# CHEMISTRY 260

**Spring 2016**

Book required : "Spectrometric Identification of Organic Compounds," 7th ed., by
Silverstein and Webster (older editions acceptable, but page numbers will be different)
A copy of an instrumental analysis textbook will be on library reserve.

**1. Goals**:

A. You should understand the theory, understand how the instrument works, and be able to interpret the data for:

- 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)
- We may also briefly discuss and use X-Ray Diffraction Spectrometry (XRD)

B. You should 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

**2. Methods of Evaluation.**

**(A) Exams.** There will be two exams – no final exam.

Exam I will be in 2 parts and will cover spectroscopy; it will be given the week after spring break (March 14-18). There will be no lab that week; the exam may be taken anytime that week, including the regular lab time, with the following due times:

| | |
|---|---|
| Part 1 | Due by 5:00 Thursday, March 17 |
| Part 2 | Due by 5:00 Friday, March 18 |

(Of course, each part can be taken and completed earlier).

Exam II will be in 2 parts and will cover only those topics covered since Exam I; it may be taken any time the last 6 class days (Apr. 18-22, 25), with the following due times:

| | |
|---|---|
| Part 1 | Due by 5:00 Friday, April 22 |
| Part 2 | Due by 5:00 Monday, April 25 |

(Of course, each part can be taken and completed earlier).

Any part of an exam turned in after the due time will receive no credit. Any exam must be completed and turned in no later than 5:00 of the day you take it.

For an exam, as noted on the Honor Code pledge, you may use no materials other than the exam itself. While you will take the exam on a computer, you may not use any files on the computer nor any information from the Internet.

**(B) Problem Set.** There will be a spectroscopy problem set assigned; this is to be treated like a take-home exam. It will be due by 4:00, Friday, March 4. 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. You will have a choice of problems, so find ones you can work out without asking for assistance. The problems should be solved on separate sheets of paper, with some of your reasoning shown at the beginning of each one, then a structure, and finally verification clearly shown at the end of each one.

**(C) Reports.** For each technique we cover in lab, you will write a report (a total of 5). See the lab section, below, for more information. Reports turned in late will lose points, unless arrangements are made in advance.

**3. Writing.**

"Of all those arts in which the wise excel, Nature's chief masterpiece is writing well."
– Andre Breton

This course is not designed to teach you to write; the expectation is that as a sophomore, you are already writing at the college level. The goals for a writing-intensive courses such as this one are:

(1) To give you practice and experience writing in a particular discipline;
(2) To help you learn the material by writing about it – "Writing to Learn."

Chemistry 260 is different from most other science classes; as this is a writing-intensive class, your grade on lab reports and exams won't be based just on how much you show you know, but also on how you write what you know. If a lab report is not well written, is not in proper English and scientific style, and is not well organized, it will not receive a high grade, regardless of the correctness of its content. This applies to exams too, but to a lesser extent – if an exam is so poorly written that it detracts from its readability and my understanding of what you said, you may lose points for writing, but writing comprises a larger portion of the grade on the lab reports,

as you have the opportunity to spell check, proof read, have others proof read, and seek assistance from the writing center.

**(D) Video Project.** 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. Participation by all persons in the group is necessary to earn that portion of the grade (see below). The video may be turned in either electronically or on media, and should be in Quicktime format. It is due by 4:00, April 26 (Reading Day).

**4. Honor Code.** The Honor Code applies to the exams, the problem set, and the lab reports. A lab report itself must be your work alone. The lab report for Experiment 2 (Report 2) 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.

| | | |
|---|---|---|
| **5. Grading:** | Exam I | 25 % |
| | Exam II | 25 % |
| | Problem set | 8 % |
| | Lab | 40 % |
| | Video | 2 % |

# CHEMISTRY 260 LAB

**1. Before the lab.** You should read the experiment and review your class notes.

**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. .

**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 2, 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 date and time noted on the schedule. The report should include:

a. A title page with:
    (1) Your name and the names of the others who worked on the experiment.
    (2). Date of experiment and date of report.
    (3). Number and title of experiment.
b. Explanation of the technique and theory.
c. Brief description of the problem investigated.
d. Data (normally organized into tables).
e. Graphs, equations, diagrams, etc., where appropriate.
f. Results
g. 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. They must be printed in black ink, with 12-point type, and on one side of the paper only, Normally, you should avoid writing anything in – use the word processor instead.

**7. Sources.** I expect the theory section of your report to be your words, not those of some author of a book or from the internet. The way for you to understand a technique is for you to explain it in your own words. You may use a reference, hard copy or on line, for diagrams and figures (note that you must cite it), but your grade will be lowered if it's evident that your report is not in your own words.

**8. 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. 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).

**9. Writing the reports.** For the first two reports, I will critique a draft which you will rewrite. You may use the writing center. 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. How Experiments and Reports Go Together.**

| **Report Number** | **Experiment Number** | **Technique** |
|---|---|---|
| 1 | 1-A and 1-B | uv-vis spectroscopy |
| 2 | 2 | NMR and IR spectroscopy, mass spectrometry |
| 3 | 3 | atomic absorption spectroscopy |
| 4 | 4 | gas chromatography |
| 5 | 5-A and 5-B | liquid chromatography |

# SCHEDULE

| Date | Experiment |
|---|---|
| Jan. 28 | --- |
| Feb. 4 | 1-A and 1-B (UV-VIS) |
| Feb. 11 | 2 (IR-NMR-MS) |
| Feb. 18 | --- |
| Feb. 25 | --- |
| Mar. 3 | 3 (AA) |
| Mar. 10 | --- (spring break) |
| Mar. 17 | --- |
| Mar. 24 | 4 (GC) |
| Mar. 31 | 5-A (TLC) |
| Apr. 7 | 5-B (HPLC) |
| Apr. 14 | --- |
| Apr. 21 | --- |

| Date | Work due |
|---|---|
| Jan. 28 | Draft of theory portion of **Report 1** due |
| Feb. 4 | |
| Feb. 11 | Draft of procedure/results/discussion portions of **Report 1** due |
| Feb. 18 | ►**Report 1** due<br>Draft of theory portion of **Report 2** due* |
| Feb. 25 | Draft of procedure/results/discussion portions of **Report 2** due |
| Mar. 3 | ►**Report 2** due by 4:00<br>*Problem set* due by Fri, Mar. 4, 4:00 |
| Mar. 10 | --- |
| Mar. 14-18 | *Exam I* (see due times on page 2 above) |
| Mar. 24 | ►**Report 3** due |
| Mar. 31 | ►**Report 4** due |
| Apr. 7 | --- |
| Apr. 14 | ►**Report 5** due |
| Apr. 18-22 | *Exam II* (see due times on page 2 above)<br>*Videos* due by Tues., Apr. 26, 4:00 |

(Unless otherwise stated above, reports are due in class on the dates listed above)

* This is a long report and you will be collaborating with another author. Start it well before the draft is due – you don't have to wait until you've done the experiment to begin writing the theory portion of the report.

## EXPERIMENT 1-A

## VISIBLE SPECTROSCOPY

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

Chemicals: 0.20-M $NiCl_2$

Apparatus: Ultrospec 2100 spectrophotometer, plastic cuvettes

Procedure:

Prepare a series of dilutions of the nickel solution: 0.10 M, 0.050 M, and 0.025 M.

Scan the visible region using the 0.20-M solution to obtain a spectrum. Select a wavelength at which to measure the absorbance of all the solutions.

At the selected wavelength, 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 1-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 1-B**

**VISIBLE SPECTROSCOPY**
**TWO-COMPONENT MIXTURE**

For this experiment, assume you are analyzing Kool-Aid in a quality control laboratory.

Objective: To determine the concentration of the red and blue dyes in grape Kool-Aid.

Chemicals: FD&C Blue Dye 1, FD&C Red Dye 40, grape Kool-Aid

Apparatus: Ultrospec 2100 spectrophotometer, plastic cuvettes

Procedure: Weigh the Kool-Aid powder and prepare a solution as directed on the package.

Scan the Kool-Aid solution in the visible region.

Decide on a concentration for the red dye and the blue dye. Prepare that concentration and scan the solution in the visible region. The absorbance (Y-axis) for each dye should be roughly on the same scale as the Kool-Aid absorbance. If your dye solution is too concentrated, you can dilute it; if it is too dilute, you will have to make up a new solution.

Once you have the proper concentration of each dye, prepare a series of 3 dilutions of each one (like you did for the nickel solution the previous week).

Select a wavelength at which to measure the absorbance of each dye.

At the selected wavelength, measure the absorbance of each of the solutions and the Kool-Aid. 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 each dye in Kool-Aid. Given the mass of Kool-Aid used, determine the amount of each dye in the dry Kool-Aid powder.

In your report for this experiment, you will need to include the spectrum of each dye, the wavelength selected, the Beer's Law plot, all the absorbance readings, and the concentration of each dye in the Kool-Aid as prepared and the mass of each dye in the Kool-Aid powder.

Also, address this question: If the absorbance spectra for red and blue dye had overlapped considerably, could you have determined the concentrations the same way?

**EXPERIMENT 2**

# 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 in $CDCl_3$

Apparatus: Anasazi EFT-60 NMR spectrometer, NMR tubes
Perkin-Elmer Spectrum Two IR spectrometer

Procedure:

You will work in groups of 2 or 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 with about 2.5 cm of $CDCl_3$ (deuterochloroform) with TMS. Add a few drops of sample. Obtain the proton NMR spectrum, including the integration.

For IR: 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. 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.

In your report, include a discussion of the theory of mass spectrometry along with that of FT-NMR spectrometry and IR spectroscopy.

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 3

## ATOMIC ABSORPTION SPECTROSCOPY

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

Chemicals: lead nitrate or copper nitrate

Apparatus: Buck 200A atomic absorption spectrometer

Procedure:

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).

The wavelength for copper, 324.7 nm, will have been set. 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.

# EXPERIMENT 4

# GAS CHROMATOGRAPHY

Objective: To determine the retention times of substances and to analyze a mixture of them

Chemicals: various organic compounds – specific ones announced in lab

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. Calculate the number of theoretical plates and HETP using your last peak, and $\alpha$ (separation) and R (resolution) using your last two peaks.

Show the structures of the compounds and explain why they eluted in the order you found.

## EXPERIMENT 5-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 5-B is finished.

## EXPERIMENT 5-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 75 % 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. Show the structures of the compounds and explain why they eluted in the order you found.

# 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

$y = b_0 + b_1 x_1$

$y = 0.01335 + 0.0994\ x_1$

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 |
|---|---|---|
| $s^2_{reg} / s^2_{res}$ | 920.67339 | 100.00 % |
| $s^2_{reg} / s^2_{pe}$ | 1772.26188 | 100.00 % |
| $s^2_{lof} / s^2_{pe}$ | 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 |
|---|---|---|
| $s^2_{reg} / s^2_{res}$ | 3.58590 | 94.98 % |
| $s^2_{reg} / s^2_{pe}$ | 1361.98028 | 100.00 % |
| $s^2_{lof} / s^2_{pe}$ | 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 |
|---|---|---|
| $s^2_{reg} / s^2_{res}$ | 3630.85538 | 100.00 % |
| $s^2_{reg} / s^2_{pe}$ | 1834.74795 | 100.00 % |
| $s^2_{lof} / s^2_{pe}$ | 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 % |