

CHEMISTRY 260

Spring 2009

Book required : "Spectrometric Identification of Organic Compounds," 7th ed., by
Silverstein and Webster

You might want a copy of an instrumental analysis textbook – any will do, and there are a couple on library reserve.

Goals:

A. For the following techniques, which we will concentrate on, you should understand the theory, understand how the instrument works, and be able to interpret the data:

- Ultraviolet and visible spectroscopy
- Infrared spectroscopy (IR)
- Nuclear magnetic resonance (NMR) spectrometry
- Mass spectrometry (MS)
- Atomic absorption spectroscopy (AA)
- Gas chromatography (GC)
- Thin-layer chromatography (TLC)
- High-performance liquid chromatography (HPLC)

B. You should know what the following techniques are and what they are used for:

- Thermal analysis
- Nuclear methods of analysis
- Electrochemistry

C. You should also be able to:

- Identify an organic compound from its spectra
- Understand how statistics is used in analytical chemistry and interpret a statistical analysis of data
- Write a scientific report

For each technique we cover in lab, you will write a report (a total of 5). See Chemistry 260 Lab, below. You will be graded on the material in your reports, but as this is a writing-intensive course, a significant part of the grade on your reports will be based on your writing. A report which is not well written, in proper English and scientific style, will not receive a high grade, regardless of its content.

There will be two exams. Exam I will cover spectroscopy; it will be given the week after spring break (March 16-20). There will be no lab that week; the exam may be taken anytime that week, including the regular lab time. It may be taken in one part or two parts. Exam II will cover only those topics covered since Exam I; it may be taken any time the last 7 class days (Apr. 20-28). It may also be taken as a whole or in two parts. Although writing won't be strictly graded on the exams, if your writing is very poor, your exam grade may be lowered.

There will be a spectroscopy problem set assigned; this is to be treated like a take-home exam. The assignment will be given before spring break and will be due Friday, March 20 (the Friday after spring break). You may use only your book(s) and lecture notes for the problem set. This is not a writing assignment, so you don't have to compose a report.

There will be a final project – you will divide into 2 groups; each group will choose one of the instruments and prepare a video illustrating the theory, the operation, and what the results are used for. This will not be graded, but is necessary for passing the course.

The Honor Code applies to the exams, the problem set, and the lab reports. It is permissible to discuss a lab report prior to writing it up, but the report itself must be your work alone. The lab report for Experiment II (Report II) will be written jointly by the students who worked together on the experiment. These students will turn in one report and all students in the group will receive the same grade on the report. See the Honor Code handout for more information.

Grading:	Exam I	25 %
	Exam II	25 %
	Problem set	10 %
	Lab	40 %

CHEMISTRY 260 LAB

- 1. Before the lab.** You should come into the lab with some knowledge of the experiment to be performed. This means having read the experiment and reviewed your class notes and the relevant textbook sections.
- 2. During the lab.** The experiment must be performed and completed during the lab period. Work efficiently and make good use of your time. You are expected to be present in the lab until the experiment is finished and to participate in all parts. Most of the glassware and chemicals needed can be obtained from the stockroom. Chemical preparation will be carried out in the prep room; use a hood when necessary. The instruments are expensive and must be handled carefully. If an instrument is broken, this not only involves the expense of repairing it, but may also entail down time before service can be arranged.
- 3. After the lab.** Before leaving the lab, all equipment must be cleaned and put away. Any equipment obtained from the stockroom must be returned.
- 4. Safety.** Proper safety precautions must be observed at all times. This means wearing approved safety glasses, no eating or drinking in the lab or instrument room, wearing proper protective clothing (e.g., no sandals), not wearing contact lenses, and no unauthorized experimentation.
- 5. Reports.** All data collected in lab should be recorded in a notebook. All people in a lab will work on the same experiment and instrument. This means everybody must participate in each part of the procedure -- preparation, instrument operation, and clean-up. The report itself is to be your work alone -- any collaboration is a violation of the Honor Code. (For Experiment II, two students will work together to prepare a single report; both students will receive the same grade.) In most cases, there will be only one original record of the data obtained directly from the instrument. If so, one person should turn in the original with his or her report and the others should mention whose report includes this record. If desired, you may copy or photocopy the data for your own report.
- 6. Report style.** A written report is required for each technique investigated. The report is due at the beginning of the lab as noted on the schedule. The report should include:
 - a. Your name and the names of the others who worked on the experiment.
 - b. Date of experiment and date of report.
 - c. Number and title of experiment.
 - d. Technique and theory.
 - e. Description of apparatus used.
 - f. Brief description of the problem investigated.
 - g. Data (normally organized into tables).

- h. Graphs, equations, diagrams, etc., where appropriate.
- i. Results
- j. Discussion and conclusions.

Reports must be written in proper scientific English -- using passive voice and past tense where appropriate. Reports should be word processed and double-spaced; they must be neat and legible with correct spelling, grammar, and punctuation. Normally, you should avoid writing anything in -- use the word processor instead.

7. Grading. Reports will be graded on a 100-point basis. You will be graded on the report itself (style, organization, completeness), your data, your results. The most important aspect is your writing and your explanation of theory and instrumentation. Some of the grading will, by the nature of the reports, be subjective.

Your lab grade will be computed by averaging the report grades and an evaluation of your performance in lab (participation, handling of equipment, clean-up, safety procedures).

8. Writing the reports. For the first two reports, I will critique a draft which you will rewrite. For the first three reports, I will post a general outline to our class conference. After that, however, I expect you to write your reports without assistance from me. You may use the writing center and you may have someone else in the class look over your report. I will make available in my office sample reports from previous years. You may look at these reports before writing your own to see the style and the major components; you may look at them after you've written your paper to see if you omitted anything, or put something in the wrong place, or to perhaps find a diagram you'd like to include in your paper. However, it is an Honor Code violation to use the sample reports, directly or indirectly (paraphrasing), to write your report. Thus, you may not take notes from these sample papers. They are not intended to be used to write your paper.

9. Numbering of Experiments and Reports.

Report Number	Experiment Number	Technique
I	I-A and I-B	uv-vis spectroscopy
II	II	NMR and IR spectroscopy, mass spectrometry
III	III	atomic absorption spectroscopy
IV	IV	gas chromatography
V	V-A and V-B	liquid chromatography

SCHEDULE

Date	Experiment	Reports
Jan. 29	I-A (UV-VIS)	
Feb. 5	I-B (UV-VIS)	>Draft of theory/instrumentation parts of Report I due
Feb. 12	II (IR-NMR-MS)	>Draft of procedure/ results/discussion parts of Report I due
Feb. 19	---	> Report I due >Draft of theory/ instrumentation parts of Report II due
Feb. 26	---	>Draft of procedure/results/discussion parts of Report II due
Mar. 5	III (AA)	> Report II due Friday, Mar. 6, 4:00
Mar. 12	--- (spring break)	
Mar. 19	---	> <i>Exam I</i> this week (finish by Friday, Mar. 20, 4:00) > <i>Problem set</i> due by Friday, Mar. 20, 4:00
Mar. 26	IV (GC)	> Report III due
Apr. 2	V-A (TLC)	> Report IV due
Apr. 9	V-B (HPLC)	
Apr. 16	---	> Report V due
Apr. 23	---	> <i>Exam II</i> this week (finish by Tuesday, Apr. 28, 4:00) > <i>Videos</i> due by Wednesday, Apr. 29, 4:00

(Reports are due at the beginning of lab time on Thursday unless otherwise specified)

EXPERIMENT I-A

VISIBLE SPECTROSCOPY

Objective: To determine the concentration of a transition metal ion (Ni^{2+}) in a solution using visible spectroscopy

Chemicals: 0.2-M NiCl_2

Apparatus: Shimadzu 1601 spectrophotometer, plastic cuvettes

Procedure:

Prepare a series of dilutions of the nickel solution: 0.1 M, 0.05 M, and 0.025 M.

Select "Spectrum" mode on the instrument. Place the 0.2-M nickel solution in a cuvette in the front cell holder and an empty cuvette in the rear holder. Select absorbance for the Y-axis with a maximum of 1.00. Obtain the spectrum of the nickel solution by scanning from 700 nm to 400 nm. Determine the wavelength of maximum absorbance using the zoom and peak functions.

Select "Photometric" mode on the instrument and go to the wavelength you found. Measure the absorbance of each of the nickel solutions, including the unknown. From the standard solutions, construct a Beer's Law curve of absorbance vs. concentration. If a point appears to be too far off the line, discard it.

Using the standard curve, determine the concentration of the unknown nickel solution.

No report is due until you finish Experiment I-B. In your report for this experiment, you will need to include the spectrum of nickel, the wavelength selected, the Beer's Law plot, all the absorbance readings, and the concentration of the unknown.

EXPERIMENT I-B

SIMULTANEOUS MULTI-COMPONENT ANALYSIS
USING UV SPECTROSCOPY

Objective: To determine the concentration of two components (pheniramine maleate phenylephrine hydrochloride) and in a mixture (Dristan) using uv spectroscopy

Chemicals: pheniramine maleate (PAM), 80 $\mu\text{g/mL}$ in 0.010-M HCl
phenylephrine hydrochloride (PEH), 200 $\mu\text{g/mL}$ in 0.010-M HCl
0.010-M HCl
commercial Dristan[®] sample

Apparatus: Shimadzu 1601 spectrophotometer, quartz cuvettes.

Procedure:

Prepare a series of dilutions using 0.010-M HCl: PAM -- 40, 20, 10, and 5 $\mu\text{g/mL}$; PEH -- 100, 50, 25, and 12.5 $\mu\text{g/mL}$. Also, dilute the Dristan[®] sample 1:200 with the 0.010-M HCl (this means 1 mL diluted to 200 mL total volume).

Select "Spectrum" mode on the instrument. Scan each of the standard substances. Select two wavelengths to use where each substance is at or near its maximum.

Select "Photometric" mode on the instrument. Measure the absorbances of all the solutions at each of the two wavelengths (4 solutions X 2 substances X 2 wavelengths); also measure the absorbance of the diluted Dristan[®] solution at each wavelength. For each substance, use Excel to construct a Beer's Law curve at each wavelength (2 substances X 2 wavelengths); determine the absorptivity (slope) of each curve.

Solve for the concentrations of the unknown solution using the matrix method in Excel.

Once you have the concentrations in the diluted Dristan[®], you need to calculate what the concentrations were before dilution and then convert from $\mu\text{g/mL}$ to %:

Multiply by 200 to account for the dilution. Divide by 10^6 to convert from $\mu\text{g/mL}$ to g/mL (since 1 mL of dilute aqueous solution has a mass of 1 g). Multiply by 100 to convert to %.

In the report, include all spectra, Beer's Law curves, and absorbance readings. Show how you solved the equations using matrices. Give the concentrations in the original Dristan[®]. Discuss the theory of uv-visible absorption and the instrument.

EXPERIMENT II

QUALITATIVE ANALYSIS
USING NMR AND IR SPECTROSCOPY
AND MASS SPECTROMETRY

Objective: To determine the identity of organic compounds using IR, NMR, and mass spectrometry

Chemicals: unknown organic compounds
TMS

Apparatus: Anasazi EFT-60 NMR spectrometer, NMR tubes
Perkin-Elmer Paragon 500 IR spectrometer, salt plates

Procedure:

You will work in groups of 3 for this lab. Your group of students will be given 2-3 pure, liquid, organic unknowns; they will contain only carbon, hydrogen, oxygen, and/or nitrogen.

For NMR: Fill an NMR tube about 1/3 full with your unknown; add TMS. Obtain the proton NMR spectrum, including the integration.

For IR: Place a drop of the unknown between two salt plates. Obtain an infrared spectrum.

You will be provided with a mass spectrum for your unknowns. You will be provided with a carbon-13 NMR spectrum for one of your unknowns.

Determine the identity of your unknowns, including structure and proper name. Each peak in the NMR spectra should be assigned and the shift and area explained. The major peaks in the IR and mass spectra should also be assigned.

In your report, include a discussion of the theory and instrumentation of FT-NMR spectrometry and IR spectroscopy. It is not necessary to discuss the instrumentation of mass spectrometry since you did not use this instrument, but you should discuss the theory. Be sure that once you have identified your unknowns, you verify it! You must compare the shift, area, and multiplicity of each NMR peak with the predicted values and you must assign the major mass and IR spectral peaks.

Each group of students will work together to identify their unknowns and to write up the report. Only one report will be accepted from the group and all students in the group will receive the same grade.

EXPERIMENT III

ATOMIC ABSORPTION SPECTROSCOPY

Objective: To determine the concentration of a metal ion (Cu^{2+} or Pb^{2+}) using atomic absorption spectroscopy

Chemicals: lead nitrate or copper nitrate

Apparatus: Buck 200A atomic absorption spectrometer

Procedure:

Lead or copper may be used. By diluting the standard provided, prepare standard solutions of 1, 2, and 5 ppm.

Prepare standard addition solutions. Mix equal quantities of your unknown with each of the standards and deionized water (0 ppm), giving 4 standard addition solutions (e.g., one such solution might be 1 mL of unknown + 1 mL of 1 ppm).

Locate the wavelength (283.3 nm for lead or 324.7 nm for copper). Set zero while aspirating deionized water. Aspirate the standards, unknown, and standard addition solutions and record the absorbances.

Construct a standard curve and determine the concentration of the unknown. Also construct a plot of the standard addition solutions (absorbance vs. ppm added) and determine the concentration of the unknown from this graph.

In your report, include the 2 plots, absorbances, and the concentration of the unknown from the 2 methods. Discuss the theory and instrumentation.

EXPERIMENT IV

GAS CHROMATOGRAPHY

Objective: To determine the retention times of substances (hexane, toluene, 1-propanol) and to analyze a mixture of them

Chemicals: hexane, toluene, 1-propanol

Apparatus: Gow-Mac gas chromatograph with DC-stationary phase packed column and thermal conductivity detector
computer data acquisition system

Procedure:

The temperature and flow rate will be set. Inject 1 μL of the mixture, along with 5 μL of air.

Inject 1 μL of each pure substance, plus air. For your report, you will need to decide which peak in the mixture corresponds to which substance by matching the retention times. From the areas in the mixture, determine the volume in μL of each substance and calculate the composition of the mixture.

In your report, include the retention times and areas of the standards and the composition of the mixture. Discuss the theory and instrumentation. Calculate the number of theoretical plates and HETP using your last peak, and α (separation) and R (resolution) using your last two peaks.

EXPERIMENT V-A

THIN-LAYER CHROMATOGRAPHY

Objective: To determine the R_f factors of several vitamins and to determine their polarity and whether they are water-soluble or fat-soluble

Chemicals: vitamin standards; may include ascorbic acid (vitamin C), nicotinamide (a B vitamin), vitamin A (as the acetate or aldehyde), and tocopherol (vitamin E); a mixture of the vitamin standards

developing solution (ethyl acetate/ethanol 2:1 or 3:1)

Apparatus: TLC plates, polyester coated with silica gel
TLC developing tank
uv lamp

Procedure:

Prepare a solution of each vitamin as 0.01 g/mL in ethanol.

Place the developing solution in the tank and allow the atmosphere to become saturated.

Mark a starting point on a TLC plate with a pencil. Spot the plate with the solutions, including the mixture, using a capillary tube. Allow the solvent to dry and add a second spot on top of the first. When the solvent has dried again, place the plate in the developing solution in the tank. Allow the solvent to move as far as it can, remove the plate, mark the solvent front with pencil, and allow the solvent to dry. View the plate under a uv lamp. The plates have been treated with a fluorescent material, so the vitamins will appear as dark spots against a bright background. Circle each of the spots and calculate R_f values.

In your report, show the structures of the vitamins. Discuss the differences in R_f values in terms of the polarities of the vitamins and the mobile phase. (The stationary phase, silica gel, is quite polar; the mobile phase is only slightly polar.). Discuss how the polarity of a vitamin determines whether it is fat-soluble or water-soluble.

No report is necessary until Experiment V-B is finished.

EXPERIMENT V-B

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Objective: To determine the retention times of substances (toluene, benzaldehyde, DET) and to analyze a mixture containing them

Chemicals: 1.0 % toluene in methanol
0.01 % benzaldehyde in methanol
0.2% N,N-diethyl-m-toluamide (DET) in methanol
methanol, water

Apparatus: ISCO 2361 HPLC
chart recorder

Procedure:

Different concentrations are necessary because benzaldehyde absorbs strongly at 254 nm, DET absorbs moderately, and toluene absorbs weakly. Run the mixture of all 3 at a flow rate of 1 mL/min using a mobile phase of 70 % methanol/water.

Run each individual standard. From the retention times, identify each component in the mixture. Determine the % of each component in the mixture:

$$\frac{\text{area of standard}}{\text{area of unknown}} = \frac{\% \text{ of standard}}{\% \text{ of unknown}}$$

Note that the percentages will not add to 100%; they should be close to the percentages of the standards.

In your report, discuss liquid chromatography in general and specifically the two types you used, TLC and HPLC. Discuss the instrumentation used for HPLC.

STATISTICS

EVALUATION OF DATA

This is not an experiment and there is no report for it, but you can use the techniques discussed in class to analyze two sets of data to help you understand the use of statistics in analytical chemistry.

The first set of data was used to fit a first-order (linear) model. The second set of data was used to fit both a first-order and a second-order (quadratic) model. You should understand how the following were obtained and what they mean:

- (a) the coefficients,
- (b) their significance levels,
- (c) the 5 sums of squares (corrected, regression, residual, lack of fit, and pure error),
- (d) the significances of regression and lack of fit.

For the second data set, you should be able to decide which model fits the data better.

DATA SET 1

pH	Absorbance
2.0	0.212
2.0	0.200
3.0	0.319
3.0	0.328
4.0	0.411
4.0	0.400
5.0	0.515
5.0	0.500

DATA SET 2

pH	Temp.	Absorbance
1.0	10.0	0.288
1.0	40.0	0.462
1.0	70.0	0.510
1.0	100.0	0.432
4.0	10.0	0.429
4.0	40.0	0.533
4.0	70.0	0.507
4.0	100.0	0.432
7.0	10.0	0.514
7.0	40.0	0.542
7.0	70.0	0.440
7.0	100.0	0.207
10.0	10.0	0.537
10.0	40.0	0.488
10.0	70.0	0.310
10.0	100.0	0.002
4.0	40.0	0.537
4.0	70.0	0.510
7.0	40.0	0.534
7.0	70.0	0.425

DATA SET 1

First-order Model

$$y = b_0 + b_1x_1$$

$$y = 0.01335 + 0.0994 x_1$$

Analysis of Variance (ANOVA):

Source	SS	df	Variance (s^2)
Corrected for mean	0.099475	7	
Regression	0.0988036	1	0.0988036
Residual	0.0006439	6	0.000107317
Lack of fit	0.0004209	2	0.00021045
Pure error	0.000223	4	0.00005575

F ratio	Value	Significance
$s^2_{\text{reg}} / s^2_{\text{res}}$	920.67339	100.00 %
$s^2_{\text{reg}} / s^2_{\text{pe}}$	1772.26188	100.00 %
$s^2_{\text{lof}} / s^2_{\text{pe}}$	3.77489	88.01 %

t test	Value	Significance
b_0	1.1091	69.02 %
b_1	30.3426	100.00 %

DATA SET 2

First-order Model

$$y = b_0 + b_1x_1 + b_2x_2$$

$$y = 0.60204 - 0.010103 x_1 - 0.0021532 x_2$$

Analysis of Variance (ANOVA):

Source	SS	df	Variance (s^2)
Corrected for mean	0.3603469	19	
Regression	0.1069154	2	0.05345773
Residual	0.2534315	17	0.01490774
Lack of fit	0.2532745	13	0.01948265
Pure error	0.0001570	4	0.00003925

F ratio	Value	Significance
$s_{\text{reg}}^2 / s_{\text{res}}^2$	3.58590	94.98 %
$s_{\text{reg}}^2 / s_{\text{pe}}^2$	1361.98028	100.00 %
$s_{\text{lof}}^2 / s_{\text{pe}}^2$	496.37334	100.00 %

t test	Value	Significance
b_0	8.105094	100.00 %
b_1	1.137582	72.89 %
b_2	2.424400	97.32 %

DATA SET 2

Second-order Model

$$y = b_0 + b_1x_1 + b_{11}x_1^2 + b_2x_2 + b_{22}x_2^2 + b_{12}x_1x_2$$

$$y = 0.12962 + 0.07348 x_1 - 0.0033948 x_1^2 + 0.0102695 x_2 - 0.000070893 x_2^2 - 0.00084081 x_1x_2$$

Analysis of Variance (ANOVA):

Source	SS	df	Variance (s^2)
Corrected for mean	0.63034695	19	
Regression	0.36006927	5	0.072043855
Residual	0.00027767	14	0.000019834
Lack of fit	0.00012067	10	0.000012067
Pure error	0.00015700	4	0.000039250

F ratio	Value	Significance
$s_{\text{reg}}^2 / s_{\text{res}}^2$	3630.85538	100.00 %
$s_{\text{reg}}^2 / s_{\text{pe}}^2$	1834.74795	100.00 %
$s_{\text{lof}}^2 / s_{\text{pe}}^2$	0.307449	5.93 %

t test	Value	Significance
b_0	25.58306	100.00 %
b_1	52.15072	100.00 %
b_{11}	29.64093	100.00 %
b_2	72.88069	100.00 %
b_{22}	61.89774	100.00 %
b_{12}	85.38265	100.00 %