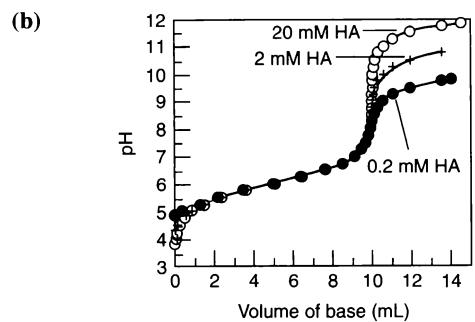
(b) mol nitrogen = mol $\text{NH}_3 = 0.104_6 \text{ mmol}$

$$(0.104_6 \times 10^{-3} \text{ mol N})(14.0067 \text{ g/mol}) \\ = 1.46_5 \text{ mg of nitrogen}$$

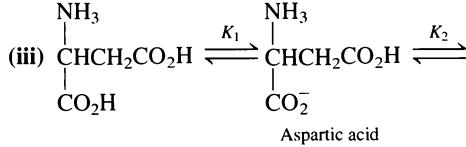
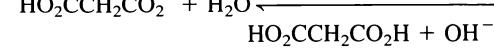
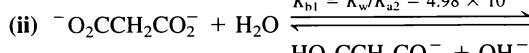
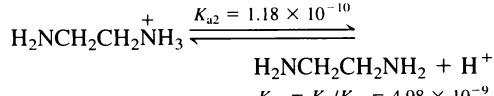
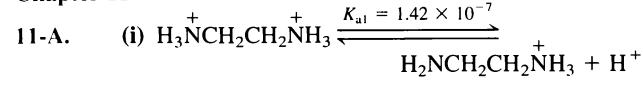
$$(\text{c}) (256 \mu\text{L}) \left(\frac{1 \text{ mL}}{1000 \mu\text{L}} \right) (37.9 \text{ mg protein/mL}) \\ = 9.70_2 \text{ mg protein}$$

$$(\text{d}) \text{ wt\% N} = \frac{1.46_5 \text{ mg N}}{9.70_2 \text{ mg protein}} \times 100 = 15.1 \text{ wt\%}$$

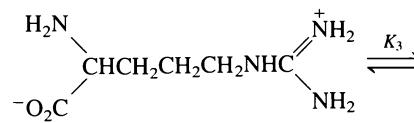
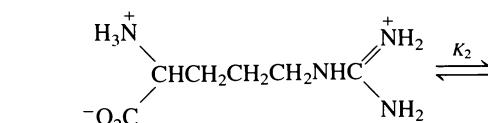
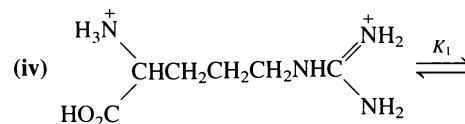
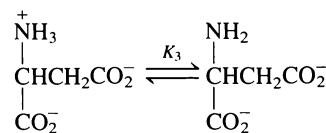
10-G. (a) Your spreadsheet should reproduce the results in Figure 10-11.



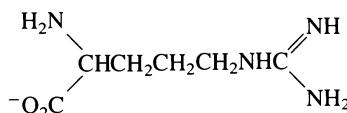
Chapter 11



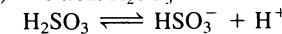
Aspartic acid



Arginine



11-B. (a) We treat H_2SO_3 as a weak monoprotic acid:



$$\frac{x^2}{0.050 - x} = K_1 = 1.39 \times 10^{-2} \Rightarrow x = 2.03 \times 10^{-2} \text{ M}$$

$$[\text{HSO}_3^-] = [\text{H}^+] = 2.03 \times 10^{-2} \text{ M} \Rightarrow \text{pH} = 1.69$$

$$[\text{H}_2\text{SO}_3] = 0.050 - x = 0.030 \text{ M}$$

$$[\text{SO}_3^{2-}] = \frac{K_2[\text{HSO}_3^-]}{[\text{H}^+]} = K_2 = 6.73 \times 10^{-8} \text{ M}$$

(b) HSO_3^- is the intermediate form of a diprotic acid.

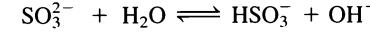
$$\text{pH} \approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2) = \frac{1}{2}(1.857 + 7.172) = 4.51$$

$$[\text{H}^+] = 10^{-\text{pH}} = 3.1 \times 10^{-5} \text{ M}; \quad [\text{HSO}_3^-] \approx 0.050 \text{ M}$$

$$[\text{H}_2\text{SO}_3] = \frac{[\text{H}^+][\text{HSO}_3^-]}{K_1} = \frac{(3.1 \times 10^{-5})(0.050)}{1.39 \times 10^{-2}} = 1.1 \times 10^{-4} \text{ M}$$

$$[\text{SO}_3^{2-}] = \frac{K_2[\text{HSO}_3^-]}{[\text{H}^+]} = \frac{(6.73 \times 10^{-8})(0.050)}{3.1 \times 10^{-5}} = 1.1 \times 10^{-4} \text{ M}$$

(c) We treat SO_3^{2-} as if it were monobasic:



$$\frac{x^2}{0.050 - x} = K_{b1} = \frac{K_w}{K_{a2}} = 1.49 \times 10^{-7} \Rightarrow x = 8.62 \times 10^{-5}$$

$$[\text{HSO}_3^-] = 8.62 \times 10^{-5} \text{ M};$$

$$[\text{H}^+] = \frac{K_w}{x} = 1.16 \times 10^{-10} \text{ M} \Rightarrow \text{pH} = 9.94$$

$$[\text{SO}_3^{2-}] = 0.050 - x = 0.050 \text{ M}$$

$$[\text{H}_2\text{SO}_3] = \frac{[\text{H}^+][\text{HSO}_3^-]}{K_1} = 7.2 \times 10^{-13} \text{ M}$$

11-C. (a)

	pH 9.00	pH 11.00
Principal species:		
Secondary species:		

Reasoning: The acid dissociation constants are $\text{p}K_1 = 9.30$ and $\text{p}K_2 = 11.06$. pH 9.00 is below $\text{p}K_1$, so H_2A is the predominant form. If H_2A is predominant, HA^- must be the second most abundant species. pH 11.00 is just below $\text{p}K_2 = 11.06$. At pH = 11.06, HA^- and A^{2-} would be present in equal concentration. At pH 11.00, the more acidic HA^- will be slightly more abundant than the basic form, A^{2-} .

$$(b) \text{HC}^-: \text{pH} \approx \frac{1}{2}(\text{p}K_2 + \text{p}K_3) = \frac{1}{2}(8.36 + 10.74) = 9.55$$

$$11-\text{D. (a)} \underbrace{(50.0 \text{ mL})(0.050 \text{ M})}_{\text{mmol H}_2\text{A}} = \underbrace{V_{e1}(0.100 \text{ M})}_{\text{mmol OH}^-}$$

$$\Rightarrow V_{e1} = 25.0 \text{ mL}$$

$$V_{e2} = 2V_{e1} = 50.0 \text{ mL}$$

(b) 0 mL: Treat H_2A as a monoprotic weak acid.

$$\begin{array}{ccccccc} \text{H}_2\text{A} & \xrightleftharpoons{K_1} & \text{H}^+ & + & \text{HA}^- \\ 0.050 & 0 & -x & & x & & \\ \frac{x^2}{0.050 & 0 & -x} & & & & & & \\ \Rightarrow x = 7.75 \times 10^{-3} & & & & & & \Rightarrow \text{pH} = 2.11 \end{array}$$

12.5 mL: We are halfway to the first equivalence point and there is a 1:1 mixture of H_2A and HA^- : $\text{pH} = \text{p}K_1 = 2.85$.

25.0 mL: This is the first equivalence point. H_2A has been converted into HA^- , the intermediate form of a diprotic acid.

$$\begin{aligned} \text{pH} &\approx \frac{1}{2}(\text{p}K_1 + \text{p}K_2) = \frac{1}{2}(2.847 + 5.696) \\ &= 4.27 \end{aligned}$$

37.5 mL: Half of HA^- has been converted into A^{2-} , so $\text{pH} = \text{p}K_2 = 5.70$.

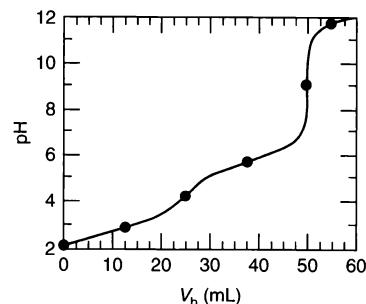
50.0 mL: H_2A has been converted into A^{2-} at a formal concentration of

$$\begin{aligned} F &= \frac{(50.0 \text{ mL})(0.050 \text{ M})}{100.0 \text{ mL}} = 0.025 \text{ M} \\ \text{A}^{2-} + \text{H}_2\text{O} &\rightleftharpoons \text{HA}^- + \text{OH}^- \\ \frac{x^2}{0.025 & 0 & -x} &= K_{b1} = \frac{K_w}{K_{a2}} \end{aligned}$$

$$\begin{aligned} \Rightarrow x &= 1.12 \times 10^{-5} \\ \Rightarrow \text{pH} &= -\log\left(\frac{K_w}{x}\right) = 9.05 \end{aligned}$$

55.0 mL: There is an excess of 5.0 mL of OH^- :

$$\begin{aligned} [\text{OH}^-] &= \frac{(5.0 \text{ mL})(0.100 \text{ M})}{105.0 \text{ mL}} \\ &= 0.00476 \text{ M} \Rightarrow \text{pH} = 11.68 \end{aligned}$$



Chapter 12

$$12-\text{A. (a)} \text{PbI}_2(s) \xrightleftharpoons{x} \text{Pb}^{2+} + 2\text{I}^-$$

$$x(2x)^2 = K_{sp} = 7.9 \times 10^{-9} \Rightarrow 2x = [\text{I}^-] = 2.5 \text{ mM}$$

The observed concentration of dissolved iodine in pure water is approximately 3.8 mM, or 50% higher than predicted. One reason for this is that there are more species in solution than just Pb^{2+} and I^- , such as PbI^+ . A second reason is that as PbI_2 dissolves, it increases its own solubility by adding ions to the solution to create ionic atmospheres around the dissolved ions and decreasing their attraction for each other. When KNO_3 is added, the number of ions in the ionic atmosphere increases further, thereby decreasing the attraction of Pb^{2+} and I^- for each other, and increasing the solubility of PbI_2 .

$$\begin{aligned} (b) \mu &= \frac{1}{2}\{[\text{Pb}^{2+}] \cdot (+2)^2 + [\text{I}^-] \cdot (-1)^2\} \\ &= \frac{1}{2}\{(0.0010 \cdot 4) + (0.0020 \cdot 1)\} = 0.0030 \text{ M} \end{aligned}$$

$$12-\text{B. (a)} \text{HgBr}_2(s) \rightleftharpoons \text{Hg}^{2+} + 2\text{Br}^- \Rightarrow$$

$$K_{sp} = [\text{Hg}^{2+}] \gamma_{\text{Hg}^{2+}} [\text{Br}^-]^2 \gamma_{\text{Br}^-}^2$$

$$1.3 \times 10^{-19} = (x)(1)(2x)^2(1)^2$$

$$1.3 \times 10^{-19} = 4x^3$$

$$x = [\text{Hg}^{2+}] = \sqrt[3]{\frac{1.3 \times 10^{-19}}{4}} = 3.2 \times 10^{-7} \text{ M}$$

The value of K_{sp} comes from Appendix A. To find the cube root of a number, you can raise the number to the 1/3 power on your calculator.

(b) The concentration of Br^- is 0.050 M from NaBr . The ionic strength is 0.050 M. The activity coefficients in Table 12-1 are $\gamma_{\text{Hg}^{2+}} = 0.465$ and $\gamma_{\text{Br}^-} = 0.805$.

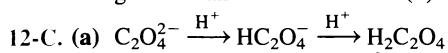
$$\text{HgBr}_2(s) \rightleftharpoons \text{Hg}^{2+} + 2\text{Br}^- \Rightarrow$$

$$K_{sp} = [\text{Hg}^{2+}] \gamma_{\text{Hg}^{2+}} [\text{Br}^-]^2 \gamma_{\text{Br}^-}^2$$

$$1.3 \times 10^{-19} = [\text{Hg}^{2+}](0.465)(0.050)^2(0.805)^2$$

$$[\text{Hg}^{2+}] = \frac{1.3 \times 10^{-19}}{(0.465)(0.050)^2(0.805)^2} = 1.7 \times 10^{-16} \text{ M}$$

- (c) If the equilibrium $\text{HgBr}_2(s) + \text{Br}^- \rightleftharpoons \text{HgBr}_3^-$ also occurs, additional mercuric ion would be in solution in the form HgBr_3^- . The solubility of HgBr_2 would be greater than that calculated in (b).



The species are Na^+ , $\text{C}_2\text{O}_4^{2-}$, HC_2O_4^- , $\text{H}_2\text{C}_2\text{O}_4$, Cl^- , H^+ , OH^- , and H_2O .

- (b) Charge balance: $[\text{Na}^+] + [\text{H}^+] = 2[\text{C}_2\text{O}_4^{2-}] + [\text{HC}_2\text{O}_4^-] + [\text{Cl}^-] + [\text{OH}^-]$

(c) One mass balance states that the total concentration of Na^+ must be $2 \times \frac{5.00 \text{ mmol}}{0.100 \text{ L}} \Rightarrow [\text{Na}^+] = 0.100 \text{ M}$. A second mass balance states that the total moles of oxalate are $\frac{5.00 \text{ mmol}}{0.100 \text{ L}} \Rightarrow 0.050 \text{ M} = [\text{C}_2\text{O}_4^{2-}] + [\text{HC}_2\text{O}_4^-] + [\text{H}_2\text{C}_2\text{O}_4]$. A third mass balance is that $[\text{Cl}^-] = 0.025 \text{ M}$.

- (d) We are adding 2.50 mmol H^+ to 5.00 mmol of the base, oxalate. The predominant species are $\text{C}_2\text{O}_4^{2-}$ and HC_2O_4^- , with a negligible amount of $\text{H}_2\text{C}_2\text{O}_4$. The pH is going to be near pK_2 for oxalic acid, which is 4.27. Therefore $[\text{H}^+] \approx 10^{-4.27} \text{ M}$ and $[\text{OH}^-] = K_w/[\text{H}^+] \approx 10^{-9.73} \text{ M}$ in this solution. The charge and mass balances can be simplified by ignoring $[\text{H}_2\text{C}_2\text{O}_4]$, $[\text{H}^+]$, and $[\text{OH}^-]$ in comparison with the concentrations of major species:

Charge balance: $[\text{Na}^+] \approx 2[\text{C}_2\text{O}_4^{2-}] + [\text{HC}_2\text{O}_4^-] + [\text{Cl}^-]$

Mass balance: $0.050 \text{ M} \approx [\text{C}_2\text{O}_4^{2-}] + [\text{HC}_2\text{O}_4^-]$

- 12-D. (a) *Pertinent reactions:* Two given in the problem plus $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$.

Charge balance: Invalid because pH is fixed

Mass balance: $[\text{Ag}^+] = [\text{CN}^-] + [\text{HCN}] \quad (\text{A})$

Equilibrium constants:

$$K_{\text{sp}} = [\text{Ag}^+][\text{CN}^-] = 2.2 \times 10^{-16} \quad (\text{B})$$

$$1/K_a = \frac{[\text{HCN}]}{[\text{CN}^-][\text{H}^+]} = 1.6 \times 10^9 \quad (\text{C})$$

$$K_w = [\text{H}^+][\text{OH}^-] = 1.0 \times 10^{-14} \quad (\text{D})$$

Count equations and unknowns: There are four equations (A–D) and four unknowns: $[\text{Ag}^+]$, $[\text{CN}^-]$, $[\text{HCN}]$, and $[\text{OH}^-]$. $[\text{H}^+]$ is known.

Solve: Putting $[\text{H}^+] = 10^{-9.00} \text{ M}$ into Equation C gives

$$1.6 \times 10^9 = \frac{[\text{HCN}]}{[\text{CN}^-][1.0 \times 10^{-9}]} \Rightarrow [\text{HCN}] = 1.6[\text{CN}^-]$$

Substituting into Equation A gives

$$\begin{aligned} [\text{Ag}^+] &= [\text{CN}^-] + [\text{HCN}] \\ &= [\text{CN}^-] + 1.6[\text{CN}^-] = 2.6[\text{CN}^-] \end{aligned}$$

Substituting into Equation B gives

$$K_{\text{sp}} = 2.2 \times 10^{-16} = [\text{Ag}^+][\text{CN}^-] = (2.6[\text{CN}^-])[\text{CN}^-] \Rightarrow [\text{CN}^-] = 9.20 \times 10^{-9} \text{ M}$$

$$\begin{aligned} [\text{Ag}^+] &= K_{\text{sp}}/[\text{CN}^-] = 2.2 \times 10^{-16}/9.20 \times 10^{-9} \\ &= 2.39 \times 10^{-8} \text{ M} \end{aligned}$$

$$\begin{aligned} [\text{HCN}] &= 1.6[\text{CN}^-] = 1.6(9.20 \times 10^{-9}) \\ &= 1.47 \times 10^{-8} \text{ M} \end{aligned}$$

- (b) *Mass balance:* All species are derived from $\text{AgCN}(s)$, so the moles of silver must equal the moles of cyanide: $[\text{Ag}^+] + [\text{AgCN}(aq)] + [\text{Ag}(\text{CN})_2^-] + [\text{AgOH}(aq)]$

mol silver

$$\begin{aligned} &= [\text{CN}^-] + [\text{HCN}] + [\text{AgCN}(aq)] + 2[\text{Ag}(\text{CN})_2^-] \\ &\quad \text{mol cyanide} \\ &\text{which simplifies to } [\text{Ag}^+] + [\text{AgOH}(aq)] \\ &= [\text{CN}^-] + [\text{HCN}] + [\text{Ag}(\text{CN})_2^-]. \end{aligned}$$

- 12-E. At pH 2.00:

$$\alpha_{\text{HA}} = \frac{10^{-2.00}}{10^{-2.00} + 10^{-3.00}} = 0.90_9$$

$$\alpha_{\text{A}^-} = \frac{10^{-3.00}}{10^{-2.00} + 10^{-3.00}} = 0.090_9$$

$$\frac{[\text{HA}]}{[\text{A}^-]} = \frac{0.90_9}{0.090_9} = 10.0$$

The results for all three pH values are

pH	α_{HA}	α_{A^-}	$[\text{HA}]/[\text{A}^-]$
2.00	0.90 ₉	0.090 ₉	10.0
3.00	0.50 ₀	0.50 ₀	1.0 ₀
4.00	0.090 ₉	0.90 ₉	0.10 ₀

Of course, you already knew these results from the Henderson-Hasselbalch equation.

Chapter 13

- 13-A. A monodentate ligand binds to a metal ion through one ligand atom. A multidentate ligand binds through more than one ligand atom. A chelating ligand is a multidentate ligand. Desferrioxamine B has six ligand oxygen atoms from three hydroxamate groups.

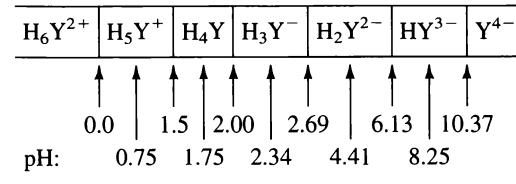
- 13-B. (a) $\text{M}^{n+} + \text{Y}^{4-} \rightleftharpoons \text{MY}^{n-4}$

$$K_f = [\text{MY}^{n-4}] / ([\text{M}^{n+}][\text{Y}^{4-}])$$

- (b) At low pH, H^+ competes with M^{n+} in binding to the ligand atoms of EDTA.

- (c) The auxiliary complexing agent prevents the metal ion from precipitating with hydroxide at high pH. EDTA displaces the auxiliary complexing agent from the metal ion.

- (d) The dividing line between each region is a pK_a value. The midpoint of each region is the average of the two surrounding pK_a values. The pH at the dividing line between two regions is the pH at which the concentrations of the species in the neighboring regions are equal. For example, at pH 2.69, $[\text{H}_3\text{Y}^-] = [\text{H}_2\text{Y}^{2-}]$. The pH at the center of a region is the pH containing the “pure” species in that region. For example, the pH of a solution made by dissolving a salt of H_2Y^{2-} (such as $\text{Na}_2\text{H}_2\text{Y}$) is 4.41.



- 13-C. (a) Only a small amount of indicator is employed. Most of the Mg^{2+} is not bound to indicator. The free Mg^{2+} reacts with EDTA before MgIn reacts. Therefore the concentration of MgIn is constant until all of the Mg^{2+}

has been consumed. Only when MgIn begins to react does the color change.

- (b) (i) Between pH 2.85 and pH 6.70, the predominant indicator species is H_3In^{3-} , which is (ii) yellow. The metal-indicator complex is (iii) red. At pH 8, the predominant indicator species is the violet H_2In^{4-} , so the titration color change is (iv) violet \rightarrow red.

13-D. (a) $(25.0 \text{ mL})(0.050 \text{ M}) = 1.25 \text{ mmol EDTA}$

(b) $(5.00 \text{ mL})(0.050 \text{ M}) = 0.25 \text{ mmol Zn}^{2+}$

(c) $\text{mmol Ni}^{2+} = \text{mmol EDTA} - \text{mmol Zn}^{2+} = 1.25 - 0.25 = 1.00 \text{ mmol Ni}^{2+}$

$[\text{Ni}^{2+}] = (1.00 \text{ mmol})/(50.0 \text{ mL}) = 0.020 \text{ M}$

13-E. (a) At pH 5.00:

$$K'_f = \alpha_{\text{Y}^{4-}} K_f = (2.9 \times 10^{-7})(10^{10.65}) = 1.3 \times 10^4$$



Initial concentration (M):	0	0	0.010
Final concentration (M):	x	x	$0.010 - x$

$$\frac{[\text{CaY}^{2-}]}{[\text{Ca}^{2+}][\text{EDTA}]} = \frac{0.010 - x}{x^2} = K'_f = 1.3 \times 10^4$$

$$\Rightarrow x = [\text{Ca}^{2+}] = 8.4 \times 10^{-4} \text{ M}$$

(b) fraction of bound calcium

$$= \frac{[\text{CaY}^{2-}]}{[\text{CaY}^{2-}] + [\text{Ca}^{2+}]} = \frac{[0.010 - 0.00084]}{0.010} = 0.92$$

92% of Ca is bound to EDTA at the equivalence point at pH 5.00.

(c) At pH 9.00: $K'_f = (0.041)(10^{10.65}) = 1.8 \times 10^9$

$$\frac{[\text{CaY}^{2-}]}{[\text{Ca}^{2+}][\text{EDTA}]} = \frac{0.010 - x}{x^2} = 1.8 \times 10^9$$

$$\Rightarrow x = [\text{Ca}^{2+}] = 2.4 \times 10^{-6} \text{ M}$$

fraction of bound calcium

$$= \frac{[\text{CaY}^{2-}]}{[\text{CaY}^{2-}] + [\text{Ca}^{2+}]} = \frac{[0.010 - 2.4 \times 10^{-6}]}{0.010} = 0.9998$$

99.98% of Ca is bound to EDTA at the equivalence point at pH 9.00.

13-F. At pH 10.0, $K'_f = (0.30)(10^{10.65}) = 1.34 \times 10^{10}$

At $V_{\text{EDTA}} = 5.00 \text{ mL}$, calculations for Ca^{2+} are identical with those of Mg^{2+} :

$$\text{initial mmol Ca}^{2+} = (0.050 \text{ M Ca}^{2+})(50.0 \text{ mL}) = 2.50 \text{ mmol}$$

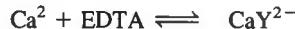
$$\text{mmol remaining} = (0.900)(2.50 \text{ mmol}) = 2.25 \text{ mmol}$$

$$[\text{Ca}^{2+}] = \frac{2.25 \text{ mmol}}{55.0 \text{ mL}} = 0.0409 \text{ M}$$

$$\Rightarrow \text{pCa}^{2+} = -\log[\text{Ca}^{2+}] = 1.39$$

$V_{\text{EDTA}} = 50.00 \text{ mL}$ is the equivalence point:

$$[\text{CaY}^{2-}] = \frac{2.50 \text{ mmol}}{100.0 \text{ mL}} = 0.0250 \text{ M}$$



Initial concentration (M):	—	—	0.0250
Final concentration (M):	x	x	$0.0250 - x$

$$\frac{[\text{CaY}^{2-}]}{[\text{Ca}^{2+}][\text{EDTA}]} = K'_f = 1.34 \times 10^{10}$$

$$\frac{0.0250 - x}{x^2} = 1.34 \times 10^{10}$$

$$\Rightarrow x = 1.37 \times 10^{-6} \text{ M} \Rightarrow \text{pCa}^{2+} = -\log x = 5.86$$

At $V_{\text{EDTA}} = 51.00 \text{ mL}$, there is 1.00 mL of excess EDTA:

$$[\text{EDTA}] = \frac{0.050 \text{ mmol}}{101.0 \text{ mL}} = 0.000495 \text{ M}$$

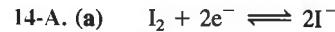
$$[\text{CaY}^{2-}] = \frac{2.50 \text{ mmol}}{101.0 \text{ mL}} = 0.0248 \text{ M}$$

$$\frac{[\text{CaY}^{2-}]}{[\text{Ca}^{2+}][\text{EDTA}]} = K'_f = 1.34 \times 10^{10}$$

$$\frac{[0.0248]}{[\text{Ca}^{2+}][0.000495]} = 1.34 \times 10^{10}$$

$$\Rightarrow [\text{Ca}^{2+}] = 3.7 \times 10^{-9} \text{ M} \Rightarrow \text{pCa}^{2+} = 8.43$$

Chapter 14



Oxidant



Reducant



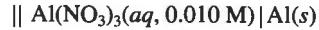
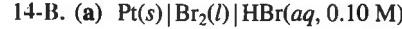
$$N = 1.00 \text{ g } \text{S}_2\text{O}_3^{2-}/(112.13 \text{ g/mol}) = 8.92 \text{ mmol } \text{S}_2\text{O}_3^{2-}$$

$$q = nNF$$

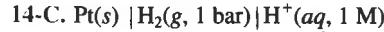
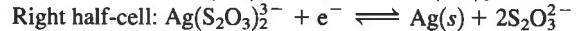
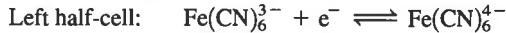
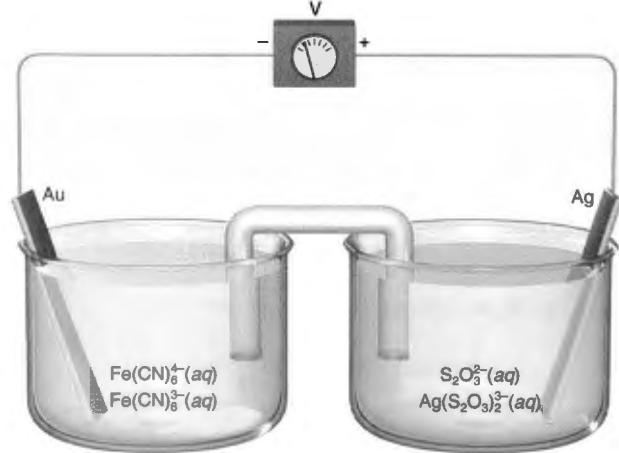
$$= (1)(8.92 \times 10^{-3} \text{ mol})(9.649 \times 10^4 \text{ C/mol}) = 861 \text{ C}$$

(e) current (A) = coulombs/s = $861 \text{ C}/60 \text{ s} = 14.3 \text{ A}$

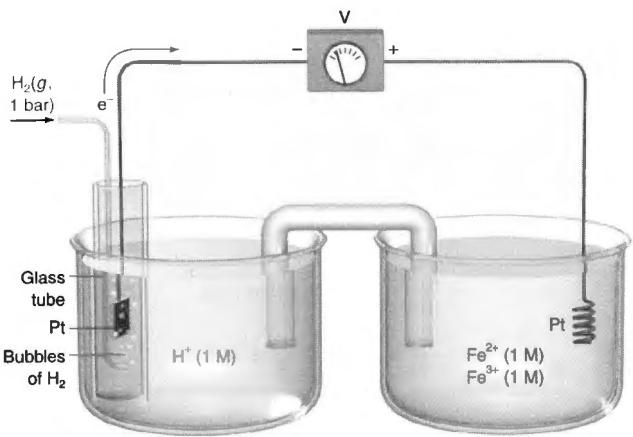
(f) work = $E \cdot q = (0.200 \text{ V})(861 \text{ C}) = 172 \text{ J}$



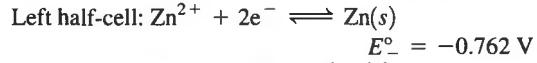
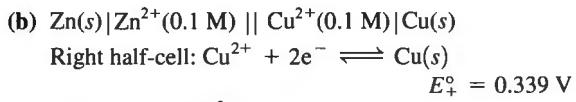
(b)



E° for the reaction $\text{Fe}^{3+} + \text{e}^- \rightleftharpoons \text{Fe}^{2+}$ is 0.771 V. That is, Fe is positive with respect to Pt in the cell. Therefore electrons flow from Pt to Fe through the meter.

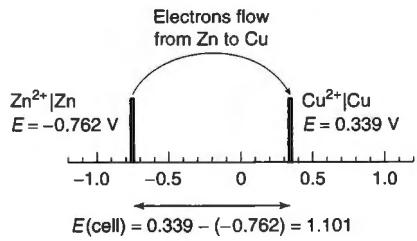


14-D. (a) $E = E^\circ - \left(\frac{0.059\ 16}{3}\right) \log\left(\frac{P_{AsH_3}}{[H^+]^3}\right)$
 $E = -0.238 - \left(\frac{0.059\ 16}{3}\right) \log\left(\frac{0.010\ 0}{(10^{-3.00})^3}\right) = -0.376\ V$



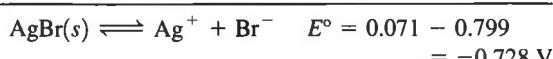
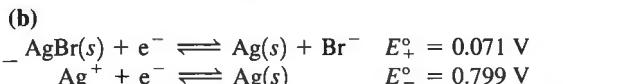
$$E = \left\{ 0.339 - \left(\frac{0.059\ 16}{2}\right) \log\left(\frac{1}{0.1}\right) \right\} - \left\{ -0.762 - \left(\frac{0.059\ 16}{2}\right) \log\left(\frac{1}{0.1}\right) \right\} = 1.101\ V$$

The positive voltage tells us that electrons are transferred from Zn to Cu. The net reaction is $Cu^{2+} + Zn(s) \rightleftharpoons Cu(s) + Zn^{2+}$.



14-E. (a) Right half-cell: $Cu^{2+} + 2e^- \rightleftharpoons Cu(s)$
 $E_+^\circ = 0.339\ V$
 Left half-cell: $Zn^{2+} + 2e^- \rightleftharpoons Zn(s)$
 $E_-^\circ = -0.762\ V$

$$E^\circ = E_+^\circ - E_-^\circ = 1.101\ V$$
 $K = 10^{nE^\circ/0.059\ 16} = 10^{2(1.101)/0.059\ 16} = 1.7 \times 10^{37}$



$$K_{sp} = 10^{1E^\circ/0.059\ 16} = 5 \times 10^{-13}$$

14-F. (a) $E = E_+ - E_-$
 $= \left\{ E_+^\circ - (0.059\ 16) \log\left(\frac{[Fe^{2+}]}{[Fe^{3+}]}\right) \right\} - 0.197$
 $0.771\ V \quad ? \quad \underbrace{\log\left(\frac{[Fe^{2+}]}{[Fe^{3+}]}\right)}_{?} \quad \text{Reference electrode voltage}$

$$0.703 = \left\{ 0.771 - 0.059\ 16 \log\left(\frac{[Fe^{2+}]}{[Fe^{3+}]}\right) \right\} - 0.197$$

$$0.059\ 16 \log\left(\frac{[Fe^{2+}]}{[Fe^{3+}]}\right) = -0.129$$

$$\Rightarrow \log\left(\frac{[Fe^{2+}]}{[Fe^{3+}]}\right) = -2.18$$

$$\Rightarrow \frac{[Fe^{2+}]}{[Fe^{3+}]} = 10^{-2.18} = 6.6 \times 10^{-3}$$

- (b)** **(i)** A potential of 0.523 V vs S.H.E. lies to the right of the origin in Figure 14-13. The Ag|AgCl potential lies 0.197 V to the right of the origin. The difference between these two points is $0.523 - 0.197 = 0.326\ V$.
(ii) A potential of 0.222 V vs S.C.E. lies 0.222 V to the right of S.C.E. Because S.C.E. lies 0.241 V to the right of S.H.E. in Figure 14-13, the distance from S.H.E. is $0.222 + 0.241 = 0.463\ V$.

Chapter 15

15-A. (a) At 0.10 mL:

$$\text{initial } Cl^- = 2.00\ \text{mmol; added } Ag^+ = 0.020\ \text{mmol}$$

$$[Cl^-] = \frac{(2.00 - 0.020)\ \text{mmol}}{(40.0 + 0.10)\ \text{mL}} = 0.049\ 4\ M$$

$$[Ag^+] = K_{sp}/[Cl^-] = (1.8 \times 10^{-10})/(0.049\ 4) = 3.6 \times 10^{-9}\ M$$

In a similar manner, we find

$$2.50\ mL: [Cl^-] = 0.035\ 3\ M \quad [Ag^+] = 5.1 \times 10^{-9}\ M$$

$$5.00\ mL: [Cl^-] = 0.022\ 2\ M \quad [Ag^+] = 8.1 \times 10^{-9}\ M$$

$$7.50\ mL: [Cl^-] = 0.010\ 5\ M \quad [Ag^+] = 1.7 \times 10^{-8}\ M$$

$$9.90\ mL: [Cl^-] = 0.000\ 401\ M \quad [Ag^+] = 4.5 \times 10^{-7}\ M$$

(b) At V_e : $[Ag^+][Cl^-] = x^2 = K_{sp}$
 $\Rightarrow [Cl^-] = [Ag^+] = \sqrt{K_{sp}} = 1.3 \times 10^{-5}\ M$

(c) At 10.10 mL: There is 0.10 mL of excess Ag⁺. Therefore
 $[Ag^+] = \frac{(0.10\ mL)(0.200\ M)}{50.1\ mL} = 4.0 \times 10^{-4}\ M$

$$\text{At 12.00 mL: } [Ag^+] = \frac{(2.00\ mL)(0.200\ M)}{52.0\ mL} = 7.7 \times 10^{-3}\ M$$

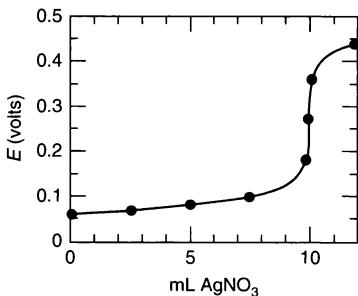
(d) At 0.10 mL:

$$E = 0.558 + (0.059\ 16) \log[Ag^+]$$

$$E = 0.558 + (0.059\ 16) \log(3.6 \times 10^{-9}) = 0.059\ V$$

In a similar manner, we find the following results:

mL AgNO ₃	E (V)	mL AgNO ₃	E (V)
0.10	0.059	9.90	0.182
2.50	0.067	10.00	0.270
5.00	0.079	10.10	0.357
7.50	0.099	12.00	0.433



15-B. Cl^- diffuses into the NaNO_3 , and NO_3^- diffuses into the NaCl . The mobility of Cl^- is greater than that of NO_3^- so the NaCl region is depleted of Cl^- faster than the NaNO_3 region is depleted of NO_3^- . The NaNO_3 side becomes negative, and the NaCl side becomes positive.

15-C. (a) Ammonium electrode:

$$E = \frac{0.059\ 16}{n} \log\left(\frac{[\text{NH}_4^+]_{\text{outer}}}{[\text{NH}_4^+]_{\text{inner}}}\right),$$

where $n = +1$. The concentration $[\text{NH}_4^+]_{\text{inner}}$ is fixed. If $[\text{NH}_4^+]_{\text{outer}}$ increases by a factor of 10, E increases by $(0.059\ 16/1) \log 10 = 0.059\ 16$ V.

$$\text{Fluoride electrode: } E = \frac{0.059\ 16}{n} \log\left(\frac{[\text{F}^-]_{\text{outer}}}{[\text{F}^-]_{\text{inner}}}\right),$$

where $n = -1$. If $[\text{F}^-]_{\text{outer}}$ increases by a factor of 10, E changes by $(-0.059\ 16) \log 10 = -0.059\ 16$ V.

$$\text{Sulfide electrode: } E = \frac{0.059\ 16}{n} \log\left(\frac{[\text{S}^{2-}]_{\text{outer}}}{[\text{S}^{2-}]_{\text{inner}}}\right),$$

where $n = -2$. If $[\text{S}^{2-}]_{\text{outer}}$ increases by a factor of 10, E changes by $(-0.059\ 16/2) \log 10 = -0.029\ 58$ V.

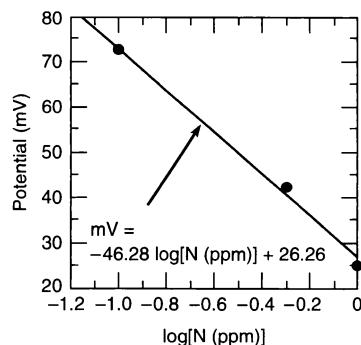
(b) A hydrophobic cation is required in the membrane to balance the charge of $\text{L}(\text{CO}_3^{2-})(\text{H}_2\text{O})$ and retain $\text{L}(\text{CO}_3^{2-})(\text{H}_2\text{O})$ in the membrane. Therefore we choose $(\text{C}_{12}\text{H}_{25})_3\text{NCH}_3^+$.

15-D. (a) Uncertainty in pH of standard buffers, junction potential, alkaline or acid errors at extreme pH values, and equilibration time for electrode.

$$(4.63)(0.059\ 16\text{ V}) = 0.274\text{ V}$$

(c) Na^+ competes with H^+ for cation-exchange sites on the glass surface. The glass responds as if some H^+ were present, and the apparent pH is lower than the actual pH.

15-E. (a)



(b) Plugging the experimental values of potential into the equation of the calibration curve in (a) gives

$$106 = -46.28 \log[\text{N}] + 26.26 \Rightarrow [\text{N}] = 0.019\ \text{ppm}$$

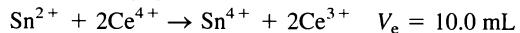
$$115 = -46.28 \log[\text{N}] + 26.26 \Rightarrow [\text{N}] = 0.012\ \text{ppm}$$

(Note that the unknown values lie beyond the calibration points, which is not good practice. It would be better to obtain more calibration points at lower concentrations to include the full range of unknown points.)

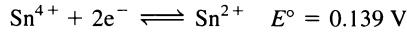
$$(c) 56 = -46.28 \log[\text{N}] + 26.26 \Rightarrow [\text{N}] = 0.23\ \text{ppm}$$

Chapter 16

16-A. Titration reaction:



Indicator electrode half-reactions:



Indicator electrode Nernst equations:

$$E_+ = 0.139 - \frac{0.059\ 16}{2} \log\left(\frac{[\text{Sn}^{2+}]}{[\text{Sn}^{4+}]}\right) \quad (\text{A})$$

$$E_- = 1.47 - 0.059\ 16 \log\left(\frac{[\text{Ce}^{3+}]}{[\text{Ce}^{4+}]}\right) \quad (\text{B})$$

Representative calculations:

At 0.100 mL: The ratio $[\text{Sn}^{2+}]/[\text{Sn}^{4+}]$ is 9.90/0.100

$$E_+ = 0.139 - \frac{0.059\ 16}{2} \log\left(\frac{[\text{Sn}^{2+}]}{[\text{Sn}^{4+}]}\right)$$

$$= 0.139 - \frac{0.059\ 16}{2} \log\left(\frac{9.90}{0.100}\right) = 0.080\ \text{V}$$

$$E = E_+ - E_- = 0.080 - 0.241 = -0.161\ \text{V}$$

At 10.00 mL: To add Nernst equations A and B, the factor in front of the log term needs to be the same in both. Therefore we multiply equation A by 2 before carrying out the addition:

$$2E_+ = 2(0.139) - 0.059\ 16 \log\left(\frac{[\text{Sn}^{2+}]}{[\text{Sn}^{4+}]}\right)$$

$$E_+ = 1.47 - 0.059\ 16 \log\left(\frac{[\text{Ce}^{3+}]}{[\text{Ce}^{4+}]}\right)$$

$$3E_+ = 1.748 - 0.059\ 16 \log\left(\frac{[\text{Sn}^{2+}][\text{Ce}^{3+}]}{[\text{Sn}^{4+}][\text{Ce}^{4+}]}\right) \quad (\text{C})$$

At the equivalence point, $[\text{Ce}^{3+}] = 2[\text{Sn}^{4+}]$ and $[\text{Ce}^{4+}] = 2[\text{Sn}^{2+}]$. Putting these equalities into Equation C makes the log term 0.

Therefore $3E_+ = 1.748$ and $E_+ = 0.583\ \text{V}$.

$$E = E_+ - E_- = 0.583 - 0.241 = 0.342\ \text{V}$$

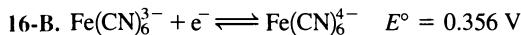
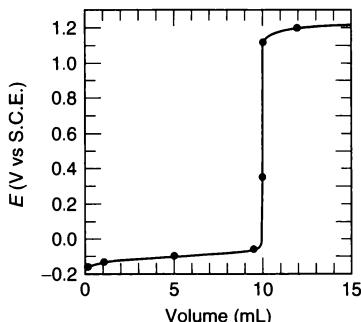
At 10.10 mL: The ratio $[\text{Ce}^{3+}]/[\text{Ce}^{4+}]$ is 10.00/0.10

$$E_+ = 1.47 - 0.059\ 16 \log\left(\frac{[\text{Ce}^{3+}]}{[\text{Ce}^{4+}]}\right)$$

$$E_+ = 1.47 - 0.059\ 16 \log\left(\frac{10.00}{0.10}\right) = 1.35_2\ \text{V}$$

$$E = E_+ - E_- = 1.35_2 - 0.241 = 1.11\ \text{V}$$

mL	E (V)	mL	E (V)
0.100	-0.161	10.00	0.342
1.00	-0.130	10.10	1.11
5.00	-0.102	12.00	1.19
9.50	-0.064		



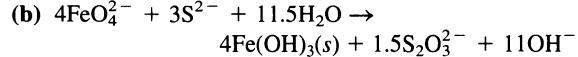
The end point will be between 0.356 and 0.77 V. Methylene blue with $E^\circ = 0.53 \text{ V}$ is closest to the midpoint of the steep part of the titration curve. The color change would be from blue to colorless.

16-C. (a) 50.00 mL contains exactly 1/10 of the $\text{KIO}_3 = 0.102 \text{ g} = 0.477 \text{ g/mol KIO}_3$. Each mole of iodate makes three moles of triiodide, so $\text{I}_3^- = 3(0.477 \text{ g/mol}) = 1.432 \text{ g/mol}$.

(b) Two moles of thiosulfate react with one mole of I_3^- . Therefore there must have been $2(1.432 \text{ g}) = 2.865 \text{ g}$ of thiosulfate in 37.66 mL; so the concentration is $(2.865 \text{ g})/(37.66 \text{ mL}) = 0.07608 \text{ M}$.

(c) 50.00 mL of KIO_3 make 1.432 g of I_3^- . The unreacted I_3^- requires 14.22 mL of sodium thiosulfate = $(14.22 \text{ mL})(0.07608 \text{ M}) = 1.082 \text{ g}$, which reacts with $\frac{1}{2}(1.082 \text{ g}) = 0.541 \text{ g}$ of I_3^- . The ascorbic acid must have consumed the difference = $1.432 \text{ g} - 0.541 \text{ g} = 0.891 \text{ g}$. Each mole of ascorbic acid = 0.891 g , which has a mass of $(0.891 \text{ g})/(176.13 \text{ g/mol}) = 0.005 \text{ mol}$. Ascorbic acid in the unknown = $100 \times (0.005 \text{ mol})/(1.223 \text{ g}) = 12.8 \text{ wt\%}$.

Chapter 17



(c) The anode reaction produces 6e^- for each FeO_4^{2-} . Reaction with S^{2-} requires 4FeO_4^{2-} to consume 3S^{2-} . The number of electrons required for oxidation of each sulfide is

$$\left(\frac{6\text{e}^-}{\text{FeO}_4^{2-}}\right)\left(\frac{4\text{FeO}_4^{2-}}{3\text{S}^{2-}}\right) = \frac{8\text{e}^-}{\text{S}^{2-}}$$

or 8 mol e^- per mol S^{2-} .

A current of 16.0 A (= 16.0 C/s) for 1.00 h (3 600 s) provides a charge of $q = It = (16.0 \text{ C/s})(3 600 \text{ s}) = 5.76 \times 10^4 \text{ C}$.

$$N = \text{mol e}^- = \frac{q}{nF} = \frac{5.76 \times 10^4 \text{ C}}{(1 \text{ charge/electron})(96 485 \text{ C/mol})}$$

$$= 0.597 \text{ mol e}^-$$

which will react with

$$\frac{0.597 \text{ mol e}^-}{8 \text{ mol e}^-/\text{mol S}^{2-}} = 0.0746 \text{ mol S}^{2-}$$

$$(d) \frac{0.0746 \text{ mol S}^{2-}}{0.0100 \text{ mol S}^{2-}/\text{L}} = 7.46 \text{ L}$$

- 17-B. (a) The glucose monitor has a test strip with two carbon indicator electrodes and a silver-silver chloride reference electrode. Indicator electrode 1 is coated with glucose oxidase and a mediator. When a drop of blood is placed on the test strip, glucose from the blood is oxidized near indicator electrode 1 by mediator to gluconolactone and the mediator is reduced. The enzyme glucose oxidase catalyzes the oxidation. Reduced mediator is reoxidized at the indicator electrode whose potential is kept at +0.2 V with respect to the $\text{Ag}|\text{AgCl}$ reference electrode. Electric current between indicator electrode 1 and the reference electrode is proportional to the rate of oxidation of mediator, which is proportional to the concentration of glucose plus any interfering species in the blood. Indicator electrode 2 has mediator but no glucose oxidase. Current measured between indicator electrode 2 and the reference electrode is proportional to the concentration of interfering species in the blood. The difference between the two currents is proportional to the concentration of glucose in the blood.
- (b) In the absence of a mediator, the rate of oxidation of glucose depends on the concentration of O_2 in the blood. If $[\text{O}_2]$ is low, the current will be low and the monitor will give an incorrect, low reading for the glucose concentration. A mediator such as 1,1'-dimethylferrocinium ion replaces O_2 in the glucose oxidation and is subsequently reduced at the indicator electrode. The concentration of mediator is constant and high enough so that variations in electrode current are due mainly to variations in glucose concentration. Also, lowering the required electrode potential for oxidation of the mediator reduces the possible interference by other species in the blood.

17-C. See solution to 5-C.

- 17-D. (a) Faradaic current arises from redox reactions at the electrode. It is what we are trying to measure. Charging current comes from the flow of ions toward or away from the electrode as electrons flow into or out of the electrode when a potential step is applied. Charging current is unrelated to electrochemical reactions.
- (b) Charging current decays more rapidly than faradaic current after a potential step. By waiting 1 s after a step before measuring current, we find that charging

current has largely decayed and there is still significant faradaic current.

- (c) Square wave polarography is much faster than other forms of polarography. It gives increased signal because current is measured as the difference between an oxidation current and a reduction current in each cycle. The derivative peak shape makes it easier to resolve closely spaced signals.
- (d) In anodic stripping voltammetry, analyte is reduced and concentrated at the working electrode at a controlled potential for a constant time. The potential is then ramped in a positive direction to reoxidize analyte, during which current is measured. The height of the oxidation wave is proportional to the concentration of analyte. Stripping is the most sensitive polarographic technique because analyte is concentrated from a dilute solution. The longer the period of concentration, the more sensitive is the analysis.

Chapter 18

$$\begin{aligned}
 18\text{-A. (a)} \quad v &= c/\lambda = (2.998 \times 10^8 \text{ m/s})/(100 \times 10^{-9} \text{ m}) \\
 &= 2.998 \times 10^{15} \text{ s}^{-1} = 2.998 \times 10^{15} \text{ Hz} \\
 \tilde{v} &= 1/\lambda = 1/(100 \times 10^{-9} \text{ m}) = 10^7 \text{ m}^{-1} \\
 (10^7 \text{ m}^{-1})\left(\frac{1 \text{ m}}{100 \text{ cm}}\right) &= 10^5 \text{ cm}^{-1} \\
 E &= h\nu = (6.626 2 \times 10^{-34} \text{ J} \cdot \text{s})(2.998 \times 10^{15} \text{ s}^{-1}) \\
 &= 1.986 \times 10^{-18} \text{ J} \\
 (1.986 \times 10^{-18} \text{ J}/\text{photon})(6.022 \times 10^{23} \text{ photons/mol}) \\
 &= 1196 \text{ kJ/mol}
 \end{aligned}$$

As shown in the table below, parts (b), (c), (d) are done in an analogous manner:

λ	v (Hz)	\tilde{v} (cm $^{-1}$)	E (kJ/mol)	Spectral region	Molecular process
100 nm	2.998×10^{15}	10^5	1.196×10^3	ultraviolet	electronic excitation
500 nm	5.996×10^{14}	2×10^4	239.3	visible	electronic excitation
10 μm	2.998×10^{13}	1 000	11.96	infrared	molecular vibration
1 cm	2.998×10^{10}	1	0.011 96	microwave	molecular rotation

$$18\text{-B. (a)} \quad A = \epsilon bc = (1.05 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1})(1.00 \text{ cm})(2.33 \times 10^{-4} \text{ M}) = 0.245$$

$$(b) \quad T = 10^{-A} = 0.569 = 56.9\%$$

$$(c) \quad \text{Doubling } b \text{ will double } A \Rightarrow A = 0.489 \Rightarrow$$

$$T = 10^{-A} = 32.4\%$$

$$(d) \quad \text{Doubling } c \text{ also doubles } A \Rightarrow A = 0.489 \Rightarrow$$

$$T = 10^{-A} = 32.4\%$$

$$(e) \quad A = \epsilon bc = (2.10 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1})(1.00 \text{ cm})(2.33 \times 10^{-4} \text{ M}) = 0.489$$

Curve	Absorption peak (nm)	Predicted color (Table 18-1)	Observed color
A	760	green	green
B	700	green	blue green
C	600	blue	blue
D	530	violet	violet
E	500	red or purple-red	red
F	410	green-yellow	yellow

18-C. (a) Don't touch the cuvet with your fingers. Wash the cuvet as soon as you are finished with it and drain out the rinse water. Use matched cuvets. Place the cuvet in the instrument with the same orientation each time. Cover the cuvet to prevent evaporation and to keep dust out.

(b) If absorbance is too high, too little light reaches the detector for accurate measurement. If absorbance is too low, there is too little difference between sample and reference for accurate measurement.

$$18\text{-D. (a)} \quad \epsilon = \frac{A}{cb} = \frac{0.267 - 0.019}{(3.15 \times 10^{-6} \text{ M})(1.000 \text{ cm})} = 7.87 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$$

$$(b) \quad c = \frac{A}{\epsilon b} = \frac{0.175 - 0.019}{(7.87 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1})(1.000 \text{ cm})} = 1.98 \times 10^{-6} \text{ M}$$

Chapter 19

19-A. The source contains a visible lamp and an ultraviolet lamp, only one of which is used at a time. Grating 1 apparently selects some limited range of wavelengths to leave the source. In the monochromator, grating 2 is the principal element that selects the wavelength to reach the exit slit. The width of the exit slit determines how wide a range of wavelengths leaves the monochromator. The rotating chopper is a mirror that alternately directs the monochromatic light through the sample or reference cuvets in the sample compartment. The rotating chopper after the sample compartment directs light from each path to the detector, which is a photomultiplier tube.

19-B. (a) We use Equations 19-5 with $b = 0.100 \text{ cm}$:

$$\begin{aligned} D &= b(\epsilon'_X \epsilon''_Y - \epsilon'_Y \epsilon''_X) \\ &= (0.100)[(720)(274) - (212)(479)] \\ &= 9.57_3 \times 10^3 \text{ (the units are } M^{-2} \text{ cm}^{-1}) \\ [X] &= \frac{1}{D}(A' \epsilon''_Y - A'' \epsilon'_Y) \\ &= \frac{(0.233)(274) - (0.200)(212)}{9.57_3 \times 10^3} = 2.24 \text{ mM} \\ [Y] &= \frac{1}{D}(A'' \epsilon'_X - A' \epsilon''_X) \\ &= \frac{(0.200)(720) - (0.233)(479)}{9.57_3 \times 10^3} = 3.38 \text{ mM} \end{aligned}$$

(b) There would still be an isosbestic point at 465 nm because $\epsilon_{\text{HIn}}^{465} = \epsilon_{\text{In}^-}^{465}$.

19-C. (a) $(163 \times 10^{-6} \text{ L})(1.43 \times 10^{-3} \text{ M Fe(III)}) = 2.33 \times 10^{-7} \text{ mol Fe(III)}$

(b) $1.17 \times 10^{-7} \text{ mol apotransferrin} / 2.00 \times 10^{-3} \text{ L} \Rightarrow 5.83 \times 10^{-5} \text{ M apotransferrin}$

(c) Prior to the equivalence point, all added Fe(III) binds to the protein to form a red complex whose absorbance is shown in the figure. After the equivalence point, no more protein binding sites are available. The slight increase in absorbance arises from the color of the iron reagent in the titrant.

19-D. (a) In electronic transitions, energy is absorbed or liberated when an electron is promoted to a higher energy level, or falls from a higher energy level. In vibrational transitions, the amplitude of vibrations increases or decreases. A rotational transition causes the molecule to rotate faster or slower.

(b) A molecule in an excited state can collide with other molecules and transfer kinetic energy without emitting a photon. The molecule that absorbs the energy in the collision ends up moving faster or vibrating with greater amplitude or rotating faster.

(c) Fluorescence is a transition from an excited singlet electronic state to the ground singlet state. Phosphorescence is a transition from an excited triplet state to the ground singlet state. Fluorescence is at higher energy than phosphorescence and fluorescence occurs faster than phosphorescence.

(d) Fluorescence involves a set of transitions opposite those for absorption. Instead of going from the ground vibrational state of S_0 to various states of S_1 in absorption, fluorescence takes a molecule from the ground vibrational state of S_1 to various states of S_0 . Absorption has a series of peaks from λ_0 to higher energy. Fluorescence has a series of peaks from λ_0 to lower energy.

(e) Photochemistry is the breaking of chemical bonds initiated by absorption of a photon. Chemiluminescence is the emission of light as a result of a chemical reaction.

19-E. (a) At low concentration, fluorescence is proportional to analyte concentration. As concentration increases, neighboring unexcited molecules absorb some of the fluorescence before it can leave the cuvet. Some excited neighbors return to the ground state by emission of heat, rather than light. Therefore fluorescence becomes less efficient as concentration increases. Eventually, a point is reached at which self-absorption is so likely that further increase in analyte concentration decreases the fluorescence.

(b) Each molecule of analyte bound to antibody 1 also binds one molecule of antibody 2 that is linked to one enzyme molecule. Each enzyme molecule catalyzes many cycles of reaction in which a colored or fluorescent product is created. Therefore each analyte molecule results in many product molecules.

Chapter 20

20-A. In atomic absorption spectroscopy, light of a specific frequency is passed through a flame containing free atoms. Absorbance of light is measured and is proportional to the concentration of atoms. In atomic emission spectroscopy, no lamp is used. The intensity of light emitted by excited atoms in the flame is measured and is proportional to the concentration of atoms.

20-B. (a) Atoms in a furnace are confined in a small volume for a relatively long time. The detection limit is small because the concentration of atoms in the gas phase is relatively high. A large sample volume is not required because gaseous atoms do not rapidly escape from the furnace. In a flame or plasma, the volume of gas phase is relatively large, so the concentration of atoms is relatively low. A great deal of sample is required because the atoms are rapidly moving through and escaping from the flame or plasma.

(b) For a body mass of 55 kg, the tolerable weekly intake of tin is $(14 \text{ mg Sn/kg body mass})(55 \text{ kg}) = 770 \text{ mg}$. One kilogram of tomato juice contains 241 mg Sn, so the number of kilograms of tomato juice containing 770 mg is $(770 \text{ mg})/(241 \text{ mg/kg}) = 3.2 \text{ kg}$.

20-C. (a) First find the frequency of the light:

$$\begin{aligned} \nu &= c/\lambda \\ &= (2.998 \times 10^8 \text{ m/s})/(400 \times 10^{-9} \text{ m}) \\ &= 7.495 \times 10^{14} \text{ s}^{-1} \end{aligned}$$

$$\begin{aligned}\text{energy} &= h\nu \\ &= (6.626 \times 10^{-34} \text{ J} \cdot \text{s}) (7.495 \times 10^{14} \text{ s}^{-1}) \\ &= 4.966 \times 10^{-19} \text{ J}\end{aligned}$$

$$\begin{aligned}\text{(b)} \quad \frac{N^*}{N_0} &= \left(\frac{g^*}{g_0} \right) e^{-\Delta E/kT} \\ &= \left(\frac{1}{1} \right) e^{-(4.966 \times 10^{-19} \text{ J}) / [(1.381 \times 10^{-23} \text{ J/K})(2500 \text{ K})]} \\ &= 5.7 \times 10^{-7}\end{aligned}$$

20-D. *Absorption* is measured in the presence and absence of a strong magnetic field. The Zeeman effect splits the analyte absorption peak when a magnetic field is applied and very little light is absorbed at the hollow-cathode lamp wavelength. The difference between absorption with and without the magnetic field is due to analyte. *Emission* is measured at the peak wavelength and at the surrounding baseline at slightly lower and higher wavelengths. Emission due to analyte is the peak emission minus the average baseline intensity of the two off-wavelength measurements.

- 20-E.** (a) *Spectral interference* arises from overlap of analyte absorption or emission lines with absorptions or emissions from other elements or molecules in the sample or the flame.
 (b) *Chemical interference* is caused by any substance that decreases the extent of atomization of analyte.
 (c) *Ionization interference* is a reduction of the concentration of free atoms by ionization of the atoms.
 (d) La^{3+} acts as releasing agent by binding tightly to PO_4^{3-} and freeing Pb^{2+} .

20-F. The six peaks that we see for Hg correspond to the six natural isotopes at their relative abundances. For example, ^{202}Hg is most abundant and ^{200}Hg is the second most abundant. We see only three of the four isotopes of Pb because ^{204}Pb has only 1.4% abundance and it is hidden beneath ^{204}Hg . The coincidence of ^{204}Hg and ^{204}Pb is an example of isobaric interference. There are gaps at masses of 203 and 205 because neither Hg nor Pb has isotopes at these masses.

Chapter 21

21-A. 1-C, 2-D, 3-A, 4-E, 5-B

21-B. Your answer to part (a) could be different from mine because of the size of the figure in your textbook. However, your answers to parts (b) through (e) should be similar to mine.

- (a) t_r : octane, 78.7 mm; nonane, 101.8 mm
 $w_{1/2}$: octane, 9.1 mm; nonane, 12.4 mm units
 (b) octane: $N = \frac{5.55(78.7)^2}{(9.1)^2} = 411$
 nonane: $N = \frac{5.55(101.8)^2}{(12.4)^2} = 374$
 (c) octane: $H = L/N = (1.00 \text{ m}/411) = 2.4 \text{ mm}$
 nonane: $H = (1.00 \text{ m}/374) = 2.7 \text{ mm}$

(d) Using the width at the baseline, we find

octane $w = 15.4 \text{ mm}$; nonane $w = 21.4 \text{ mm}$

$$\text{resolution} = \frac{\Delta t_r}{w_{av}} = \frac{101.8 - 78.7}{\frac{1}{2}(15.4 + 21.4)} = 1.26$$

Using the width at half-height, we find

$$\text{resolution} = \frac{0.589\Delta t_r}{w_{1/2av}} = \frac{0.589(101.8 - 78.7)}{\frac{1}{2}(9.1 + 12.4)} = 1.27$$

$$\text{(e)} \quad \frac{\text{large load}}{\text{small load}} = \left(\frac{\text{large column radius}}{\text{small column radius}} \right)^2$$

$$\frac{27.0 \text{ mg}}{3.0 \text{ mg}} = \left(\frac{\text{large column radius}}{\text{small column radius}} \right)^2 \Rightarrow$$

$$2.0 \text{ mm}$$

$$\text{large column radius} = 6.0 \text{ mm}$$

$$\text{large column diameter} = 12.0 \text{ mm}$$

Flow rate should be proportional to the cross-sectional area of the column, which is proportional to the square of the radius.

$$\frac{\text{large column flow rate}}{\text{small column flow rate}} = \left(\frac{\text{large column radius}}{\text{small column radius}} \right)^2$$

$$= \left(\frac{6.0 \text{ mm}}{2.0 \text{ mm}} \right)^2 = 9.0$$

If the small column flow rate is 7.0 mL/min, the large column flow rate should be $(9.0)(7.0 \text{ mL/min}) = 63 \text{ mL/min}$.

21-C. (a) Molecules in the gas phase move faster than molecules in liquid. Therefore longitudinal diffusion in the gas phase is much faster than longitudinal diffusion in the liquid phase, so band broadening by this mechanism is more rapid in gas chromatography than in liquid chromatography.

(b) (i) Optimum flow rate gives minimum plate height (23 cm/s for He). (ii) When flow is too fast, there is not adequate time for solute to equilibrate between the phases as the mobile phase streams past the stationary phase, so the band is broadened. (iii) When flow is too slow, plate height increases (that is, band broadening gets worse) because solute spends a long time on the column and is broadened by longitudinal diffusion.

(c) There is no broadening by multiple flow paths in an open tubular column.

(d) The longer the column, the better the resolution. We can use a longer open tubular column than a packed column because particles in the packed column resist flow and require high pressures for high flow rate.

21-D. (a) In a total ion chromatogram, the mass spectrometer is set to respond to a wide range of m/z values. Any compound eluted from the column will give a mass spectral signal, so all compounds are observed in the chromatogram. In the selected ion chromatogram, the spectrometer is set to respond to just one value (or perhaps a few values) of m/z . Only those compounds that produce ions with the set value of m/z are observed. The selected ion chromatogram has a higher signal-to-noise ratio than that of the total ion chromatogram because more time is spent collecting data at the chosen value of m/z .

- (b)** The mass spectrometer is set to respond only to m/z 413 in trace *h*. Prior to the elution of noscapine, none of the components of the mixture produce ions with m/z 413. The chromatogram is flat until noscapine is eluted. Even though six other compounds are eluted, and acetylcodeine partly overlaps noscapine, only noscapine is observed.

- 21-E. (a)** The even mass of the molecular ion at m/z 194 tells us that there is an even number of N atoms. The intensity ratio $(M+1)/M = 8.8\%$ suggests that the number of C atoms is $8.8\%/1.1\% = 8$. Formulas with 8 C atoms, an even number of N atoms, and a nominal mass of 194 are $C_8H_{22}O_6$, $C_8H_{18}O_5$, $C_8H_6O_4N_2$, $C_8H_{22}O_3N_2$, $C_8H_{10}O_2N_4$, $C_8H_{26}ON_4$, and $C_8H_{14}N_6$. However, there are no possible structures you could draw for $C_8H_{22}O_3N_2$ or $C_8H_{26}ON_4$ if C makes 4 bonds, H makes 1 bond, O makes 2 bonds, and N makes 3 bonds. There are too many H atoms in these two formulas.
- (b)** Calculate the exact mass by adding the masses of the atoms and subtracting the mass of one electron, because the species is a cation:

$$\begin{array}{rcl} C_5H_8O^+: & & C_6H_{12}^+: \\ \begin{array}{rcl} 5C & 5 \times 12.000 & 00 \\ 8H & +8 \times & 1.007 & 825 \\ 1O & +1 \times & 15.994 & 91 \\ -e^- & -1 \times & 0.000 & 55 \end{array} & & \begin{array}{rcl} 6C & 6 \times 12.000 & 00 \\ 12H & 12 \times & 1.007 & 825 \\ -e^- & -1 \times & 0.000 & 55 \end{array} \\ \hline & & 84.056 & 96 \end{array}$$

$$\begin{array}{rcl} & & C_6H_{12}^+: \\ & & 84.093 & 35 \end{array}$$

Difference = $84.093\ 35 - 84.056\ 96 = 0.036\ 39$ Da
 The difference 0.036 39 in exact mass between $C_5H_8O^+$ and $C_6H_{12}^+$ is 4.5 times greater than the difference in mass between atrazine H^+ and cymoxanil NH_4^+ ($216.109\ 1 - 216.101\ 0 = 0.008\ 1$), which were resolved by the spectrometer in the figure. Therefore we expect $C_5H_8O^+$ and $C_6H_{12}^+$ to be resolved.

- (d)** Split injection is the ordinary mode for open tubular columns. Splitless injection is useful for trace and quantitative analysis. On-column injection is useful for thermally sensitive solutes that might decompose during a high-temperature injection.

- (e)** **(i)** carbon atoms bearing hydrogen atoms; **(ii)** all analytes; **(iii)** molecules with halogens, conjugated $C=O$, CN , NO_2 ; **(iv)** P and S; **(v)** P and N; **(vi)** S; **(vii)** all analytes

- (f)** A *reconstructed total ion chromatogram* is created by summing all ion intensities (above a selected value of m/z) in each mass spectrum at each time interval in a chromatography experiment. The technique responds to essentially everything eluted from the column and has no selectivity at all. In *selected ion monitoring*, intensities at just one or a few values of m/z are monitored. Only species with ions at those m/z values are detected, so the selectivity is much greater than that of the reconstructed total ion chromatogram. Signal is increased because ions are collected at each m/z for a longer time than would be allowed if the entire spectrum were being scanned. Noise is decreased because other eluates are less likely to contribute signal intensity at the selected value of m/z .

Selected reaction monitoring is most selective. One ion from the first mass separator is passed through a collision cell where it breaks into product ions that are separated by a second mass separator. The intensities of one or a few of these product ions are plotted as a function of elution time. The selectivity is high because few species from the column produce the first selected ion and even fewer break into the same fragments in the collision cell. This technique is so selective that it can transform a poor chromatographic separation into a highly specific determination of one component with virtually no interference.

- (g)** Quadrupole Q1 selects only the parent ion caffeine $^+$ ($C_8H_{10}N_4O_2^+$) at m/z 194. This ion fractures from collisions in Q2. Only the daughter ion at m/z 109 is selected by Q3 for monitoring. Few other components of the sample produce an ion at m/z 194 and even fewer would produce a daughter ion from m/z 194 that appears at m/z 109. Selected reaction monitoring is highly specific for one desired substance and shows almost no response to other substances.

- 22-B.** Solvent is competing with solute for adsorption sites. The strength of the solvent-adsorbent interaction is independent of solute.

- 22-C. (a)** In normal-phase chromatography, the stationary phase is more polar than the mobile phase. In reversed-phase chromatography, the stationary phase is less polar than the mobile phase.

Chapter 22

- 22-A. (a)** Low-boiling solutes are separated well at low temperature, and the retention of high-boiling solutes is reduced to a reasonable time at high temperature.
- (b)** An open tubular column gives higher resolution than a packed column. The narrower the column, the higher the resolution that can be attained. A packed column can handle much more sample than an open tubular column, which is critical for preparative separations in which we are trying to isolate some quantity of the separated components.
- (c)** Diffusion of solute in H_2 and He is more rapid than in N_2 . Therefore equilibration of solute between mobile phase and stationary phase is faster. The column can be run faster without excessive broadening from the finite rate of mass transfer between the mobile and stationary phases.

- (b)** Reversed-phase chromatography uses bonded phases to retain solutes. Increasing the fraction of organic solvent in the mobile phase increases the eluent strength and decreases retention time. Hydrophilic interaction chromatography uses strongly polar bonded phases to retain solutes. Increasing the fraction of organic solvent in the mobile phase decreases the eluent strength and increases retention time.
- (c)** In isocratic elution, the composition of eluent is constant. In gradient elution, the composition of the eluent is changed—usually continuously—from low eluent strength to high eluent strength.
- (d)** In normal-phase chromatography, polar solvent must compete with analyte for polar sites on the stationary phase. The more polar the solvent, the better it binds to the stationary phase and the greater is its ability to displace analyte from the stationary phase.
- (e)** In reversed-phase chromatography, nonpolar analyte adheres to the nonpolar stationary phase. A polar solvent does not compete with analyte for nonpolar sites on the stationary phase. Making the solvent less polar gives it greater ability to displace analyte from the stationary phase.
- (f)** In hydrophilic interaction chromatography, polar analyte is retained by the polar stationary phase and the thin layer of water coating the stationary phase. Nonpolar solvent does not compete with polar analyte for retention sites. Making the solvent less polar gives it less ability to displace analyte from the stationary phase.
- (g)** A guard column is a small, disposable column containing the same stationary phase as the main column. Sample and solvent pass through the guard column first. Any irreversibly bound impurities stick to the guard column, which is eventually thrown away. This guard column prevents junk from being irreversibly bound to the expensive main column and eventually ruining it.
- (h)** Electrospray introduces solution-phase ions into the gas phase. Atmospheric pressure chemical ionization produces new ions.
- (i)** In a macroporous particle, solute can diffuse to the middle of the particle and must diffuse back out to enter the mobile phase. The larger the particle, the longer the diffusion path and the less efficient the column. Efficiency is measured by plate height, with smaller plate height being more efficient. In a superficially porous particle, solute only diffuses into the porous layer, which is $0.5\text{ }\mu\text{m}$ thick in this case. With a smaller diffusion distance, a large superficially porous particle behaves like a small macroporous particle.
- 22-D. (a)** Analytes are released into the chromatography column over a long period of time (possibly many minutes) from the heated fiber or the heated absorption tube. If analytes were not cold trapped on the column prior to chromatography, they would be eluted in extremely broad bands instead of sharp peaks.
- (b)** Solid-phase extraction uses a short column containing a chromatographic stationary phase. The column carries out gross separations of one type of analyte from other types of analyte (for example, separating nonpolar from polar analytes). Large particle size allows sample to drain through the solid-phase extraction column without applying high pressure. In chromatography, small particle size increases the efficiency of separation, but high pressure is necessary to force solvent through the column.
- (c)** Strongly polar molecules such as sugars wash through the solid-phase extraction column and are lost. Strongly hydrophobic compounds such as fats are retained on the column and separated from the caffeine and theobromine when they are eluted with methanol.
- (d)** The nominal mass of chloramphenicol, $\text{C}_{11}\text{H}_{11}\text{N}_2\text{O}_5\text{Cl}_2$, is $(11 \times 12) + (12 \times 1) + (2 \times 14) + (5 \times 16) + (2 \times 35) = 322$. The base peak at m/z 321 must be $[\text{M} - \text{H}]^-$, which is $[\text{C}_{11}\text{H}_{11}\text{N}_2\text{O}_5^{35}\text{Cl}_2]^-$. The 60% abundant ion at m/z 323 is $[\text{C}_{11}\text{H}_{11}\text{N}_2\text{O}_5^{35}\text{Cl}^{37}\text{Cl}]^-$.

Chapter 23

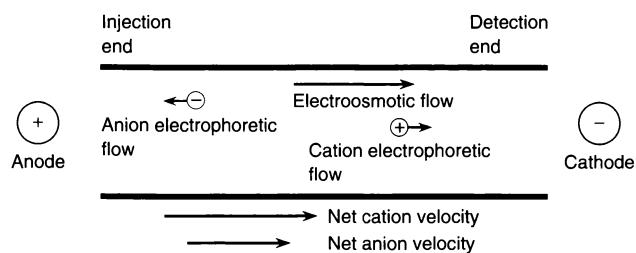
- 23-A. (a)** Deionized water has been passed through ion-exchange columns to convert cations to H^+ and anions to OH^- , making H_2O . Nonionic impurities (such as neutral organic compounds) are not removed by this process.
- (b)** Gradient elution with increasing concentration of H^+ is required to displace more and more strongly bound cations from the column.
- (c)** Cations from a large volume of water are collected on a small ion-exchange column and then eluted in a small volume of concentrated acid. If the acid were not very pure, impurities in the acid could be greater than the concentration of trace species collected from the large volume of water.
- (d)** The stationary phase contains both fixed positive charge and fixed negative charge. The fixed positive charge is an anion exchanger, and the fixed negative charge is a cation exchanger.
- (e)** In the charged aerosol detector, eluate evaporates to leave an aerosol of nonvolatile solute. The fine particles mix with a stream of N_2^+ ions formed in a high-voltage discharge, and the aerosol particles become positively charged. Excess N_2^+ is separated from the aerosol by an electric field, and the charged aerosol flows to a collector. The chromatogram displays charge reaching the collector as a function of time.
- 23-B. (a)** The separator is a column that separates ions by ion exchange. The suppressor exchanges the counterion to reduce the conductivity of eluent and allow analytes to be detected by their electrical conductivity.

- (b) The anion exchanger in the separator column retains sulfate better than it retains nitrate, probably because sulfate has a greater negative charge. Sulfate emerges from the column after nitrate.
- (c) We detect ions in the eluate by their electrical conductivity. It is hard to measure the conductivity of low concentrations of analyte ions in the presence of a moderate concentration of KOH. By converting KOH to H₂O, eluate conductivity is near zero except when analyte emerges from the column.

- 23-C. (a) total volume = $\pi(0.80 \text{ cm})^2(20.0 \text{ cm}) = 40.2 \text{ mL}$
- (b) If the void volume (= volume of mobile phase excluded from gel) is 18.2 mL, the stationary phase plus its included solvent must occupy $40.2 - 18.2 = 22.0 \text{ mL}$.
- (c) If pores occupy 60.0% of the stationary phase volume, the pore volume is $(0.600)(22.0 \text{ mL}) = 13.2 \text{ mL}$. Large molecules excluded from the pores are eluted in the void volume of $x = 18.2 \text{ mL}$. The smallest molecules (which can enter all of the pores) are eluted in a volume of $y = 18.2 + 13.2 = 31.4 \text{ mL}$. (In fact, there is usually some adsorption of solutes on the stationary phase, so retention volumes in real columns can be greater than 31.4 mL.)

- 23-D. (a) 25 μm diameter: volume = $\pi r^2 \times \text{length}$
 $= \pi(12.5 \times 10^{-6} \text{ m})^2(5 \times 10^{-3} \text{ m}) = 2.5 \times 10^{-12} \text{ m}^3$
 $(2.5 \times 10^{-12} \text{ m}^3)\left(\frac{1 \text{ L}}{10^{-3} \text{ m}^3}\right)$
 $= 2.5 \times 10^{-9} \text{ L} = 2.5 \text{ nL}$
- 50 μm diameter:
volume = $\pi(25 \times 10^{-6} \text{ m})^2(5 \times 10^{-3} \text{ m}) = 9.8 \text{ nL}$
- (b) Capillary electrophoresis eliminates peak broadening from (1) the finite rate of mass transfer between the mobile and stationary phases and (2) multiple flow paths around stationary phase particles.

23-E. (a)



Net flow of cations and anions is to the right, because electroosmotic flow is stronger than electrophoretic flow at high pH

- (b) At pH 3, net flow of cations is to the *right* and net flow of anions is to the *left*.
- (c) With no analyte present, the constant concentration of chromate in background buffer gives a steady ultraviolet absorbance at 254 nm. When Cl⁻ emerges, it displaces some chromate anion (to maintain electro-neutrality). Because Cl⁻ does not absorb at 254 nm, the absorbance *decreases*.

- 23-F. (a) In the absence of micelles, all neutral molecules move with the electroosmotic speed of bulk solvent and arrive at the detector at time t_0 . Negative micelles migrate upstream and arrive at time $t_{mc} > t_0$. Neutral molecules partition between bulk solvent and micelles, so they arrive between t_0 and t_{mc} . The more time a neutral molecule spends in micelles, the closer is its migration time to t_{mc} .
- (b) (i) pK_a for C₆H₅NH₃⁺ is 4.60. At pH 10, the predominant form is neutral. (ii) Anthracene arrives at the detector last, so it spent more time inside micelles and therefore must have been more soluble in the micelles.

Answers to Problems

Complete solutions to all problems can be found in the

Solutions Manual for Exploring Chemical Analysis.

Numerical answers and other short answers are given here.

Chapter 1

- 1-2. (a) milliwatt = 10^{-3} watt
(b) picometer = 10^{-12} meter
(c) kilohm = 10^3 ohm
(d) microcoulomb = 10^{-6} coulomb
(e) terajoule = 10^{12} joule
(f) nanosecond = 10^{-9} second
(g) femtogram = 10^{-15} gram
(h) decipascal = 10^{-1} pascal
- 1-3. (a) 100 fJ or 0.1 pJ (b) 43.172 8 nC
(c) 299.79 THz (d) 0.1 nm or 100 pm
(e) 21 TW (f) 0.483 amol or 483 zmol
- 1-4. (a) 7.457×10^4 W (b) 7.457×10^4 J/s
(c) 1.782×10^4 cal/s (d) 6.416×10^7 cal/h
- 1-5. (a) 0.025 4 m, 39.37 inches
(b) 0.214 mile/s, 770 mile/h
(c) 1.04×10^3 m, 1.04 km, 0.643 mile
- 1-6. (a) 1.74×10^{-22} N (b) 5×10^{-4} K
(c) $60 \text{ Be} = 8.979 \times 10^{-25}$ kg;
force = 8.8×10^{-24} N = 8.8 yN
- 1-8. 1.10 M
- 1-9. 0.054 8 ppm, 54.8 ppb
- 1-10. 4.4×10^{-3} M, 6.7×10^{-3} M
- 1-11. (a) 70.5 g (b) 29.5 g (c) 0.702 mol
- 1-12. 6.18 g
- 1-13. (a) 1.7×10^3 L (b) 2.4×10^5 g
- 1-14. 8.0 g
- 1-15. (a) 55.6 mL (b) 1.80 g/mL
- 1-16. 5.48 g
- 1-17. 10^{-3} g/L, 10^3 $\mu\text{g}/\text{L}$, 1 $\mu\text{g}/\text{mL}$, 1 mg/L
- 1-18. 7×10^{-10} M
- 1-19. 15 days for chunk white tuna; 3.5 days for chunk light tuna
- 1-20. (a) 804 g solution, 764 g ethanol
(b) 16.6 M
- 1-21. (a) 0.228 g Ni (b) 1.06 g/mL
- 1-22. 1.235 M
- 1-23. Dilute 8.26 mL of 12.1 M HCl to 100.0 mL.
- 1-24. (a) 3.40 M (b) 14.7 mL
- 1-25. Shredded Wheat: 3.6 Cal/g, 102 Cal/oz; doughnut: 3.9, 111; hamburger: 2.8, 79; apple: 0.48, 14
- 1-27. (a) $K = 1/[\text{Ag}^+]^3 [\text{PO}_4^{3-}]$
(b) $K = P_{\text{CO}_2}^6/P_{\text{O}_2}^{15/2}$
- 1-28. (a) $P_A = 0.028$ bar, $P_E = 48$ bar
(b) 1.2×10^{10}
- 1-29. unchanged
- 1-30. 4.5×10^3

- 1-31. (a) 3.6×10^{-7} (b) 3.6×10^{-7} M (c) 3.0×10^4

- 1-33. If mean concentration is assumed to be 2.3 mg nitrate nitrogen/L, flow $\approx 5\ 000$ tons/yr.

Chapter 2

- 2-7. 5.403 1 g
2-8. 14.85 g
2-9. 0.296 1 g
2-10. 9.980 mL
2-11. 5.022 mL
2-12. 15.631 mL
2-13. 0.70%

Chapter 3

- 3-1. (a) 1.237 (b) 1.238 (c) 0.135 (d) 2.1
(e) 2.00
- 3-2. (a) 0.217 (b) 0.216 (c) 0.217 (d) 0.216
- 3-3. (a) 4 (b) 4 (c) 4
- 3-4. (a) 12.3 (b) 75.5 (c) 5.520×10^3
(d) 3.04 (e) 3.04×10^{-10} (f) 11.9
(g) 4.600 (h) 4.9×10^{-7}
- 3-5. (a) 12.01 (b) 10.9 (c) 14 (d) 14.3
(e) -17.66 (f) 5.97×10^{-3} (g) 2.79×10^{-5}
- 3-6. (a) 208.233 (b) 560.594
- 3-7. 389.977
- 3-9. (b) 25.031, systematic; ± 0.009 , random
(c) 1.98 and 2.03, systematic; ± 0.01 and ± 0.02 , random
(d) random (e) random
(f) mass is systematically low because empty funnel was not dried; also, there is always random error, but we do not know how much in one experiment.
- 3-10. (a) $3.124 (\pm 0.005)$ or $3.123_6 (\pm 0.005_2)$
(b) $3.124 (\pm 0.2\%)$ or $3.123_6 (\pm 0.1\%)$
- 3-11. (a) 2.1 ± 0.2 (or $2.1 \pm 11\%$)
(b) 0.151 ± 0.009 (or $0.151 \pm 6\%$)
(c) $0.22_3 \pm 0.02_4 (\pm 11\%)$
(d) $0.097_1 \pm 0.002_2 (\pm 2.3\%)$
- 3-12. (a) $21.0_9 (\pm 0.1_6)$ or $21.1 (\pm 0.2)$; relative uncertainty = $\pm 0.8\%$
(b) $27.4_3 (\pm 0.8_6)$; relative uncertainty = $\pm 3.1\%$
(c) $(14.9 \pm 1.3) \times 10^4$ or $(15 \pm 1) \times 10^4$; relative uncertainty = $\pm 9\%$
- 3-13. (a) $10.18 (\pm 0.07) (\pm 0.7\%)$
(b) $174 (\pm 3) (\pm 2\%)$
(c) $0.147 (\pm 0.003) (\pm 2\%)$
(d) $7.86 (\pm 0.01) (\pm 0.1\%)$
(e) $2\ 185.8 (\pm 0.8) (\pm 0.04\%)$

- 3-14. (a) 6.0 ± 0.2 ($\pm 4\%$)
 (b) $1.30_8 \pm 0.09_2$ ($\pm 7.0\%$)
 (c) $(1.30_8 \pm 0.09_2) \times 10^{-11}$ ($\pm 7.0\%$)
 (d) $2.7_2 \pm 0.7_8$ ($\pm 29\%$)

- 3-15. (a) $(3.8 \pm 0.4) \times 10^{-6}$ M (b) 12%
 (c) Relative error =
$$\frac{\text{uncertainty in } [\text{H}^+]}{[\text{H}^+]}$$

 $= 2.303$ (uncertainty in pH)

- 3-16. $(6.3 \pm 1.5) \times 10^{-9}$ M, $\pm 23\%$

- 3-17. 78.112 ± 0.005

- 3-18. 95.978 ± 0.009

- 3-19. (a) 58.443 ± 0.002 g/mol (b) $0.450\ 7$ ($\pm 0.000\ 5$) M

- 3-20. (a) $0.020\ 77 \pm 0.000\ 03$ M (b) yes

- 3-21. 1.235 ± 0.002 M

- 3-22. (a) 16.6 mL (b) 0.169 (± 0.002) M

- 3-23. Formula in cell F3:
 $=B3*\$A\$4+C3*\$A\$6+D3*\$A\$8+E3*\$A\10

- 3-25. Method 2 is more accurate. The relative uncertainty in mass in Method 1 is far greater than any other uncertainty in either procedure. Method 1 gives $0.002\ 72_6 \pm 0.000\ 01_8$ M AgNO₃. Method 2 gives $0.002\ 726 \pm 0.000\ 006_5$ M AgNO₃.

Chapter 4

- 4-2. 0.683, 0.955, 0.997

- 4-3. $F_{\text{calculated}} (= 2.8_2) < F_{\text{table}} (= 3.18)$, so difference is *not* significant.

- 4-4. (a) $1.527\ 67$ (b) $0.001\ 26$, $0.082\ 5\%$
 (c) $1.527\ 93 \pm 0.000\ 10$

- 4-5. (b) larger

- 4-6. 108.6_4 , 7.1_4 , $108.6_4 \pm 6.8_1$

- 4-7. (a) $\bar{x}_1 = 0.027\ 5_6$, $s_1 = 0.000\ 4_{88}$; $\bar{x}_2 = 0.026\ 9_0$,
 $s_2 = 0.000\ 4_{06}$

- (b) $F_{\text{calculated}} (= 1.44) < F_{\text{table}} (= 6.39)$, so difference is *not* significant.

- (c) $s_{\text{pooled}} = 0.000\ 4_{49}$; $t = 2.32 > t_{\text{table}} (95\%) = 2.306$, so difference is *significant*.

- 4-8. $2.299\ 47 \pm 0.001\ 15$, $2.299\ 47 \pm 0.001\ 71$

- 4-9. $F_{\text{calculated}} (= 3.56) < F_{\text{table}} (= 6.39)$, so difference is *not* significant.

- $s_{\text{pooled}} = 8.9_0$; $t = 1.67 < t_{\text{table}} (95\%) = 2.306$, so the difference is *not* significant.

- 4-10. (a) $F_{\text{calculated}} (= 5.2_7) > F_{\text{table}} (\approx 2.04)$, so difference is *significant*.

- (b) $t_{\text{calculated}} (= 18.2$ for 40 degrees of freedom) $> t_{\text{table}} (= 2.021)$, so difference is *significant*.

- (c) $F_{\text{calculated}} (= 1.3_3) < F_{\text{table}} (\approx 2.04)$, so difference is *not* significant.

- (d) $s_{\text{pooled}} = 0.001\ 08$; $t (= 1.39) < t_{\text{table}} (\approx 2.01$ for 47 degrees of freedom); difference is *not* significant.

- 4-11. $F_{\text{calculated}} (= 1.5_6) < F_{\text{table}} (= 9.12)$, so standard deviations are *not* significantly different. $s_{\text{pooled}} = 9.20$;
 $t_{\text{calculated}} (= 2.75) > t_{\text{table}} (= 2.365$ for 95% confidence and 7 degrees of freedom), so difference is *significant*.

- 4-12. $F_{\text{calculated}} (= 3.7_6) < F_{\text{table}} (= 6.26)$, so standard deviations are *not* significantly different. $s_{\text{pooled}} = 0.000\ 021_3$;

$t_{\text{calculated}} (= 2.48) > t_{\text{table}}$ ($= 2.262$ for 95% confidence and $5 + 6 - 2 = 9$ degrees of freedom), so difference is *significant*.

- 4-13. (a) $F_{\text{calculated}} (= 20.2) > F_{\text{table}} (= 5.05)$, so standard deviations are *significantly* different. Degrees of freedom = $5.49 \approx 5$. $t_{\text{calculated}} (= 0.80) < t_{\text{table}} (= 2.571$ for 95% confidence and 5 degrees of freedom), so difference is *not* significant.

- (b) $F_{\text{calculated}} (= 9.00) > F_{\text{table}} (= 5.05)$, so standard deviations are *significantly* different. Degrees of freedom = $6.10 \approx 6$. $t_{\text{calculated}} (= 5.4_2) > t_{\text{table}} (= 2.447$ for 95% confidence and 6 degrees of freedom), so difference is *significant*.

- (c) $F_{\text{calculated}} (= 4.9_4) < F_{\text{table}} (= 5.05)$, so standard deviations are *not* significantly different. $s_{\text{pooled}} = 0.01_{55}$. $t_{\text{calculated}} (= 2.6_8) > t_{\text{table}} (= 2.228$ for 95% confidence and 10 degrees of freedom), so difference is *significant*.

- (d) Buret and pipet have similar accuracy, but flask delivers a low value. Buret and pipet are within Class A tolerance. Flask is out of tolerance or neither student can read meniscus correctly.

- 4-14. $G_{\text{calculated}} (= 1.98) > G_{\text{table}} (= 1.822)$; discard 0.195.

- 4-15. outlier = 0.169; $G_{\text{calculated}} (= 2.26) > G_{\text{table}} (= 2.176)$; discard 0.169.

- 4-16. $y = -2x + 15$

- 4-17. $-1.299 (\pm 0.001) \times 10^4$, $3 (\pm 3) \times 10^2$

- 4-18. (a) $2.0_0 \pm 0.3_8$ (b) $2.0_0 \pm 0.2_6$

- 4-19. (a) $y (\pm 0.005_7) = 0.021\ 7_7 (\pm 0.000\ 1_9)x + 0.004_6 (\pm 0.004_4)$

- (c) protein = $23.0_7 \pm 0.2_9$ μg

- 4-21. It does not appear to be either CuCO₃ or CuCO₃ · xH₂O. The 99% confidence intervals for the class and instructor data exceed 51.43 wt% Cu expected in CuCO₃. If the material were a hydrate, the Cu content would be even less.

- 4-22. ⁸⁷Sr/⁸⁶Sr ratios agree with each other within their 95% confidence limits at both sites for the LGM time. Therefore LGM dust could have come from the same source at both sites. The isotope ratios are not the same at the two sites at the EH time. Therefore EH dust came from different sources for each site.

$$\text{pg/g} = 10^{-12} \text{ g Sr per g ice} = \text{parts per trillion.}$$

Chapter 5

- 5-8. c

- 5-9. 50% of red wells should be green and 8% of green wells should be red. It would be worse if there were a 50% false negative rate.

- 5-10. $F_{\text{calculated}} (= 1.9_4) < F_{\text{table}} (= 4.74)$, so standard deviations are *not* significantly different. The means are significantly different because $t_{\text{calculated}} (= 2.47) > t_{\text{table}} (= 2.262)$ for 95% confidence and 9 degrees of freedom.

- 5-11. (a) 0.003_{12} (b) 8.6×10^{-8} M (c) 2.9×10^{-7} M

- 5-12. One observation (day 101) is outside the action line.

None of the other criteria are violated.

- 5-13. yes: 7 consecutive measurements all above or all below the center line
- 5-14. (a) 22.2 ng/mL: precision = 23.8%, accuracy = 6.6%
88.2 ng/mL: precision = 13.9%, accuracy = -6.5%
314 ng/mL: precision = 7.8%, accuracy = -3.6%
(b) signal detection limit = 129.6; detection limit = 4.8×10^{-8} M; quantitation limit = 1.6×10^{-7} M
- 5-15. 96%, 0.064 $\mu\text{g/L}$ (= 3s)
- 5-16. 0.644 mM
- 5-17. 1.21 mM
- 5-18. (a) Figure 5-7 (b) x -intercept of standard addition graph = -8.72 ppb (c) Sr = 116 ppm
- 5-19. (a) 8.72 ± 0.43 ppb (b) 116 ± 6 ppm
- 5-20. 313 ppb
- 5-21. Standard addition line: $y = 42.852x + 1.088$
Pb in 4.60 mL = 0.02541 ± 0.00098 ppm
(a) and (b) Pb in 1.00 mL = $0.116_9 \pm 0.004_5$ ppm
- 5-22. 11.9 μM
- 5-23. 7.49 $\mu\text{g/mL}$
- 5-24. 0.47 mmol
- 5-25. Repeat the test on a preserved, duplicate sample taken at the same time as the first sample.

Chapter 6

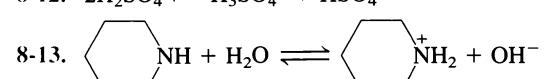
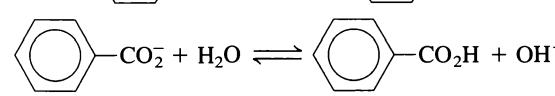
- 6-2. 43.2 mL, 270.0 mL
- 6-3. 4.300×10^{-2} M
- 6-4. 0.149 M
- 6-5. 32.0 mL
- 6-6. (a) 0.045 00 M (b) 36.42 mg/mL
- 6-7. 947 mg
- 6-8. 1.72 mg
- 6-9. (a) 0.020 34 M (b) 0.125 7 g (c) 0.019 83 M
- 6-10. (a) 0.105 3 mol/kg solution
(b) 0.286₉ mol/kg solution
- 6-11. (a) 0.001 492 8 ($\pm 0.06\%$) mol
(b) 0.001 434 ($\pm 0.14\%$) mol
(c) $\frac{\text{volumetric uncertainty}}{\text{gravimetric uncertainty}} = 2.2$; largest uncertainty in gravimetric delivery is mass of AgNO₃; largest uncertainty in volumetric delivery is pipet volume
- 6-12. 89.07 wt%
- 6-13. 9.066 mM
- 6-14. 3.555 mM
- 6-15. 30.5 wt%
- 6-16. 0.020 6 ($\pm 0.000 7$) M
- 6-17. (a) 7.1×10^{-5} M
(b) 1.0×10^{-3} g/100 mL
- 6-18. (a) $6.6_9 \times 10^{-5}$ M
(b) 14.4 ppm
- 6-19. 8.5 zM, no
- 6-20. 1 400 ppb, 76 ppb, 0.98 ppb; AgBr is best choice
- 6-21. (a) $[\text{Hg}_2^{2+}] = 6.8_8 \times 10^{-7}$ M; $[\text{IO}_3^-] = 1.3_8 \times 10^{-6}$ M
(b) $[\text{Hg}_2^{2+}] = 1.3 \times 10^{-14}$ M
- 6-22. I⁻ before Br⁻ before Cl⁻ before CrO₄²⁻
- 6-23. 0.106 0 M, 11.55 M
- 6-24. (a) $x = 0.000 945 4$

- 6-25. (a) SO₄²⁻ [from soil] + Ba²⁺ [from BaCl₂(s)] \longrightarrow BaSO₄(s) (b) 0.11₈ mmol (c) 0.11₈ mmol (d) 1.1 wt%

Chapter 7

- 7-2. 2
- 7-3. 0.085 38 g
- 7-4. 50.79 wt% Ni
- 7-5. 7.22 mL
- 7-6. 8.665 wt% K
- 7-7. 0.339 g
- 7-8. (a) 5.5 mg/100 mL (b) 5.834 mg, yes
- 7-9. Ba, 47.35 wt%; K, 8.279 wt%; Cl, 31.95 wt%
- 7-10. (a) 19.98 wt%
- 7-11. 11.69 mg CO₂, 2.051 mg H₂O
- 7-12. (a) 51.36 wt% C, 3.639 wt% H (b) C₆H₅
- 7-13. C₄H₉NO₂
- 7-14. 104.1 ppm C
- 7-15. (a) 0.027 36 M (b) systematic
- 7-16. 75.40 wt% Al₂O₃
- 7-17. C₈H_{9.06 \pm 0.17}N_{0.997 \pm 0.010}
- 7-18. 12.4 wt% S
- 7-19. (a) 2.270 wt% S (b) 0.32 million tons SO₂
(c) 0.56 million tons ash
- 7-20. (a) 98.3, 104.0, 98.6, 97.6, <0.3, 36.5, 6.4, <0.3, 4.2
(b) Fe³⁺, Pb²⁺, Cd²⁺, and In³⁺ are gathered quantitatively. (c) 10 times
- 7-21. $s_{\text{pooled}} = 0.036_{36}$ and $t = 5.1_3 \Rightarrow$ difference is significant above 99% confidence level

Chapter 8

- 8-1. (a) HCN/CN⁻, HCO₂H/HCO₂⁻
(b) H₂O/OH⁻, HPO₄²⁻/PO₄³⁻
(c) H₂O/OH⁻, HSO₃⁻/SO₃²⁻
- 8-2. $[\text{H}^+] > [\text{OH}^-]$, $[\text{OH}^-] > [\text{H}^+]$
- 8-3. (a) 4 (b) 9 (c) 3.24 (d) 9.76
- 8-4. (a) 7.46 (b) 2.9×10^{-7} M
- 8-5. 2.5×10^{-5} M
- 8-6. 7.8
- 8-7. See Table 8-1.
- 8-8. weak acids: carboxylic acids, ammonium salts, aqueous metal ion with charge ≥ 2
weak bases: carboxylate anions and amines
- 8-9. (a) 0.010 M, 2.00 (b) $2.8_6 \times 10^{-13}$ M, pH 12.54
(c) 0.030 M, 1.52 (d) 3.0 M, -0.48
(e) 1.0×10^{-12} M, 12.00
- 8-10. (b) trichloroacetic acid
- 8-11. (b) sodium 2-mercaptoethanol
- 8-12. $2\text{H}_2\text{SO}_4 \rightleftharpoons \text{H}_3\text{SO}_4^+ + \text{HSO}_4^-$
- 8-13. 
- 

- 8-14. $\text{OCl}^- + \text{H}_2\text{O} \rightleftharpoons \text{HOCl} + \text{OH}^-$, 3.3×10^{-7}
 8-15. 9.78
 8-16. $2.2 \times 10^{-12} \text{ M}$
 8-17. $3.02, 9.51 \times 10^{-2}$
 8-18. $5.41, 2.59 \times 10^{-5}$
 8-19. $3.14, 7.29 \times 10^{-4} \text{ M}$, 0.084 M , 0.085 M
 8-20. 5.00
 8-21. 3.70
 8-22. 7.30
 8-23. $5.51, 3.1 \times 10^{-6} \text{ M}$, 0.060 M
 8-24. (a) 3.03, 0.094 (b) 7.00, 0.999
 8-25. 5.50
 8-26. $K_a = 2.71 \times 10^{-11}$, $K_b = 3.69 \times 10^{-4}$
 8-28. $11.28, 0.058 \text{ M}$, $1.9 \times 10^{-3} \text{ M}$
 8-29. pH = 8.88, 0.007 56%; pH = 8.38, 0.023 9%;
 pH = 7.00, 0.568%
 8-30. 10.95
 8-31. 9.97, 0.003 6
 8-32. 3.4×10^{-6}
 8-33. 2.2×10^{-7}
 8-35. 2.93, 0.118

Chapter 9

- 9-3. 4.13
 9-4. (b) 1/1 000 (c) $\text{p}K_a - 4$
 9-5. pH 10, red; pH 8, orange; pH 6, yellow
 9-6. (a) 1.5×10^{-7} (b) 0.15
 9-7. (a) 14 (b) 1.4×10^{-7}
 9-8. (a) 2.33×10^{-7} (b) 1.00 (c) 23.3
 9-9. 3.59
 9-10. (a) 8.37 (b) 0.423 g (c) 8.33 (d) 8.41
 9-11. (b) 7.18 (c) 7.00 (d) 6.86 mL
 9-12. (a) 2.56 (b) 2.86
 9-13. 3.38 mL
 9-14. 13.7 mL
 9-15. 4.68 mL
 9-16. (a) citric acid or acetic acid
 (b) imidazole hydrochloride
 (c) CAPS
 (d) CHES, boric acid, or ammonia
 9-17. (ii)
 9-18. (a) NaOH
 9-19. (b) HCl
 9-20. (a) 90.8 mL HCl
 9-21. (a) red (b) orange (c) yellow (d) red
 9-22. (a) $p = 0.940$ 6 mol, $q = 0.059$ 35 mol
 (b) $\Delta(\text{pH}) = -0.072$
 9-23. 9.13

Chapter 10

- 10-6. $V_e = 10.0 \text{ mL}$; pH = 13.00, 12.95, 12.68, 11.96, 10.96,
 7.00, 3.04, 1.75
 10-7. $V_e = 12.5 \text{ mL}$; pH = 1.30, 1.35, 1.60, 2.15, 3.57, 7.00,
 10.42, 11.12
 10-8. $V_e = 5.00 \text{ mL}$; pH = 2.66, 3.40, 4.00, 4.60, 5.69, 8.33,
 10.96, 11.95

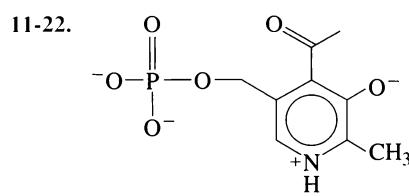
- 10-9. $V_e = 5.00 \text{ mL}$; pH = 6.32, 10.63, 11.23, 11.85
 10-10. 2.80, 3.65, 4.60, 5.56, 8.65, 11.96
 10-11. 8.18
 10-12. 3.72
 10-13. 0.091 8 M
 10-14. (a) 0.025 92 M (b) 0.020 31 M
 (c) 9.69 (d) 10.07
 10-15. $V_e = 10.0 \text{ mL}$; pH = 11.00, 9.95, 9.00, 8.05, 7.00, 5.02,
 3.04, 1.75
 10-16. $V_e = 47.79 \text{ mL}$; pH = 8.74, 5.35, 4.87, 4.40, 3.22, 2.58
 10-17. (a) 2.2×10^9
 (b) 10.92, 9.57, 9.35, 8.15, 5.53, 2.74
 10-18. (a) 9.44 (b) 2.55 (c) 5.15
 10-19. $V_e = 10.0 \text{ mL}$; pH = 9.85, 7.95, 6.99, 6.04, 5.00, 4.22,
 3.45, 2.17
 10-20. no
 10-21. yellow, green, blue
 10-23. (a) colorless \rightarrow pink (b) systematically requires
 too much NaOH
 10-24. cresol red (orange \rightarrow red) or phenolphthalein
 (colorless \rightarrow pink)
 10-25. 10.727 mL
 10-26. 0.063 56 M
 10-27. (a) 0.087 99 (b) 25.74 mg (c) 2.860 wt%
 10-28. (a) 5.62 (b) methyl red
 10-31. tribasic, 0.015 3 M

Chapter 11

- 11-2. $K_{a2} = 1.03 \times 10^{-2}$, $K_{b2} = 1.78 \times 10^{-13}$
 11-3. 7.09×10^{-3} , 6.33×10^{-8} , 4.2×10^{-13}
 11-4. Two pK values apply to the carboxylic acid and ammonium groups of all amino acids. Some amino acids have a substituent that is an acid or base, which is responsible for a third pK value.
 11-5. piperazine: $K_{b1} = 5.38 \times 10^{-5}$, $K_{b2} = 2.15 \times 10^{-9}$
 phthalate: $K_{b1} = 2.56 \times 10^{-9}$, $K_{b2} = 8.93 \times 10^{-12}$
 11-7. 2.49×10^{-8} , 5.78×10^{-10} , 1.34×10^{-11}
 11-8. 1.62×10^{-5} , 1.54×10^{-12}
 11-9. (a) 1.95, 0.089 M, 1.12×10^{-2} M, 2.01×10^{-6} M
 (b) 4.27, 3.8×10^{-3} M, 0.100 M, 3.8×10^{-3} M
 (c) 9.35, 7.04×10^{-12} M, 2.23×10^{-5} M, 0.100 M
 11-10. (a) 11.00, 0.099 0 M, 9.95×10^{-4} M, 1.00×10^{-9} M
 (b) 7.00, 1.0×10^{-3} M, 0.100 M, 1.0×10^{-3} M
 (c) 3.00, 1.00×10^{-9} M, 9.95×10^{-4} M, 0.099 0 M
 11-11. 11.60 , $[\text{B}] = 0.296 \text{ M}$, $[\text{BH}^+] = 3.99 \times 10^{-3} \text{ M}$,
 $[\text{BH}_2^{2+}] = 2.15 \times 10^{-9} \text{ M}$
 11-12. 7.53 , $[\text{BH}_2^{2+}] = 9.48 \times 10^{-4} \text{ M}$, $[\text{BH}^+] \approx 0.150 \text{ M}$,
 $[\text{B}] = 9.49 \times 10^{-4} \text{ M}$
 11-13. 5.60
 11-14. (a) $\text{p}K_1$: $[\text{H}_2\text{A}] = [\text{HA}^-]$; $\frac{1}{2}(\text{p}K_1 + \text{p}K_2)$: $[\text{H}_2\text{A}] = [\text{A}^{2-}]$;
 $\text{p}K_2$: $[\text{HA}^-] = [\text{A}^{2-}]$
 (b) monoprotic: $\text{p}K_a$: $[\text{HA}] = [\text{A}^-]$
 triprotic: $\text{p}K_1$: $[\text{H}_3\text{A}] = [\text{H}_2\text{A}^-]$; $\frac{1}{2}(\text{p}K_1 + \text{p}K_2)$:
 $[\text{H}_3\text{A}] = [\text{HA}^{2-}]$; $\text{p}K_2$: $[\text{H}_2\text{A}^-] = [\text{HA}^{2-}]$;
 $\frac{1}{2}(\text{p}K_2 + \text{p}K_3)$: $[\text{H}_2\text{A}^-] = [\text{A}^{3-}]$;
 $\text{p}K_3$: $[\text{HA}^{2-}] = [\text{A}^{3-}]$

- 11-15. (a) HA (b) A^- (c) (i) 1.0, (ii) 0.10
 11-16. (a) 4.00 (b) 8.00 (c) H_2A
 (d) HA^- (e) A^{2-}
- 11-17. pH Dominant form Second form
- | | | |
|------------|--------------|--------------|
| 2 | H_3PO_4 | $H_2PO_4^-$ |
| 3, 4 | $H_2PO_4^-$ | H_3PO_4 |
| 5, 6, 7 | $H_2PO_4^-$ | HPO_4^{2-} |
| 8, 9 | HPO_4^{2-} | $H_2PO_4^-$ |
| 10, 11, 12 | HPO_4^{2-} | PO_4^{3-} |
| 13 | PO_4^{3-} | HPO_4^{2-} |
- 11-18. (a) 9.00 (b) 9.00 (c) BH^+ (d) 1.0×10^3
- 11-19. (a) 6.85, 9.93 (b) 9.93 (c) 6.85
 (d) pH Dominant form Second form
- | | | |
|---------|-------------|-------------|
| 4, 5, 6 | BH_2^{2+} | BH^+ |
| 7, 8 | BH^+ | BH_2^{2+} |
| 9 | BH^+ | B |
| 10 | B | BH^+ |

- (e) 1.2×10^2 (f) 7.1×10^4
- 11-20. glutamic acid at pH 9.00:
 predominant $(NH_3^+)(RCO_2^-)(CO_2^-)$ (R = substituent);
 secondary $(NH_2)(RCO_2^-)(CO_2^-)$
 glutamic acid at pH 10.00:
 predominant $(NH_2)(RCO_2^-)(CO_2^-)$;
 secondary $(NH_3^+)(RCO_2^-)(CO_2^-)$
 tyrosine at pH 9.00:
 predominant $(NH_2)(ROH)(CO_2^-)$;
 secondary $(NH_3^+)(ROH)(CO_2^-)$
 tyrosine at pH 10.00:
 predominant $(NH_2)(ROH)(CO_2^-)$;
 secondary $(NH_2)(RO^-)(CO_2^-)$
- 11-21. (a) 11.00 (b) 10.5



- 11-23. 5.41, $[H_3Arg^{2+}] = 1.3 \times 10^{-5} M$, $[H_2Arg^+] = 0.050 M$,
 $[HArg] = 1.3 \times 10^{-5} M$, $[Arg^-] = 3 \times 10^{-12} M$
- 11-24. $HCit^{2-}$
- 11-25. $V_{el} = 10.0 \text{ mL}$, $V_{e2} = 20.0 \text{ mL}$
 $pH = 2.51, 4.00, 6.00, 8.00, 10.46, 12.21$
- 11-26. $V_{el} = 10.0 \text{ mL}$, $V_{e2} = 20.0 \text{ mL}$
 $pH = 11.49, 10.00, 8.00, 6.00, 3.54, 1.79$
- 11-27. (a) phenolphthalein (colorless → red)
 (b) *p*-nitrophenol (colorless → yellow)
 (c) bromothymol blue (yellow → green)
 or bromocresol purple (yellow → yellow + purple)
 (d) thymolphthalein (colorless → blue)
- 11-28. $V_{el} = 40.0 \text{ mL}$, $V_{e2} = 80.0 \text{ mL}$
 $pH = 11.36, 9.73, 7.53, 5.33, 3.41, 1.85$

- 11-29. $V_{el} = 20.0 \text{ mL}$, $V_{e2} = 40.0 \text{ mL}$, $V_{e3} = 60.0 \text{ mL}$
 $pH = 1.86, 2.15, 4.68, 7.20, 9.79, 11.43$
 (pH cannot rise as fast as we calculated at 42 mL
 because even adding 0.04 M OH^- to water cannot raise
 the pH as fast as we estimated. HPO_4^{2-} is too weak an
 acid to react appreciably with dilute OH^- .)
- 11-30. $V_{el} = 25.0 \text{ mL}$, $V_{e2} = 50.0 \text{ mL}$; $pH = 7.62, 5.97, 3.8, 1.9$
- 11-31. (a) 2.56, 3.46, 4.37, 8.42, 11.45 (b) second
 (c) thymolphthalein; first trace of blue
- 11-32. (a) $[CO_3^{2-}] = K_{a2} K_{a1} K_H P_{CO_2}/[H^+]^2$
 (b) $0^\circ C: 6.6 \times 10^{-5} \text{ mol kg}^{-1}$; $30^\circ C: 1.8 \times 10^{-4} \text{ mol kg}^{-1}$
 (c) $0^\circ C: [Ca^{2+}][CO_3^{2-}] = 6.6 \times 10^{-7} \text{ mol}^2 \text{ kg}^{-2}$
 (aragonite dissolves, calcite does not);
 $30^\circ C: [Ca^{2+}][CO_3^{2-}] = 1.8 \times 10^{-6} \text{ mol}^2 \text{ kg}^{-2}$
 (neither dissolves)

Chapter 12

- 12-4. (a) true (b) true (c) true
- 12-9. (a) 0.2 mM (b) 0.6 mM (c) 2.4 mM
- 12-10. (a) 0.660 (b) 0.54 (c) 0.18 (d) 0.83
- 12-11. 0.004 6
- 12-12. (a) 0.88₇ (b) 0.87₁
- 12-13. (a) 0.42₂ (b) 0.43₂
- 12-14. (a) $1.3 \times 10^{-6} M$ (b) $2.9 \times 10^{-11} M$
- 12-15. $\gamma_{H^+} = 0.86$, $pH = 2.07$
- 12-16. $[H^+] = 1.2 \times 10^{-7} M$; $pH = 6.99$
- 12-17. 11.94, 12.00
- 12-18. (a) $[Mn^{2+}] = 3.4_2 \times 10^{-5} M$, $[OH^-] = 6.8 \times 10^{-5} M$;
 $pH = 9.84$
 (b) $[Mn^{2+}] = 5.2_6 \times 10^{-5} M$, $[OH^-] = 1.05 \times 10^{-4} M$;
 $pH = 9.92$
- 12-19. $6.6 \times 10^{-7} M$
- 12-20. 1st iteration: $\mu = 0$, $[Pb^{2+}] = 2.0_8 \times 10^{-3} M$
 2nd iteration: $\mu = 6.24 \times 10^{-3} M$; $[Pb^{2+}] = 2.4_5 \times 10^{-3} M$
 3rd iteration: $\mu = 7.35 \times 10^{-3} M$; $[Pb^{2+}] = 2.4_8 \times 10^{-3} M$
 4th iteration: $\mu = 7.44 \times 10^{-3} M$; $[Pb^{2+}] = 2.4_8 \times 10^{-3} M$
- 12-21. (a) 1st iteration: $\mu = 0$, $[Ca^{2+}] = 4.9_0 \times 10^{-3} M$
 2nd iteration: $\mu = 0.019 6 M$; $[Ca^{2+}] = 7.9_2 \times 10^{-3} M$
 3rd iteration: $\mu = 0.031 7 M$; $[Ca^{2+}] = 8.7_9 \times 10^{-3} M$
 4th iteration: $\mu = 0.035 2 M$; $[Ca^{2+}] = 9.0_8 \times 10^{-3} M$
 5th iteration: $\mu = 0.036 3 M$; $[Ca^{2+}] = 9.1_8 \times 10^{-3} M$
 (If you use the extended Debye-Hückel equation instead
 of interpolation to find activity coefficients, the answer
 is $[Ca^{2+}] = 9.9 \times 10^{-3} M$
- (b) Remainder of dissolved calcium is the *ion pair*
 $CaSO_4(aq)$.
- 12-22. $[H^+] + 2[Ca^{2+}] + [Ca(HCO_3)^+] + [Ca(OH)^+] +$
 $[K^+] = [OH^-] + [HCO_3^-] + 2[CO_3^{2-}] + [ClO_4^-]$
- 12-23. $[H^+] = [OH^-] + [HSO_4^-] + 2[SO_4^{2-}]$
- 12-24. $[H^+] = [OH^-] + [H_2AsO_4^-] + 2[HAsO_4^{2-}] + 3[AsO_4^{3-}]$
- 12-25. (a) $2[Mg^{2+}] + [H^+] = [Br^-] + [OH^-]$
 (b) $2[Mg^{2+}] + [H^+] + [MgBr^+] = [Br^-] + [OH^-]$
- 12-26. $[CH_3CO_2^-] + [CH_3CO_2H] = 0.1 M$
- 12-27. (a) $0.20 M = [Mg^{2+}]$ (b) $0.40 M = [Br^-]$
 (c) $0.20 M = [Mg^{2+}] + [MgBr^+]$
 (d) $0.40 M = [Br^-] + [MgBr^+]$

- 12-28. (a) $[F^-] + [HF] = 2[Ca^{2+}]$
(b) $[F^-] + [HF] + 2[HF_2^-] = 2[Ca^{2+}]$
- 12-29. (a) $2[Ca^{2+}] = 3\{[PO_4^{3-}] + [HPO_4^{2-}] + [H_2PO_4^-] + [H_3PO_4]\}$
(b) $3\{[Fe^{3+}] + [Fe(OH)^{2+}] + [Fe(OH)_2^+] + [FeSO_4^+]\} = 2[SO_4^{2-}] + [HSO_4^-] + [FeSO_4^+]$
- 12-30. $[Y^{2-}] = [X_2Y_2^{2+}] + 2[X_2Y^{4+}]$
- 12-31. $[OH^-] = 1.48 \times 10^{-7} M; [H^+] = 6.8 \times 10^{-8} M; [Mg^{2+}] = 4.0 \times 10^{-8} M$
- 12-32. $5.8 \times 10^{-4} M$
- 12-33. (a) In the pH range 3–9, no reactions consume Ca^{2+} or SO_4^{2-} , so the solubility of $CaSO_4$ is constant.
(b) Below pH 3, SO_4^{2-} becomes protonated. Above pH 12, Ca^{2+} makes $CaOH^+$. Solubility of $CaSO_4$ should increase below pH 3 and above pH 12.
- 12-34. (a) $[Ag^+] = 2.4 \times 10^{-8} M; [CN^-] = 9.2 \times 10^{-9} M; [HCN] = 1.5 \times 10^{-8} M$
(b) $[Ag^+] = 2.9 \times 10^{-8} M; [CN^-] = 1.3 \times 10^{-8} M; [HCN] = 1.6 \times 10^{-8} M$
- 12-35. (a) $5.0 \times 10^{-9} mol$ (b) $4.0 \times 10^{-6} mol$
(c) $1.1 \times 10^{-8} mol$
- 12-36. (a) 0.98, 0.02 (b) 0.82, 0.18 (c) 0.60, 0.40
- 12-37. (a) 0.06, 0.94 (b) 0.39, 0.61 (c) 0.86, 0.14
- 12-38. (a) 0.09, 0.91 (b) 0.50, 0.50 (c) 0.67, 0.33
- 12-42. 0.63

Chapter 13

- 13-1. (a) 10.0 mL (b) 10.0 mL
- 13-8. 0.010 3 M
- 13-9. $[Ni^{2+}] = 0.012\ 4 M, [Zn^{2+}] = 0.007\ 18 M$
- 13-10. (a) 0.267₁ mmol (b) 0.152₂ mmol
(c) yellow, red, yellow, red
- 13-11. 0.024 30 M
- 13-12. $1.256 (\pm 0.003) mM$
- 13-13. 0.014 68 M
- 13-14. 0.092 6 M
- 13-15. 5.150 mg Mg, 20.89 mg Zn, 69.64 mg Mn
- 13-16. 32.7 wt% (theoretical = 32.90 wt%)
- 13-17. (a) 2.7×10^{-10} (b) 0.57
- 13-18. (a) $Co^{2+} + 4NH_3 \rightleftharpoons Co(NH_3)_4^{2+}$
(b) $Co(NH_3)_3^{2+} + NH_3 \rightleftharpoons Co(NH_3)_4^{2+}, \log K_4 = 0.64$
- 13-19. (a) 2.5×10^7 (b) $4.5 \times 10^{-5} M$
- 13-20. (a) 100.0 mL (b) 0.016 7 M (c) 0.041
(d) 4.1×10^{10} (e) $7.8 \times 10^{-7} M$
(f) $2.4 \times 10^{-10} M$
- 13-21. $pMn^{2+} = 1.70, 2.18, 2.81, 3.87, 4.87, 5.66, 6.46, 8.15, 8.45$
- 13-22. $pCa^{2+} = \infty, 10.30, 9.52, 8.44, 7.43, 6.15, 4.88, 3.20, 2.93$
- 13-23. EDTA behaves as a weak base (A^-) and the metal ion behaves as H^+ .
- 13-24. $pCu^{2+} = 1.10, 1.57, 2.21, 3.27, 6.91, 10.54, 11.24$
- 13-25. At pH 7, $pCu^{2+} = 1.10, 1.57, 2.21, 3.27, 8.47, 13.66, 14.36$
- 13-26. 5.6 g

- 13-27. (a) Reaction with NH_3 and OH^- raises the titration curve by 0.48 log units before V_e and
(b) leaves it unchanged after V_e .

Chapter 14

- 14-1. (b) $6.242 \times 10^{18} e^-/C$ (c) 96 485 C/mol
- 14-2. oxidant: Fe_2O_3 ; reductant: Al
- 14-3. oxidant: H_2O ; reductant: Na; $H_2O + e^- \rightleftharpoons OH^- + \frac{1}{2}H_2(g); Na(s) \rightleftharpoons Na^+ + e^-$
- 14-4. (a) oxidant: TeO_3^{2-} ; $TeO_3^{2-} + 3H_2O + 4e^- \rightleftharpoons Te(s) + 6OH^-$; reductant: $S_2O_4^{2-}$; $S_2O_4^{2-} + 4OH^- \rightleftharpoons 2SO_3^{2-} + 2H_2O + 2e^-$
(b) $3.02 \times 10^3 C$ (c) 0.840 A
- 14-5. (a) oxidant: C_2HCl_3 ; reductant: Fe (b) 4.5%
(c) 5.78 A
- 14-6. 0.015 9 J
- 14-7. (a) 71.5 A (b) $6.8 \times 10^6 J$
- 14-8. (b) $Hg_2Cl_2(s) + 2e^- \rightleftharpoons 2Hg(l) + 2Cl^-$
 $Zn^{2+} + 2e^- \rightleftharpoons Zn(s)$
(c) e^- flows from Zn (-0.75 V) to Pt (+0.25 V). Net reaction is
 $Hg_2Cl_2(s) + Zn(s) \rightleftharpoons 2Hg(l) + Zn^{2+} + 2Cl^-$
- 14-10. (a) 0.572 V (b) 0.568 V.
Disagreement between (a) and (b) is probably within the uncertainty of the standard potentials and solubility products.
- 14-11. (a) $Pt(s) | Cr^{2+}(aq), Cr^{3+}(aq) || Tl^+(aq) | Tl(s)$
(b) right: $E_+ = -0.336 V$; left: $E_- = -0.42 V$
 $E = E_+ - E_- = -0.336 - (-0.42) = 0.084 V$
(c) Pt (d) $Tl^+ + Cr^{2+} \rightleftharpoons Tl(s) + Cr^{3+}$
- 14-12. (a) right half-cell: $Hg_2Cl_2(s) + 2e^- \rightleftharpoons 2Hg(l) + 2Cl^-$
 $E = 0.309_4 V$
left half-cell: $2H^+ + 2e^- \rightleftharpoons H_2(g)$ $E = -0.120_7 V$
(b) anode is Pt of the hydrogen electrode
(c) 0.430 V
- 14-13. (a) right half-cell: $Al^{3+} + 3e^- \rightleftharpoons Al(s)$
 $E_+^\circ = -1.677 V$
left half-cell: $Br_2(l) + 2e^- \rightleftharpoons 2Br^-$
 $E^\circ = 1.078 V$
 $E = E_+ - E_- = -1.716_4 - 1.137_2 = -2.854 V$
(b) Electrons flow from Al to Pt: $\frac{3}{2}Br_2(l) + Al(s) \rightleftharpoons 3Br^- + Al^{3+}$
(c) Br_2
(d) 1.31 kJ
(e) $2.69 \times 10^{-8} g/s$
- 14-14. (a) $-0.357 V; 9.2 \times 10^{-7}$
(b) 0.722 V; 7×10^{48}
- 14-15. (a) $\frac{1}{2}O_2(g) + 2H^+ + 2e^- \rightleftharpoons H_2O(l)$
 $2H_2O(l) + 2e^- \rightleftharpoons H_2(g) + 2OH^-$
 $E = 2.057 V$
(b) $K = \frac{1}{P_{O_2}^{1/2} P_{H_2} [H^+]^2 [OH^-]^2} = 3.5 \times 10^{69}$
(c) 111 days, 7.94 kg O₂
- 14-16. $E^\circ = -0.330 V, K = 7 \times 10^{-12}$
- 14-17. $E^\circ = 0.023 V, K_f = 6.0$

- 14-18. Voltage is constant because none of the activities of reactants or products change during operation of the cell until one of the reactants is fully consumed.
- 14-19. (a) $E^\circ = -0.339$ V, $K = 1.9 \times 10^{-6}$ (b) -0.386 V
 (c) Electrons travel from Ag to Au. $\text{Ag}(s)$ is oxidized to $\text{Ag}(\text{S}_2\text{O}_3)_2^{3-}$
- 14-20. For $\text{Br}_2(l) \rightleftharpoons \text{Br}_2(aq)$, $E^\circ = -0.020$ V and $K = [\text{Br}_2(aq)] = 0.21$ M = 34 g/L.
- 14-21. (a) $2\text{IO}_3^- + 16\text{I}^- + 12\text{H}^+ \rightleftharpoons 6\text{I}_3^- + 6\text{H}_2\text{O}$
 (b) 0.675 V; 10^{114}
 (c) 0.178 V, reaction proceeds in the forward direction written in (a) (d) pH 8.5
- 14-22. (b) 0.044 V
- 14-23. (a) 0.747 V (b) 0.506 V
- 14-24. (a) 0.086 V (b) -0.021 V (c) 0.021 V
- 14-25. 0.684 V
- 14-26. The reaction $\text{L} + \text{Fe(III)} \rightleftharpoons \text{LFe(III)}$ has a greater formation constant than does the reaction $\text{L} + \text{Fe(II)} \rightleftharpoons \text{LFe(II)}$.
- 14-27. Probably $\text{Zn}(s) + 2\text{H}_2\text{O} \rightleftharpoons \text{Zn}^{2+}(aq) + \text{H}_2(g) + 2\text{OH}^-(aq)$, $E^\circ = -0.064$ V. This reaction is not spontaneous under standard conditions, but it is spontaneous when the concentrations of products are sufficiently low.
- 14-28. lead-acid battery: 83.42 A · h/kg;
 hydrogen-oxygen fuel cell: 2 975 A · h/kg

Chapter 15

- 15-1. (a) $\text{Cu}^{2+} + 2\text{e}^- \rightleftharpoons \text{Cu}(s)$
 (b) $E_+ = 0.339 - \frac{0.059}{2} \log\left(\frac{1}{[\text{Cu}^{2+}]}\right) = 0.309$ V
 (c) 0.112 V
- 15-2. (a) $\text{Br}_2(aq) + 2\text{e}^- \rightleftharpoons 2\text{Br}^-$, $E_+ = 1.057$ V
 (b) 0.816 V
- 15-3. 0.1 mL: $[\text{Ag}^+] = 1.1 \times 10^{-11}$ M, $E = -0.090$ V
 10.0 mL: $[\text{Ag}^+] = 2.2 \times 10^{-11}$ M; $E = -0.073$ V
 25.0 mL: $[\text{Ag}^+] = 1.05 \times 10^{-6}$ M; $E = 0.204$ V
 30.0 mL: $[\text{Ag}^+] = 0.0125$ M; $E = 0.445$ V
- 15-4. 0.1 mL, $E = 0.481$ V; 10.0 mL, 0.445 V; 20.0 mL, 0.194 V; 30.0 mL, -0.039 V
- 15-5. 1 mL, $E = 0.060$ V; 10.0 mL, 0.073 V; 50.0 mL, 0.270 V; 60.0 mL, 0.408 V
- 15-6. (a) $2\text{Cl}^- + \text{Hg}_2^{2+} \rightarrow \text{Hg}_2\text{Cl}_2(s)$; $V_e = 25.0$ mL
 (b) $E = 0.555 + \frac{0.059}{2} \log|\text{Hg}_2^{2+}|$
 (c) 0.1 mL, $E = 0.084$ V; 10.0 mL, 0.102 V; 25.0 mL, 0.372 V; 30.0 mL, 0.490 V
- 15-7. Left side is negative.
- 15-8. Right side is positive in both cells.
- 15-9. 10.67
- 15-10. (a) +0.10 (b) 13%
- 15-14. small
- 15-17. +0.029 58 V
- 15-18. 0.211 mg/L
- 15-19. (a) -0.407 V (b) 1.55×10^{-2} M

- 15-20. constant = -0.128 V; with Na^+ , $E = -0.310$ V; apparent concentration of $\text{Li}^+ = 8.4 \times 10^{-4}$ M
- 15-21. +1.2 mV, 10%
- 15-22. (a) 1.60×10^{-4} M (b) 3.59 wt% N
- 15-23. (a) nonactin-based electrode: K^+ ; crown ether-based electrode: Li^+ (b) $10^{-1.5}/10^{-1.0} = 0.3$
- 15-24. (a) $E = \text{constant} + \beta\left(\frac{0.059}{3}\right) \log([\text{La}^{3+}]_{\text{outside}})$
 (b) 19.7 mV (c) +25.1 mV (d) +100.7 mV
- 15-25. (a) $E = 51.09 (\pm 0.24) + 28.14 (\pm 0.08)$ log $[\text{Ca}^{2+}]$, ($s_y = 0.27$) (b), (c) $2.43 (\pm 0.06) \times 10^{-3}$ M
- 15-26. $1.22_1 \pm 0.02_9 = 1.19_2$ to 1.25_0 ; 1.19 is barely (not significantly) outside the 95% confidence interval.
- 15-28. (a) Below pH ≈ 4 , NO_2^- is converted to HNO_2 .
 (b) At high pH, the electrode responds to OH^- as an interfering ion. (c) ~ 4.5 (d) ~ 3 μM

Chapter 16

- 16-1. $E^\circ = 0.93_3$; $K = 6 \times 10^{15}$, or just 10^{16} on the basis of significant digits
- 16-2. (d) 0.490, 0.526, 0.626, 0.99, 1.36, 1.42, 1.46 V
- 16-3. (d) 1.58, 1.50, 1.40, 0.733, 0.065, 0.005, -0.036 V
 (e) Diphenylbenzidine sulfonic acid (violet \rightarrow colorless) or diphenylamine sulfonic acid (red-violet \rightarrow colorless) would be suitable. Other indicators such as diphenylamine (violet \rightarrow colorless) or tris(2,2'-bipyridine)iron (pale blue \rightarrow red) also would work.
- 16-4. (d) $-0.120, -0.102, -0.052, 0.21, 0.48, 0.53$ V
 (e) methylene blue (colorless \rightarrow blue)
- 16-5. 0.371, 0.439, 0.507, 1.128, 1.252, 1.266 V
- 16-6. (d) $-0.143, -0.102, -0.061, 0.096, 0.408, 0.450$ V
- 16-7. (b) 0.570, 0.307, 0.184 V
- 16-8. solid curve: tris(2,2'-bipyridine)iron (red \rightarrow pale blue), or tris(5-nitro-1,10-phenanthroline)iron (red-violet \rightarrow pale blue), or tris(2,2'-bipyridine)ruthenium (yellow \rightarrow pale blue); dashed curve: diphenylamine sulfonic acid (colorless \rightarrow red violet), or diphenylbenzidine sulfonic acid (colorless \rightarrow violet)
- 16-9. no
- 16-10. I^- reacts with I_2 to give I_3^- , which increases the solubility of I_2 and decreases its volatility.
- 16-11. Oxidation states: $\text{I}_2(0)$, $\text{HOI}(+1)$, $\text{IO}_3^- (+5)$, $\text{I}^- (-1)$
 The two balanced disproportionation reactions can occur in any combination, ranging from all of one to all of the other:
 $2\text{I}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{HOI} + 2\text{I}^- + 2\text{H}^+$
 $6\text{I}_2 + 6\text{H}_2\text{O} \rightarrow 2\text{IO}_3^- + 10\text{I}^- + 12\text{H}^+$
- 16-12. (a) colorless \rightarrow pale red (b) 35.50 mg
 (c) It does matter.
- 16-13. mol $\text{NH}_3 = 2(\text{initial mol H}_2\text{SO}_4 - \frac{1}{2} \times \text{mol thiosulfate})$
- 16-14. (a) We do not need to measure KI or H_2SO_4 accurately.
 (b) $\text{I}_3^- + \text{SO}_3^{2-} + \text{H}_2\text{O} \rightarrow 3\text{I}^- + \text{SO}_4^{2-} + 2\text{H}^+$
 (c) $[\text{SO}_3^{2-}] = 5.079 \times 10^{-3}$ M = 406.6 mg/L
 (d) no
- 16-15. (a) 7×10^2 (b) 1.0 (c) 0.34 g I_2/L

- 16-16. (a) 8 nmol (b) maybe just barely
 16-17. (a) 0.125 (b) $6.87_5 \pm 0.03_8$

Chapter 17

- 17-3. V₂
 17-6. 0.342 M
 17-7. 6.04; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$
 17-8. 12.567 5 g
 17-9. 54.77 wt%
 17-10. 151 $\mu\text{g/mL}$
 17-11. (b) 0.123 M
 17-12. (a) $5.2 \times 10^{-9} \text{ mol e}^-$ (b) 0.000 2₆ mL
 17-13. (a) 1.946 mmol H₂ (b) 0.041 09 M (c) 4.750 h
 17-14. trichloroacetic acid = 26.3 wt%; dichloroacetic acid = 49.5 wt%
 17-15. organohalide = 16.7 μM ; 592 $\mu\text{g Cl/L}$
 17-16. 96 486.6₇ $\pm 0.2_8$ C/mol
 17-17. (a) current (b) 0.25 mM
 17-18. (b) 2.89 ± 0.10 mM
 17-19. 0.010% ascorbic acid consumed in 10 min; yes
 17-21. 2.37 (± 0.02) mM
 17-22. 0.096 mM
 17-23. 0.000 35 wt%
 17-24. (a) $\text{Cu}^{2+} + 2\text{e}^- \rightarrow \text{Cu(s)}$ (b) $\text{Cu(s)} \rightarrow \text{Cu}^{2+} + 2\text{e}^-$
 17-25. 9.1 ± 1.6 ppb in blood
 17-26. (a) $\text{H}_2\text{SO}_3 < \text{pH } 1.86$; $\text{pH } 1.86 < \text{HSO}_3^- < \text{pH } 7.17$; $\text{SO}_3^{2-} > \text{pH } 7.17$
 (b) cathode: $\text{H}_2\text{O} + \text{e}^- \rightarrow \frac{1}{2}\text{H}_2(g) + \text{OH}^-$
 anode: $3\text{I}^- \rightarrow \text{I}_3^- + 2\text{e}^-$
 (c) $\text{I}_3^- + \text{HSO}_3^- + \text{H}_2\text{O} \rightarrow 3\text{I}^- + \text{SO}_4^{2-} + 3\text{H}^+$
 $\text{I}_3^- + 2\text{S}_2\text{O}_3^{2-} \rightleftharpoons 3\text{I}^- + \text{S}_4\text{O}_6^{2-}$
 (d) 3.64 mM
 17-27. (a) 31.2 $\mu\text{g nitrite/g bacon}$
 (b) 67.6 $\mu\text{g nitrate/g bacon}$

Chapter 18

- 18-1. (a) double (b) halve (c) double
 18-2. (a) 3.06×10^{-19} J/photon, 184 kJ/mol
 (b) 4.97×10^{-19} J/photon, 299 kJ/mol
 18-3. 8.266×10^{-11} m, 0.0826 6 nm
 18-4. (a) orange (b) violet-blue or violet (c) blue-green
 18-6. absorbance or molar absorptivity versus wavelength
 18-7. color of transmitted light is complement of color of absorbed light
 18-8. A, yellow; B, orange; C, violet; D, blue. Solution D has broad absorption that covers several spectral regions and leads to a blue appearance.
 18-9. (a) 1.20×10^{15} Hz, $4.00 \times 10^4 \text{ cm}^{-1}$, 7.95×10^{-19} J/photon, 479 kJ/mol (b) 1.20×10^{14} Hz, 4.000 cm^{-1} , 7.95×10^{-20} J/photon, 47.9 kJ/mol
 18-10. 2.7×10^{23} photons/s
 18-11. 0.004 4, 0.046, 0.30, 1.00, 2.00, 3.00, 4.00
 18-12. 0.480, 33.1%; 0.960, 11.0%

- 18-13. SPF = 2: $T = 0.5$, $A = 0.30$; half of the radiation is absorbed
 SPF = 10: $T = 0.10$, $A = 1.00$, 90% absorbed
 SPF = 20: $T = 0.05$, $A = 1.30$, 95% absorbed
 18-14. $3.56 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
 18-15. (a) 2.76×10^{-5} M (b) 2.24 g/L
 18-16. (a) 7.80×10^{-3} M (b) 7.80×10^{-4} M
 (c) $1.63 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$
 18-17. (a) 0.052 (b) 89%
 18-18. (a) 0.347 (b) 20.2%
 18-19. $2.30 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$
 18-20. (a) 0.613 (b) 1.22×10^{-6} M
 (c) $1.67 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$
 18-21. (a) $1.50 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ (b) 2.31×10^{-4} M
 (c) 4.69×10^{-4} M (d) 5.87×10^{-3} M
 18-22. (a) 6.97×10^{-5} M (b) 6.97×10^{-4} M
 (c) 1.02 mg
 18-23. 2.19×10^{-4} M
 18-24. (a) 1.74 ± 0.02 ppm ($\pm 0.01_7$ ppm)
 (b) 1.24×10^{-4} M
 18-25. 0.015 83 M NaNO₂ stock solution = 221.7 ppm N ($\mu\text{g N/mL}$)
 Dilute 10.00 mL stock to 1.000 L to get 2.217 ppm N.
 Dilute 5.00 mL stock to 1.000 L to get 1.109 ppm.
 A 50% dilution of 1.109 ppm would be 0.554 ppm.
 Dilute 15.00 mL (= 5.00 + 10.00 mL) stock to 1.000 L to get 3.326 ppm.
 18-26. (b) $2.2_1 \pm 0.1_4$ ppm or 2.2 ± 0.1 ppm
 (c) $4.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$
 18-27. Dissolve 4.25₁ g of 28.6 wt% NH₃ in 1.000 L to obtain 1.000 mg N/mL = 1 000 ppm N. To prepare 1.00 ppm N, dilute 1.000 mL to 1.000 L. To prepare 8.00 ppm N, dilute 8.000 mL to 1.000 L.
 18-28. (a) $4.49_3 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ (b) $1.00_6 \times 10^{-4}$ M
 (c) $5.03_0 \times 10^{-4}$ M (d) 16.1 wt%
 18-29. (a) 1.57×10^{-5} M (b) 0.180 (c) 6.60 mg
 18-30. (a) $2.42_5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ (b) 1.26 wt%

Chapter 19

- 19-2. deuterium
 19-4. (a) 2.38×10^3 lines/cm (b) 143 lines/cm
 19-5. (a) -23° , $+23^\circ$, $+52^\circ$
 19-9. $[\text{X}] = 4.42 \times 10^{-5}$ M, $[\text{Y}] = 5.96 \times 10^{-5}$ M
 19-11. $[\text{MnO}_4^-] = 8.35 \times 10^{-5}$ M, $[\text{Cr}_2\text{O}_7^{2-}] = 1.78 \times 10^{-4}$ M
 19-12. (a) [transferrin] = 8.99 mg/mL, [Fe] = 12.4 $\mu\text{g/mL}$
 (b) fraction of Fe in transferrin = 73.7%
 19-13. (a) $A = 2.080[\text{HIn}] + 14.200[\text{In}^-]$ (b) 6.79
 19-14. slope = 0.962, intercept = -3.803, $\text{p}K_{\text{HIn}} = 3.95$
 19-15. $[\text{A}] = 0.009\ 11\ \text{M}$, $[\text{B}] = 0.004\ 68\ \text{M}$
 19-16. ~2 and 0
 19-17. 1.73×10^{-5} M
 19-19. (a) [Se] in unknown solution = 0.038 4 $\mu\text{g/mL}$. Se in nuts = 3.56×10^{-4} wt% (b) $3.56 (\pm 0.07) \times 10^{-4}$ wt% (x -intercept = $0.038\ 4 \pm 0.000\ 7$)
 19-20. $[\text{In}^-]/[\text{HIn}] = 1.37$, pH = 7.64
 19-22. 630 kg CO₂, 11 kg SO₂

Chapter 20

- 20-3. emission
- 20-6. (a) 4.699×10^{-19} J (b) 3.67×10^{-6}
 (c) +8.4% (d) 0.010 3
- 20-7. (a) 6.07×10^{-19} J (b) 3.3×10^{-8}
 (c) +12% (d) 0.002 0
- 20-8. (a) 0.064_6 ppb⁻¹ (b) 0.23 ppb (c) 0.76 ppb
- 20-9. (a) slope = $23.5_9 \pm 0.2_8$ ($\mu\text{g/mL}$)⁻¹; intercept = 7.9 ± 5.2 ; $s_y = 5.3$ (b) 17.3 ± 0.3 $\mu\text{g/mL}$
- 20-10. (a) 3.00 mM (b) 3.60 mM
- 20-11. 0.120 M
- 20-12. 1.04 ppm
- 20-13. (a) 0, 10.0, 20.0, 30.0, 40.0 $\mu\text{g/mL}$
 (b) and (c) 204 ± 3 $\mu\text{g/mL}$
- 20-14. 1.64 ± 0.05 $\mu\text{g/mL}$
- 20-15. 25.6 $\mu\text{g/mL}$
- 20-16. 8.33×10^{-5} M
- 20-17. (b) $^{40}\text{Ar}^{16}\text{O}^1\text{H}^+$ interferes with $^{57}\text{Fe}^+$. $^{32}\text{S}^{16}\text{O}_2^+$ interferes with $^{64}\text{Zn}^+$. $^{23}\text{Na}^{35}\text{Cl}^+$ interferes with $^{58}\text{Ni}^+$.
- 20-18. (a) CsCl inhibits ionization of Sn.
 (b) slope = 0.782 ± 0.019 ; intercept = 0.86 ± 1.56 ; $R^2 = 0.997$
 (c) There is little interference at 189.927 nm, which is the better choice of wavelengths. At 235.485 nm, there is interference from Fe, Cu, Mn, Zn, Cr, and, perhaps, Mg.
 (d) limit of detection = 9 $\mu\text{g/L}$; limit of quantitation = 31 $\mu\text{g/L}$
 (e) 0.8 mg/kg
- 20-19. Ti/transferrin = 2.05
- 20-20. (c) near 81 $\mu\text{g/g}$

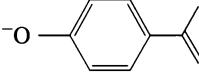
Chapter 21

- 21-2. 0.1 mm
- 21-8. (a) 4.3×10^4 (b) 3.6 μm
- 21-9. (a) $w_{1/2} = 0.8$ mm for ethyl acetate and 2.6 mm for toluene
 (b) $N = 1.1 \times 10^3$ for ethyl acetate and 1.1×10^3 for toluene
- 21-10. 6.8 cm diameter \times 25 cm long, 17 mL/min
- 21-11. (a) $w_{1/2} = 0.172$ min, $N = 3.58 \times 10^4$ (b) 0.838 mm
 (c) w (measured) = 0.311 min; $w/w_{1/2}$ (measured) = 1.81
- 21-12. (a) heptane: $w_{1/2} = 0.126$ min, 7.4×10^4 plates,
 0.40 mm plate height; $\text{C}_6\text{H}_4\text{F}_2$: $w_{1/2} = 0.119$ min,
 8.6×10^4 plates, 0.35 mm plate height
 (b) $w_{av} = 0.208$ min, resolution = 1.01
- 21-13. (a) 1.11 cm diameter \times 32.6 cm long
 (b) 0.069 mL (c) 0.256 mL/min
- 21-14. 1.47 mL/min
- 21-15. (a) 0.168_4 (b) 0.847 mM (c) 6.16 mM
 (d) 12.3 mM
- 21-16. 161 $\mu\text{g/mL}$
- 21-17. Plot area ratio versus concentration ratio.
 $y = 1.076(\pm 0.052)x + 0.008(\pm 0.033)$. Slope = $F = 1.08 \pm 0.05$. Intercept is not significantly different from zero.

- 21-18. (a) $u_{\text{optimum}} = 31.6$ mL/min
 (b) $u_{\text{optimum}} = 44.7$ mL/min, H_{optimum} increases
 (c) u_{optimum} increases, H_{optimum} decreases
- 21-19. (a) 3.25 mm (b) 615 plates (c) 0.760 min
- 21-20. molecular mass = 78.111 8, nominal mass = 78
- 21-21. 35.453
- 21-22. $\text{C}_4\text{H}_{11}\text{N}_3\text{S}^+$ (predicted mass = 133.066 81)
- 21-23. (a) $^{12}\text{C}_9\text{H}_{17}^{14}\text{N}_5^{35}\text{Cl}^+$, 230.116 68
 (b) $^{12}\text{C}_8^{13}\text{C}_1\text{H}_{17}^{14}\text{N}_5^{35}\text{Cl}^+$, 231.120 03, 9.9%
 (c) $^{12}\text{C}_9\text{H}_{17}^{14}\text{N}_5^{37}\text{Cl}^+$, 232.113 73
- 21-24. 31 = CH_2OH^+ ; 41 = C_3H_5^+ ; 43 = C_3H_7^+ ; 56 = C_4H_8^+ (loss of H_2O)
- 21-25. (a) $\text{C}_5\text{H}_5\text{N}$ (b) $\text{C}_5\text{H}_5\text{ON}_3$ or $\text{C}_6\text{H}_5\text{O}_2\text{N}$
 (c) $\text{C}_7\text{H}_4\text{O}_2\text{N}_2$, C_7O_4 , $\text{C}_6\text{H}_{12}\text{O}_4$, $\text{C}_6\text{H}_{16}\text{O}_2\text{N}_2$
 (d) $\text{C}_{11}\text{H}_{20}\text{O}$, $\text{C}_{11}\text{H}_4\text{O}_2$, $\text{C}_{11}\text{H}_8\text{N}_2$, $\text{C}_{12}\text{H}_{24}$, $\text{C}_{12}\text{H}_8\text{O}$
- 21-26. intensity ratio 36 : 37 : 38 = 100 : 0 : 31.96
- 21-27. intensity ratio 34 : 35 : 36 = 100 : 0.80 : 4.52
- 21-28. tallest peak is $^{12}\text{C}_2^{35}\text{Cl}_3^{37}\text{Cl}^+$; others are $^{12}\text{C}_2^{35}\text{Cl}_4$, $^{12}\text{C}_2^{35}\text{Cl}_2^{37}\text{Cl}_2$, $^{12}\text{C}_2^{35}\text{Cl}^{37}\text{Cl}_3$, and $^{12}\text{C}_2^{37}\text{Cl}_4$

Chapter 22

- 22-4. (b) narrow bore: 16 ng; wide bore: 5.6 μg
 (c) narrow bore: 0.16 ng; wide bore: 56 ng
- 22-5. (a) $N = 2.0 \times 10^5$; $H = 0.50$ mm; resolution = 1.3
 (b) Gas chromatography plate height \approx 50 times liquid chromatography plate height because longitudinal diffusion in gas is so much faster than in liquid.
- 22-6. $n = 12.44 \approx 12$ or 13 CH_2 groups
- 22-7. (a) nominal mass in selected ion chromatogram = 73
 (b) m/z 73 is $M - 15$ (loss of CH_3) for MTBE and $M - 29$ (loss of C_2H_5) for TAME. Loss of C_2H_5 bound to C in TAME suggests that CH_3 lost from MTBE was also bound to C, not O. If CH_3 bound to O were easily lost from MTBE and TAME, we would expect to see C_2H_5 bound to O lost from ETBE, but there is no $M - 29$ (m/z 73) for ETBE. By similar reasoning, m/z 87 for ETBE and TAME represents loss of CH_3 that was bound to C in both molecules.
- 22-8. (a) caffeine $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$ nominal mass = 194 Da;
 $(^{13}\text{CH}_3)_3\text{-caffeine} = ^{13}\text{C}_3^{12}\text{C}_5\text{H}_{10}\text{N}_4\text{O}_2$ (197 Da);
 The transition m/z 197 \longrightarrow 111 represents loss of $^{13}\text{C}^{12}\text{C}_2\text{H}_3\text{NO}_2$.
 (c) m/z 195 is $^{13}\text{C}^{12}\text{C}_7\text{H}_{10}\text{N}_4\text{O}_2$; expected intensity $(M+1)/M = 8 \times 1.1\% = 8.8\%$
- 22-9. (a) decrease (b) increase (c) increase
 (d) decrease
- 22-12. 0.19₂ min, 0.13₆ min
- 22-13. (a) $N = 1\ 500$ plates; $H = 33$ $\mu\text{m}/\text{plate} \approx 20$ particles per plate
 (b) optimum resolution ≈ 2 particles per plate; column is run for maximum speed
- 22-14. (a) RCO_2H , RNH_3^+ (b) Amine will be eluted first.
- 22-15. (a) lower (b) higher
- 22-16. use slower flow rate, longer column, or smaller particle size
- 22-17. (b) C term (finite rate of mass transfer) causes band broadening

- 22-18. 0.418 mg/mL
- 22-19. (a) 8.68×10^8 (b) $0.273 \text{ m}^2/\text{g}$
(c) particles must be very porous
- 22-21. (a) CocaineH⁺ (protonated on nitrogen) is C₁₇H₂₂NO₄, *m/z* 304.
m/z 182 is probably cocaineH⁺ minus C₆H₅CO₂H.
(b) *m/z* 304 = ¹²C₁₇H₂₂NO₄ was selected by Q1.
¹³C¹²C₁₆H₂₂NO₄ was blocked by Q1. *m/z* 304 is isotopically pure, so it gives no ¹³C-containing fragments.
(c) Q1 selects *m/z* 304, eliminating substances without a signal at *m/z* 304. Q3 selects the fragment *m/z* 182 derived from *m/z* 304, eliminating any other *m/z* 304 that does not give a fragment at *m/z* 182.
(d) phenyl group
- 22-22. *m/z* 227 is BPA that has lost one phenolic proton (C₁₅H₁₅O₂)
m/z 133 is probably C₉H₉O⁻ 
- 22-27. (d) $78 \pm 5 \mu\text{g/L}$; $192 \pm 6 \mu\text{g/L}$
- 22-28. (i) b (ii) a (iii) c
- 22-29. [NO₂⁻] = 1.8 μM; [NO₃⁻] = 384 μM
- 23-12. (b) Peak near 2 min is excess fluorescence-labeled phenytoin that exceeds capacity of antibody on the column. Peak near 8 min is fluorescence-labeled phenytoin displaced from column by free phenytoin in unknown serum.
(c) 0.08 s
(d) $F_{\text{calculated}} = 9.9 < F_{\text{table}} = 19.0$ for 2 degrees of freedom in numerator and denominator \Rightarrow standard deviations are not significantly different.
 $s_{\text{pooled}} = 0.33 \mu\text{M}$; $t_{\text{calculated}} = 0.44 < t_{\text{table}} = 2.776$, so difference is not significant
- 23-13. (a) cations < neutrals < anions
(b) low, against, never!
(c) anions < neutrals < cations
- 23-14. 420 ppm = 420 μg/mL = 6.8 mM
- 23-18. (a) longitudinal diffusion: $H \approx B/u$
(b) longitudinal diffusion and mass transfer: $H \approx B/u + Cu$
- 23-19. (a) *t* = 40.1 min, *w*_{1/2} = 0.75 min, 1.6×10^4 plates
(b) plate height = 25 μm
- 23-20. thiamine < (niacinamide + riboflavin) < niacin; thiamine is most soluble
- 23-21. light chain: 17 300; heavy chain: 23 500
- 23-22. (a) mean detection limit = 0.1 μg/L; mean quantitation limit = 0.3 μg/L
(b) 0.000 058
- 23-23. (a) pH 3.76
- 23-24. Pentylammonium cation is anchored to the stationary phase because its hydrophobic tail is soluble in the C₁₈ phase. The cationic headgroup behaves as an ion-exchange site for anions. Sulfate is retained best because it has the most negative charge.
- 23-25. (a) Filtration eliminates solids from the analysis.
(b) Anion exchange concentrates SO₄²⁻ by a factor of 100.
(c) Final product is solid BaSO₄.
(d) Na₂SO₄ is a carrier to ensure that there is enough product to handle at the end of the analysis. Without carrier, there would be too little solid to isolate.
- 23-26. Perhaps Mg²⁺ and Ca²⁺ neutralize negative charge on the capillary wall by binding to —Si—O⁻ groups. EDTA binds Mg²⁺ and Ca²⁺ more tightly than the wall does.

Chapter 23

- 23-1. (a) Mixture contains RCO₂⁻, RNH₂, Na⁺, and OH⁻; all pass directly through the cation-exchange column.
(b) Mixture contains RCO₂H, RNH₃⁺, H⁺, and Cl⁻; RNH₃⁺ is retained and the others are eluted.
- 23-3. VOSO₄ = 80.9 wt%, H₂SO₄ = 10.7 wt%, H₂O = 8.4 wt%
- 23-5. NH₃ < (CH₃)₃N < CH₃NH₂ < (CH₃)₂NH
- 23-7. 38.0 wt%
- 23-8. (a) 3.88 (b) -773 μM and +780 μM
(c) 0.053 2 mg/mL
- 23-9. The more negative the mean charge of the compound, the more it is excluded from the ion-exchange resin and the more rapidly it is eluted.
- 23-10. 4.8×10^4
- 23-11. (a) 5.7 mL (b) 11.5 mL (c) adsorption occurs

Index

Abbreviations:

b = box; d = demonstration; i = illustration; m = marginal note; p = problem; r = reference; t = table; AP = appendix; CP = color plate

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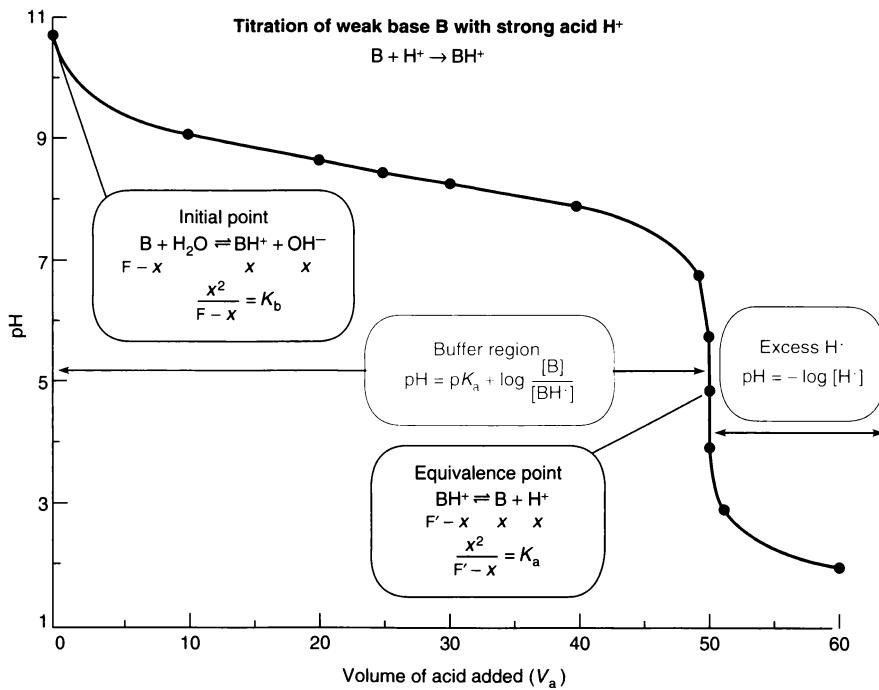
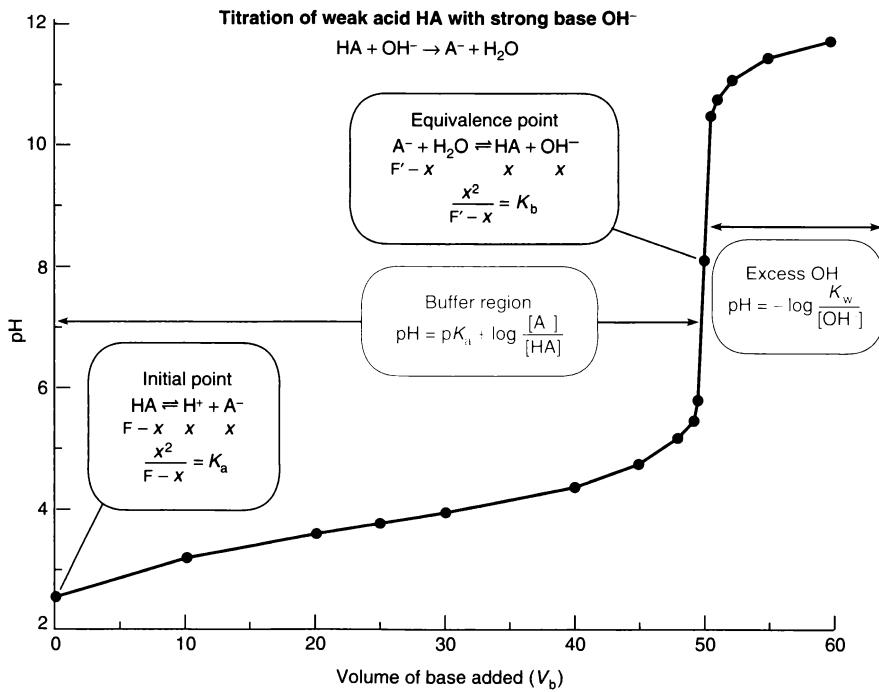
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Physical Constants (2010)^a

Term	Symbol	Value ^b
Elementary charge	e	$1.602\ 176\ 655\ (35) \times 10^{-19}\ \text{C}$
Speed of light in vacuum	c	$4.803\ 204\ 78\ (10) \times 10^{10}\ \text{esu}$
Planck's constant	h	$2.997\ 924\ 58 \times 10^8\ \text{m/s}$
		$\times 10^{10}\ \text{cm/s}$
$h/2\pi$	\hbar	$6.626\ 069\ 57\ (29) \times 10^{-34}\ \text{J}\cdot\text{s}$
		$\times 10^{-27}\ \text{erg}\cdot\text{s}$
Avogadro's number	N	$1.054\ 571\ 726\ (47) \times 10^{-34}\ \text{J}\cdot\text{s}$
Gas constant	R	$6.022\ 141\ 29\ (27) \times 10^{-27}\ \text{erg}\cdot\text{s}$
		$\times 10^{23}\ \text{mol}^{-1}$
		$\text{J}/(\text{mol}\cdot\text{K})$
		$\text{V}\cdot\text{C}/(\text{mol}\cdot\text{K})$
Faraday constant ($= Ne$)	F	$8.205\ 736\ 1\ (74) \times 10^{-2}\ \text{L}\cdot\text{bar}/(\text{mol}\cdot\text{K})$
Boltzmann's constant ($= R/N$)	k	$1.987\ 204\ 1\ (18) \times 10^7\ \text{erg}/(\text{mol}\cdot\text{K})$
Electron rest mass	m_e	$9.648\ 533\ 65\ (21) \times 10^{-5}\ \text{m}^3\cdot\text{atm}/(\text{mol}\cdot\text{K})$
Proton rest mass	m_p	$1.380\ 648\ 8\ (13) \times 10^{-2}\ \text{L}\cdot\text{atm}/(\text{mol}\cdot\text{K})$
Dielectric constant (permittivity of free space)	ϵ_0	$9.109\ 382\ 91\ (40)\ \text{cal}/(\text{mol}\cdot\text{K})$
Gravitational constant	G	$1.672\ 621\ 777\ (74) \times 10^4\ \text{C/mol}$
		$\times 10^{-23}\ \text{J/K}$
		$\times 10^{-16}\ \text{erg/K}$
		$\times 10^{-31}\ \text{kg}$
		$\times 10^{-28}\ \text{g}$
		$\times 10^{-27}\ \text{kg}$
		$\times 10^{-24}\ \text{g}$
		$\times 10^{-12}\ \text{C}^2/(\text{N}\cdot\text{m}^2)$
		$\times 10^{-11}\ \text{m}^3/(\text{s}^2\cdot\text{kg})$

a. 2010 CODATA Values from <http://physics.nist.gov/cuu/Constants/index.html> (August 2011).

b. Numbers in parentheses are the one-standard-deviation uncertainties in the last digits.

Concentrated Acids and Bases

Name	Approximate weight percent	Molecular mass	Approximate molarity	Approximate density (g/mL)	mL of reagent needed to prepare 1 L of ~1.0 M solution
Acid					
Acetic	99.8	60.05	17.4	1.05	57.3
Hydrochloric	37.2	36.46	12.1	1.19	82.4
Hydrofluoric	49.0	20.01	28.4	1.16	35.2
Nitric	70.4	63.01	15.8	1.41	63.5
Perchloric	70.5	100.46	11.7	1.67	85.3
Phosphoric	85.5	97.99	14.7	1.69	67.8
Sulfuric	96.0	98.08	18.0	1.84	55.5
Base					
Ammonia ^a	28.0	17.03	14.8	0.90	67.6
Sodium hydroxide	50.5	40.00	19.3	1.53	51.8
Potassium hydroxide	45.0	56.11	11.5	1.44	86.6

a. 28.0 wt% ammonia is the same as 56.6 wt% ammonium hydroxide.

Dissolution of Calcium Carbonate by Carbon Dioxide



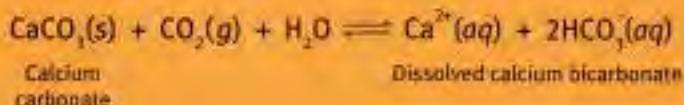
CaCl_2 (3.5 mmol) dissolved
in 250 mL H₂O

CaCO_3 , precipitated with
 K_2CO_3 (1.2 mmol)

Adding Dry Ice dissolves
 CaCO_3 precipitate.

Precipitate completely dissolved: pH = 5.6

Shells and skeletons of marine organisms such as plankton and coral are made of calcium carbonate. The front cover shows the mineral aragonite, one of the two crystalline forms of calcium carbonate. Since 1950, the burning of fossil fuels including coal, oil, wood, and natural gas, has increased atmospheric CO₂ by 25%—and this number is rising every year. A similar quantity of CO₂ has entered the ocean. This dissolved CO₂ acts as an acid and has lowered the pH of the ocean by ~0.1 unit so far. Continued burning of fossil fuels threatens sea life by dissolution of calcium carbonate shells and skeletons. Loss of marine life at the bottom of the food chain threatens the entire food chain. Box 11-1 in this book highlights chemical equilibrium aspects of ocean acidification.



FRONT COVER PHOTO: Michael Nichols/National Geographic Stock

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