CHEM 406-01, Instrumental analysis, Fall 2003

Chemistry 406
Instrumental Analysis

Text: Principles of Instrumental Analysis, 5th Ed., Skoog, Holler, and Nieman
Lecture: 8:00 - 9:30 am, Tu, Th, 207 Kennedy Hall
Lab: 1:00 – 4:00 pm, M, W, 402 Kennedy Hall
Instructor: Dr. Tanya Shtoyko, 411 Kennedy Hall; phone: 843-3959

GOALS: The goals that you should work towards in this course are;
1. To be able to assess and address the germane challenges associated with a particular analysis;
2. To be able to select an appropriate method or methods to solve a chemical problem;
3. To understand, on a detailed level, the theory and operation of modern instrumentation;
4. To gain practical laboratory experience using modern instrumentation to answer questions;
5. To be able to interpret data and to use the appropriate statistical methods in their evaluation;
6. To be able to communicate effectively the results of an investigation into a chemical problem;

EVALUATION: Your grade in this course is determined by your performance on several exams and laboratory assignments that assess what you have learned in the lab and lecture as well as your ability to evaluate and solve chemical problems. Each graded assignment is listed below along with its weight.

Lecture: 800 points

1. Three exams worth 200 points each;
2. A comprehensive final exam worth 200 points;

Lab reports: 800 points

The total number of points you attained on all graded work determines your final grade.

Grade	Total points
A	1440-1600
B	1280-1439
C	1120-1279
D	960-1119
F	below 960

POLICIES: My expectation is that you will attend all classes and labs unless directed otherwise. For both the lecture and lab, there will be regularly assigned readings and occasional problem sets. None of these assignments are graded; they are for your benefit only. The assigned readings from the text are listed on the syllabus. In addition, I will frequently assign articles for you to read. We will discuss these readings and problem sets during class and lab, and I will expect you to participate. These assignments are the minimum I believe is necessary for an average student to understand the subject material. If you are unable to attend a class or lab, it is your responsibility to determine all material discussed and assignments given.

You will be allowed to make up a missed exam with an excused absence. Normally, these reasons would include medical emergencies, a death in your family or required travel for a Rhodes' event (e.g., athletic team travel). If at all possible, please let me know ahead of time if you are not able to take an exam at its scheduled time so that another time for you to

take it can be arranged. If the absence is not excused, you will receive zero points for the exam. All graded work must be pledged to be your own or your groups' (if applicable).

Unless otherwise specified, your laboratory report/assignment is due no later than the beginning of the lab period one week after the scheduled end of the experiment. Unless there are extenuating circumstances, reports submitted after this time but before two weeks after the scheduled end of the experiment will have 10% of the possible points deducted. Reports will not be accepted after this time and zero points will be recorded for the assignment.

Course Schedule:

Topic	Chapter
Sampling	1, other
Measurement, calibration, noise	1, 5
Properties of light, optics	6
Lasers, wavelength selectors	7, other
Molecular absorption spectrophotometry	13, 14
Molecular fluorescence	15
Atomic absorption spectrophotometry	8, 9
Atomic emission spectrophotometry	10
EXAM I (200 points)	
Nuclear magnetic resonance spectroscopy	19, other
Radiochemistry	12, 32
Mass Spectrometry	11, 20
EXAM II (200 points)	
Electrochemical principles	other
Coulometry, electrogravimetry, cyclic voltammetry	22, 24, 25
Polarography	25
Chromatographic principles, band broadening	26
GC & LC Instrumentation	27, 28
Electrophoresis	30
EXAM III (200 points)	

CHEMISTRY 406 LAB
Revised schedule
Fall, 2003

Day	Date	Time	Experiment
W	9/3	1-4	Check in; safety; introduction

M	9/8	1-4	Method Validation 1: Drug determination in Dristan Nasal Spray
			(TEAMS: Carl & Shay; Philip, Kathryn, and Jesse)
W	9/10	1-4	Method Validation 1:
M	9/15	1-4	Method Validation 1
W	9/17	1-4	Method Validation 1 (**team technical report: 175 points**)
M	9/22	1-4	Project 1: Elemental Analysis (Shay, Philip, Kathryn)
W	9/24	1-4	Project 1: Elemental Analysis (Carl & Jesse)
F	9/26	1-4	Project 1
M	9/29	1-4	Project 1 (Shay, Philip, Kathryn)
W	10/1	1-4	Project 1 (Carl & Jesse)
F	10/3	1-4	Project 1
M	10/6	1-4	Project 1(Shay, Philip, Kathryn)
W	10/8	1-4	Project 1 (Carl & Jesse)
F	10/10	1-4	Project 1 (**individual project report: 175 points**)
M	10/13	1-4	Interpretation of mass spectra (Philip & Carl)
W	10/15	1-4	Interpretation of mass spectra (Kathryn, Shay, Jesse) **(individual project: 50 points)**
M	10/20		No class
W	10/22	1-4	Analysis of polymer coatings
M	10/27	1-4	Analysis of polymer coatings (Jesse, Shay, Philip)
W	10/29	1-4	Analysis of polymer coatings (Kathryn & Carl)
F	10/31	1-4	Analysis of polymer coatings (**interpretation: 75 points**)
M	11/3	1-4	Project 2: Molecular Analysis (Philip & Shay)
W	11/5	1-4	Project 2: Molecular Analysis (Carl, Kathryn, Jesse)
F	11/7	1-4	Project 2 (TEAMS: Philip& Shay; Carl, Kathryn, and Jesse)
M	11/10	1-4	Project 2 (Philip & Shay)
W	11/12	1-4	Project 2 (Carl, Kathryn, Jesse)
F	11/14	1-4	Project 2
M	11/17	1-4	Project 2 (Philip & Shay)
W	11/19	1-4	Project 2 (Carl, Kathryn, Jesse) (**team project report: 175 points**)
F	11/21	1-4	Electrochemistry of adrenaline
M	11/24	1-4	Electrochemistry (**individual formal report: 75 points**)
W	11/26		No class
M	12/1	1-4	Method Validation 2: Caffeine in beverages
			(TEAMS: Carl, Philip & Kathryn; Jesse & Shay)
W	12/3	1-4	Method Validation 2 (**team technical report: 75 points**)
M	12/8	1-4	Elective assignment
W	12/10	1-4	Check out

Method Validation 1: Drug determination in Dristan Nasal Spray

In this experiment, your team will validate a method used to determine simultaneously the concentration of two pharmacologically active compounds in Dristan Nasal Spray: phenylephrine hydrochloride (PEH) and pheniramine maleate (PAM). The method involves the measurement of the absorbance of the Dristan at two wavelengths and the determination of the concentration of each component by using Beer's law.

PEH and PAM absorb light between 240 and 300nm, and the spectrum of each overlaps significantly. Assuming only these two compounds absorb light at a given wavelength, the measured absorbance at any two wavelengths is described by Beer's law:

$$A_{\lambda 1} = \varepsilon_{\lambda 1}\, b\, C_{PAM} + \varepsilon_{\lambda 1}'\, b\, C_{PEH} \quad (1)$$

$$A_{\lambda 2} = \varepsilon_{\lambda 2}\, b\, C_{PAM} + \varepsilon_{\lambda 2}'\, b\, C_{PEH} \quad (2)$$

If the molar absorptivities of each compound are determined at both wavelengths, measuring the absorbance of the Dristan Nasal Spray at these two wavelengths allows you to solve the resulting simultaneous equations and calculate the concentration of PAM and PEH.

Validation Procedure

Initial assessment

Prepare stock solutions of PAM and PEH at concentrations of about 200µg/mL in 0.01M HCl. Prepare a diluted solution of each (again in 0.01M HCl) and obtain a spectrum in the range 240-300nm. Choose your dilution factor so that the maximum absorbance is not greater than 1.0. Once you have obtained these two spectra, select two appropriate wavelengths to be used in the analysis. Since the absorbance of the Dristan Nasal Spray is too large to conduct this analysis, it will need to be diluted.

Assess Specificity

Dristan Nasal Spray has one other compound that absorbs light in this region: benzalkonium chloride. Prepare a solution of benzalkonium chloride at the concentration in which it is present in Dristan, and then dilute it further by the same factor as the Dristan. Obtain a spectrum of the solution and then assess the extent to which benzalkonium chloride interferes with the analysis at the wavelengths chosen.

Assess Linearity

Prepare five standards for PAM and PEH evenly distributed in concentration between 50 and 150% of the expected concentration present in the diluted Dristan sample. Measure the absorbance of each solution and construct a calibration plot to determine the molar absorptivities for each compound at the two wavelengths. Repeat this procedure five times. Assess linearity by calculating r^2 as well as plotting the response factor versus concentration.

Assess Precision

Instrument Precision: Measure the absorbance of a single aliquot of a solution of PEH, PAM or Dristan a minimum of 10 times. The measurement should be conducted on a solution with an appropriate concentration and at both wavelengths.

Intra-assay Precision: Prepare five solutions of PAM, PEH or Dristan at an appropriate concentration and measure the absorbance of each solution at both wavelengths.

Assess Accuracy

Determine the concentration of PAM and PEH in the Dristan and compare these values with those on the package. This will serve as a determination of the accuracy.

Project 1: Elemental Analysis

The nature of your project will be to quantify the amount of some element in a sample using a flame atomic absorption spectrophotometer. You will design and execute a project using a standard method of analysis.

Instrument setup/checkout

Since you will be determining trace quantities of an element in your sample(s), contamination of the sample(s) is a real concern. Therefore, carefully clean all volumetric glassware that you use in this experiment. You should clean all glassware with detergent, rinse it with water, soak it in 3M nitric acid for an hour, and then rinse it completely with Millipore Milli-Q deionized water.

I will demonstrate the appropriate manner to setup the instrument and conduct an analysis for Zn (an arbitrarily chosen metal). Prepare a standard Zn stock solution (500 mg/L) in the following manner. Analytically weigh about 0.25g ($\pm$ 0.1mg) of primary grade Zn into a beaker and add 10mL of trace metal grade nitric acid to dissolve the Zn. The solution should then be quantitatively transferred to a 500mL volumetric flask and diluted to the mark with Millipore Milli-Q deionized water. This stable stock solution will be used to prepare your standard Zn solutions that will be used in the construction of your working curve. A concentration of Zn less than 10 mg/L is not stable and needs to be prepared prior to use. Prepare three standard solutions having a concentration of Zn between 1.0 and 2.0 mg/L from the standard Zn stock solution by serial dilution. This is the optimum working concentration for Zn. Aspirate each solution, measure its absorbance, and check for linearity.

Project Guidelines

1. There are several resources that describe and give references to standard methods used in atomic absorption. These include Perkin-Elmer's analytical methods and the Official Methods of Analysis (located in Burrow Library). Methods include analysis of biochemical, agricultural, environmental samples, foods, plastics and fibers, soils, metals, paints, cements, ceramics, pharmaceuticals, cosmetics, petrochemicals as well as other types of samples.

2. You will have a few restrictions in selecting your project.
- only certain elements may be determined based on our supply of hollow cathode lamps and the type of flame available;
- we must have the necessary chemicals and equipment to conduct the analysis;
- you must be able to obtain suitable samples;
- you must use the method of standard addition to determine the concentration of the element in your sample(s).

3. No later than the beginning of the second scheduled laboratory period you must propose a project. At a minimum, you must have,
- a well defined reason for undertaking the project (i.e., a well proposed question);
- verified that we have the necessary lamp, flame type, and chemicals to conduct the analysis as well as located a source for your sample(s);
- at least one literature reference for this analysis.

I must approve the project no later than the beginning of the third scheduled laboratory period.

Analysis of polymer coatings
Pyrolysis Gas Chromatography/Mass Spectrometry

Pyrolysis gas chromatography/mass spectrometry (PyGCMS) is a powerful tool used to identify components in polymers, and their relative amounts. A minute sample of a polymer is thermally degraded in a helium atmosphere (i.e., pyrolyzed), leading to bond fragmentation and the creation of small molecules that are related to the structure of the original macromolecule. These small molecules are then separated chromatographically and sent into a mass spectrometer, one of the most powerful techniques for elucidating the structure of organic compounds. We will discuss the details of this in lab, but you will use this technique to obtain information about the nature of polymer coatings. Dr. Redfearn, our resident polymer chemist and expert on PyGCMS, will assist us in this lab.

Project 2: Molecular Analysis

The nature of your project will be to determine the presence or absence of some environmentally interesting molecular species in an environmental sample (e.g., water, soil, sediment or unwashed fruits, vegetables or plants). Your team will design and execute a project using a standard method of analysis.

Project Guidelines

1. The Official Methods of Analysis (located in Burrow Library) will provide you with the appropriate procedure for the analysis of PCBs in soil, water and foods.

2. You will have a few restrictions in selecting your project.
- we must have the necessary chemicals and equipment to conduct the analysis;
- you must be able to obtain suitable samples;

3. No later than the beginning of the second scheduled laboratory period your team must propose a project. At a minimum, you must have,
- a well defined reason for undertaking the project (i.e., a well proposed question);
- selected a sample and verified that we have the necessary equipment and chemicals to conduct the analysis as well as located a source for your sample;
- at least one literature reference for this analysis.

I must approve the project no later than the beginning of the third scheduled laboratory period.

Electrochemistry of adrenaline

Adrenaline is a catecholamine vital to regulation of numerous functions in the body. The catechol portion of the molecule is easily oxidized at physiological pH. However, many molecules have chemical reactions that precede or follow oxidation or reduction, and the purpose of this project is to investigate any chemical reaction(s) coupled to the oxidation of adrenaline.

Cyclic voltammetry is a widely used electrochemical technique that provides information that can lead to an understanding of reactions that precede or follow the electron transfer step. It also permits the measurement of the formal potential of a redox couple.

This reaction is pH dependent, so you will prepare two solutions. The first solution is a 1mM adrenaline solution in 1.0M sulfuric acid and the second is a 1mM adrenaline solution in a pH 3.00 buffer. You should investigate and interpret the oxidative electrochemistry of adrenaline in each solution by obtaining cyclic voltammograms at 100mV/s.

Method Validation 2: Caffeine in beverages

High performance liquid chromatography (HPLC) is the primary technique for the separation and quantification of complex mixtures that are unsuitable for analysis using gas chromatography. In this lab, your team will assess a method of separating and determining the amount of caffeine in the beverage using HPLC.

Validation Procedure

Initial assessment

The separation of your selected soft drink will be accomplished by using a C18 column and a mobile phase of 80% 1M acetic acid and 20% methanol. You should prepare about 600mL of this solution and adjust the pH of the acetic acid with 50% NaOH to a pH of 3.4. This solution should be prepared fresh each day. To save time, you may wish to prepare a 1M acetic acid solution, mix it with the methanol, and then adjust its pH as needed. All solutions used in the HPLC (including samples and standards) should be filtered through a membrane filter.

Prepare a standard caffeine solution in the mobile phase whose concentration is approximately 1.00mg/mL.

Equilibrate the column for 15 minutes by passing the mobile phase through the column at a reasonable flow rate. The beverage components may be detected using uv absorption with a wavelength of 254nm. Inject 20µL of the beverage into the HPLC and record the chromatogram. Next, inject the same quantity of your caffeine standard into the HPLC and then identify the caffeine peak in your chromatogram. Verify that the caffeine is separated from the other components. If not, the pH of the mobile phase will need to be adjusted.

Assess Linearity

Prepare three caffeine standards in the mobile phase evenly distributed in concentration between 50 and 150% of the estimated concentration present in the beverage. Inject 20µL of the standards into the HPLC and measure the area under the peak. Duplicate each run. Using an average area for each standard, assess linearity by calculating r^2.

Assess Accuracy

Determine the concentration of caffeine in the beverage and compare this value with that from the manufacturer. This will serve as a determination of the accuracy.

To properly maintain the HPLC, it will be necessary for you to completely rinse all traces of acetic acid from the pumps and column. To do this, you will need to prepare an 80% water and 20% methanol solution that you will use to flush the HPLC. I will give you specific instructions on the operation and shut down of the HPLC.