CHEMISTRY 260

Spring 2002

Book required : "Spectrometric Identification of Organic Compounds," 5th ed., by Silverstein, Bassler, and Morrill

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:

- Fluorescence spectroscopy
- X-ray and electron spectroscopy
- Polarimetry
- 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

There will be two exams. Exam I will cover spectroscopy; it will be given the week after spring break. 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 full week of classes up until reading day. It may also be taken as a whole or in two parts.

There will be a problem set assigned from Silverstein and Bassler; this is to be treated like a take-home exam. The assignment will be given before spring break and will be due Friday, March 22 (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.

There will be a final project – each of you will choose one of the instruments and with the assistance of Academic Computing, will make a short video explaining what the instrument is and what it's used for. (Two people may have to make one of these together, depending on the number of students.) These videos will be placed on the Natural Science/Mathematics Division web page.

The Honor Code applies to the exams, the problem set, and the lab reports. It is permissible to discuss lab reports prior to writing them up, but the report itself must be your work alone. The lab report for Experiment III will be written jointly by the pair of students who worked together on the experiment. The two students will turn in one report and both students 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	8 %
	Lab	40 %
	Participation in project	2 %

As this is a writing-intensive course, conferencing and revision are important components. You should turn in a draft of your first two lab reports for me to look over before you turn in these reports for a grade; I will meet with you after I've critiqued your draft and you can revise it. You may submit rough drafts of subsequent reports if you want.

The "due" dates for rough drafts and lab reports are suggested due dates; if you will not be turning in your report by then, please talk with me. Please note that I do need several days to read and critique your rough drafts before meeting with you.

The exams must be taken during the weeks listed; the problem set must be turned in by March 22.

SCHEDULE

Jan. 31	Experiment I (UV-VIS)
Feb. 7	Experiment II (UV-VIS)
Feb. 14	Experiment III (IR-NMR-MS); rough draft due on Exp. I-II (UV-VIS)
Feb. 21	Experiment III con't. (IR-NMR-MS); report due on Exp. I-II (UV-VIS)
Feb. 28	Experiment IV (AA); rough draft due on Exp. III (IR-NMR-MS)
Mar. 7	Experiment V (GC); report due on Exp. III (IR-NMR-MS)
Mar. 14	Spring break; no lab
Mar. 21	No lab; Exam I this week; problem set due on March 22.
Mar. 28	Experiment VI (TLC); report due on Exp. V (GC)
Apr. 4	Experiment VII (HPLC)
Apr. 11	Experiment VIII (statistics); report due on Exp. VI-VII (LC)
Apr. 18	No lab; report due on Exp. VIII (statistics); videos should be complete
Apr. 25	No lab; Exam II this week.

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, performed any preliminary calculations necessary, 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 the 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 III, 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).

EXPERIMENT I

VISIBLE SPECTROSCOPY

Chemicals: 0.2-M $NiCl_2$

Apparatus: Shimazdu 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 II. 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 II

SIMULTANEOUS MULTI-COMPONENT ANALYSIS
USING UV SPECTROSCOPY

Chemicals: pheniramine maleate (PAM), 80 μg/mL in 0.010-M HCl
phenylephrine hydrochloride (PEH), 200 μg/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 μg/mL; PEH -- 100, 50, 25, and 12.5 μg/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, 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.

Once you have the concentrations in the diluted Dristan®, you need to calculate what the concentrations were before dilution and then convert from μg/mL to %:

Multiply by 200 to account for the dilution. Divide by 10^6 to convert from μg/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 III

QUALITATIVE ANALYSIS

Chemicals: unknown organic compound
TMS

Apparatus: Varian EM360A NMR spectrometer, NMR tubes
Perkin-Elmer Paragon 500 IR spectrometer, salt plates

Procedure:

Each pair of students will be given 2 pure, liquid, organic unknowns; they will contain only carbon, hydrogen, oxygen, and/or nitrogen. (If there is a group of 3, they will be given 3 unknowns.)

Fill an NMR tube about 1/3 full with your unknown; add an equal amount of TMS. Obtain the NMR spectrum, including the integration trace. Clean out the NMR tube with acetone.

Place a drop of the sample between two salt plates. Obtain an infrared spectrum. Clean the salt plates with chloroform.

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

Determine the identity of your unknowns, including structure and proper name. Each peak in the NMR spectra must be assigned and the shift and area explained. The major peaks in the IR and mass spectra must also be assigned. After you have identified the unknown, go back and explain how it would (or would not) be expected to give the observed spectra.

Predict what the C-13 NMR spectrum of your other unknown would look like.

This is a two-week experiment, with the report due after the second week.

In your report, include a discussion of the theory and instrumentation of scanning proton NMR spectrometry and IR spectroscopy. It is not necessary to discuss the instrumentation of C-13 NMR or mass spectrometry since you did not use these instruments, but you should discuss the theory. Be sure that once you have identified your unknowns, you compare the shift, area, and multiplicity of each NMR peak with the predicted values and that you assign the major mass and IR spectral peaks.

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

EXPERIMENT IV

ATOMIC ABSORPTION SPECTROSCOPY

Chemicals: lead nitrate

Apparatus: Buck 200A atomic absorption spectrometer

Procedure:

Prepare a solution of 20-ppm lead, if not already prepared. From this solution, prepare standard solutions of 1, 2, 5, and 10 ppm lead.

Prepare standard addition solutions. Mix equal quantities of your unknown with each of the standards and deionized water (0 ppm Pb), giving 5 standard addition solutions (e.g., one such solution might be 1 mL of unknown + 1 mL of 10 ppm Pb).

Locate the lead wavelength (283.3 nm). 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.

BUCK 200A ATOMIC ABSORPTION SPECTROMETER

1. Turn the instrument on and adjust the lamp current to be around 5 mA.

2. If necessary, tune in the wavelength by adjusting the wavelength selector control for maximum deflection on the energy meter.

3. In general, the lamp will not need to be aligned and the burner position will not need to be adjusted. If this is not the case, see the manual. There is a slit width control at the back of the instrument. The chart inside the lamp compartment gives the setting for each element.

4. Make sure the loop in the drain line is filled with water.

5. Turn the oxidant selector to air. Turn on the air tank main valve. If necessary, adjust the regulator for a pressure around 40 psi. The oxidant flow control on the AA should be set at around 5-6.

6. MAKE ABSOLUTELY CERTAIN THAT AIR IS FLOWING THROUGH THE BURNER BEFORE TURNING ON THE FUEL! Turn on the acetylene at the tank and turn on the fuel switch on the spectrometer. If necessary, adjust the regulator for a pressure of 5 psi. The fuel flow control on the AA should be approximately 5.

7. Ignite the flame using the lighter. If necessary, adjust the air and/or acetylene flow controls.

8. Select Auto-Zero mode. Aspirate deionized water. Press the zero button and continue aspirating the blank until the display reads zero. Aspirate the solutions and read the absorbance from the meter. NOTE: It is often better to switch to integration mode. This integrates the signal for 7 seconds, giving less noise in the reading. After switching to integration mode, aspirate a solution and press the read button. In 7 seconds, the display will show the reading for that solution. That reading will be held until you press the read button again.

9. To shut down the AA, first turn off the acetylene with the fuel switch on the spectrometer. Turn off the acetylene tank valve. Open the fuel switch until both gauges on the acetylene tank read 0, and then close the fuel switch. Turn off the air tank valve. When these gauges read 0, turn the oxidant selector to the center position.

10. Turn off the power.

EXPERIMENT V

GAS CHROMATOGRAPHY

Chemicals: methanol, ethylene glycol, aqueous mixture of the two

Apparatus: Gow-Mac 740P gas chromatograph with FID, Carbowax 20-M stationary phase in a 15 m x 0.25 mm capillary column
recording integrator

A couple of years ago, a train derailment spilled methanol and ethylene glycol in the Alcovy River, the source of drinking water for Newton County. Although we will not be measuring these substances at trace levels as were present in the water, this mixture is a good example of chromatographic separation and identification.

Inject 2 different volumes of each of the pure substances (methanol and ethylene glycol) and prepare a calibration curve for each. Inject the mixture; from the retention times identify which peak is which substance and from the areas of the peaks in the mixture and the calibration curve, determine the percentage of each substance in the aqueous 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, α (separation), and R (resolution).

EXPERIMENT VI

THIN-LAYER CHROMATOGRAPHY

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)

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.

Look up 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 VIII is finished.

EXPERIMENT VII

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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

Apparatus: ISCO 2361 HPLC
chart recorder

Procedure:

Prepare solutions of the three substances in methanol, if not already prepared -- use 0.01 % for benzaldehyde, 1.0 % for toluene, and 0.2 % for DET. (These 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. Use 10 μL as the injection volume.

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.

EXPERIMENT IX

EVALUATION OF DATA

Using the techniques discussed in class, two sets of data are to be analyzed. The first set of data is to be used to fit a first-order (linear) model. You should report the coefficients, their significance levels, the 5 sums of squares (corrected, regression, residual, lack of fit, and pure error), and the significances of regression and lack of fit.

The second set of data is to be used to fit both a first-order and a second-order (quadratic) model. Again, all of the information requested for the first data set should be reported for each mode. Also, include an evaluation as to which model fits the data better, and discuss the criteria you used to make that decision. You should also report the results of canonical analysis on the quadratic model, and what information it provides about the response surface.

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

BONUS PROJECT

You are a forensic chemist; a life insurance company has hired you to consult about the death of a policy holder. This woman was found dead in her house. She had a terminal illness and had been depressed, so the police suspect suicide; life insurance will not pay off if death was due to suicide. However, the cause of death appears natural. The only foreign substance found in her system was a substance whose IR spectrum, mass spectrum, and GC retention time match those of dextromethorphan, a cough suppressant found in over-the-counter cough medicines. Indeed, there was a half-empty bottle of cough medicine (120-mL size of Vicks Formula 44, containing 15 mg dextromethorphan /5 mL) in her house. The amount of dextromethorphan in her system was not nearly enough to be fatal, however; even the entire bottle of cough medicine, if consumed, would not have contained a lethal amount of dextromethorphan. However, the insurance company wants to make sure her death was natural and not suicide before it pays off the policy, so they've turned to you. They've requested you take an NMR spectrum of this foreign substance and compare that to the spectrum of dextromethorphan, and then that you use your chemical knowledge and judgment to decide if there's any possibility of suicide in this woman's death. If so, what technique(s) might you employ to demonstrate this to the insurance company?

The following pharmaceutical information may be useful:

Dextromethorphan is relatively safe even in overdose. CNS effects are most prevalent and include stupor, hyperexcitability, ataxia, dystonia, nystagmus, coma, toxic psychosis and changes in muscle reflexes. Respiratory depression, tachycardia, increase in baseline seizure activity, nausea and vomiting also occur. Acute overdose will probably not result in severe symptoms of intoxication unless very large amounts have been ingested.

TOXICITY : LD50 Data unknown, but toxicity begins at around 20-30 mg/kg of body weight in humans.