

CHM-4130L

**INSTRUMENTAL ANALYSIS
LABORATORY**

LABORATORY MANUAL

Florida International University
Department of Chemistry and Biochemistry
Spring 2007

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CHM4130L SPRING 2005
INSTRUMENTAL ANALYSIS LABORATORY

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Scheduled Class Time: Thursdays 14:20-6:20 PM CP 395
Office Hrs: 11:00-12:00 noon (Thursday)

Course Description:

This course is designed for professionally oriented students in the majors' program in chemistry; its aim is to develop competence in areas of chemical analysis using modern instrumentation. These topics include both training in the laboratory, and additional expertise interpreting and communicating the data from these experiments to a panel of reviewers.

Your assignments for this course include 7 hands-on experiments with a formal report and a research paper. You are also required to give an oral presentation based on the research paper. The research paper will be submitted to the instructor at the time of the presentation.

Grading:

There will be 15 points for each of the seven experiments, 30 points for the research paper and oral presentation, and 15 points for instructor's evaluation.

Lab grades are calculated as follows:

Your grade will depend on: a) your results and b) how well they are presented in your final formal laboratory report. Multiple factors such as accuracy of your determination, neatness and completeness of your written report and a strong indication of your understanding of the experiment will contribute towards your grade. In addition, part of your grade (up to 3 points) will be based on how well you answer inquires about the experimental protocol you are using and how well you understand the fundamentals of the analytical technique. I might do this at anytime during the lab session to evaluate your up-front preparation for the lab. Nevertheless, if you need to ask questions please do so, I am not grading that! Your preparation and participation of the experiments, and complying with the laboratory rules will contribute to the instructor evaluation.

LABORATORY PROCEDURE

A necessary prerequisite for adequate performance in the laboratory is your preparation prior to the laboratory period. Students must be well acquainted with the instructions for the experiment to be carried out. You only have 4 hours to complete the experiments! Therefore, make sure you have read the procedures and the instrument operating instructions **before** coming to class. Also come to class with an outline of exactly how you intend to prepare your standard solutions and run your experiments. *Be prepared, have a lined-up plan, do not procrastinate!*

The lab schedule is included in this manual (**see page 10**) so you can plan accordingly. If you are unable to attend a scheduled laboratory period due to a University valid excuse, the instructor should be notified immediately, before the lab period starts, so that suitable alternative arrangements can be made. Please keep in mind that lab make up is normally not allowed because of the Instruments and lab space limitations.

All students will work in pairs on a given experiment during each laboratory. It is extremely important to be as efficient as possible during each and every lab period. Some experiments are carried out in two lab periods (4hrs x 2). In general you will finish all preparations for the experiment during the first period (prepare stock solutions, sample solutions etc.), and conduct the instrumental analysis during the second period.

Please come to class on time. In many cases, the experiments will take the entire 4 hours allocated! **Your partner will not be allowed to work alone so be responsible with his/her time. Your will not be allowed to work if you come to the laboratory 30 min late.**

All experimental data will be recorded, as it is obtained, on a formal bound lab notebook (8.5 X 11). Upon completion of the day's lab work, **the instructor will validate your work by placing his/her initials in your lab-book even if the experiment has not been completed.** The original data sheets (or a photocopy) must be included in your formal report and will be part of your grade.

SAFETY

Remember: "SAFETY IS ALWAYS THE NUMBER ONE PRIORITY"

Should any problem arise inform the instructor immediately.

Do not put yourself in danger.

If you think something is unsafe STOP and consult the instructor before continuing.

IMPORTANT RULES

1. Please remember that we share the lab with two or more classes and therefore do not leave anything out after the lab session is finished at 6:20 pm. Place your unknowns and standard solutions vials in beakers and lock them in your drawers. **Any unattended or unlabeled container will be properly disposed without asking.**
2. Always return all chemicals to the CHM4130L cabinet immediately after use.
3. Keep your work area always clean, in particular when working with instruments or in the balance room. Your lab-book will not be validated until your work area is clean. **Point deductions will be taken off your experiment grade if this conduct is persistent.**
4. Do not leave standard solutions in volumetric flasks. Once you are done with the preparation, transfer the solution to a **labeled** vial for storage. Include the Group number and other important information in the labels; a lot of people may be doing the same experiment at the same time. Rinse the volumetric flasks thoroughly before returning them to the cabinet.
5. Yes, you must have a lab notebook and you must write everything in black or blue ink (no pencils). When correcting something do not white-out, ~~scratch the error with a single line~~ and write the correction above or below.
6. **Remember to record the unknown sample number for each experiment.**
7. Always wear a lab coat and safety glasses and please, no sandals!
8. Read the operating instructions for every instrument **before** you use it. If you have any questions about anything stop and ask!
9. Be aware of the due dates for each experiment (you will be penalized for late submissions), If you can't make it for any reason notify the instructor in advance, not after the fact.
10. Working in groups requires an even share of the responsibilities so do not drag your feet, talk to your partner. If problems arise don't wait until it's too late, talk to the instructor about it.
11. **PLAGIARIZE** (play-jâ-riz) *v.* to take and use another person's ideas, writings or inventions as one's own.
Note that plagiarism is illegal, unethical, and you can be expelled from the University for it. **Plagiarizing old laboratory reports will not be tolerated! Any evidence of such activities will result in no points for that exercise.** Further disciplinary actions will be taken at the discretion of the instructor.

REPORT WRITING

A formal report is required for each one of the experiments. Reports are due at the beginning of the next lab period after you finish an experiment. **There will be a 10% grade deduction on all late submissions** and no report will be accepted after two weeks of the experiment completion have passed. Your final report should be neatly typed or handwritten (in ink) on 8 ½ x 11 paper. However, **we strongly recommend that you use a word processor and a spreadsheet to generate your reports for your own convenience.** Graphs should be plotted on the same size paper and should be scaled so that the data occupies the majority of the plotting area. All graphs in the final report, with the exception of your raw data, **must be computer generated.** All axes should be labeled and the proper units displayed. When logarithmic scales are used please make sure the data is accurately represented in your plot. When reporting tables of data please check your significant figures, they are important. **All statistical treatment of the data must be done using a computer.**

Your final report **must** consist of the following sections:

- I. Title Page: Title of the experiment, course name and number, your name and the date.
- II. Introduction: Presents a description of the parameters to be measured and the general approach to the problem. Should also include a detailed description of the analytical technique involved showing a thorough knowledge of the concepts involved. This section should also state the **objectives** of the experiment.
- III. Experimental Section:
 1. Analytical procedure: This section should include a description of how the experiment was conducted and the equipment was used. Do not copy the procedure section from the lab manual. Write in your own words to describe how you performed the experiment. Be sure to include any modifications or deviations from the suggested protocols.
 2. Raw Data: Record all experimental data **as it is obtained.** Include the original data sheet with your report (remember an initialized copy of the data has been submitted to the instructor).
- IV. Results: Present your data in tables and graphs. Calculations and error analysis must be shown and explained. Use SI units only. If difficulties were encountered include a narrative description of the problem. All graphs, tables and sections must have a title/caption and should be referenced in your text.
- V. Discussion and Conclusions: Discuss your findings; make comparisons with known values if available. Elaborate on possible sources of errors, selectivity and sensitivity of the technique, detection limits, matrix effects, interferences, accuracy, precision, applicability, etc. Suggest any possible improvements in the experiment and present your summary conclusion.
- VI. References: Include references using the ACS format used in the journal Analytical Chemistry; i.e.: Number all references consecutively in parentheses

and include them at the end of your report in a section called "References". i.e.:
 (1) Cai, Y.; Alzaga, R.; Bayona, J.M. *Anal. Chem.* **1994**, 66, 1161-1167.

Since your final grade greatly depend on these reports please work hard on them.

Checklist for Lab Report Grades

	Max Points	Grade
<i>Cover page to include title, names, and date</i>	0.5	
Introduction		
Description of technique/Principles/Equations/Reactions	2	
Objectives		
	1	
Experimental Section		
Analytical narrative	2	
Raw data	2	
Results		
Tables of data/observations/Calculations	1	
Graphs fits/equations/error/axes/Units/SD's	2	
Analytical Result*	2	
Discussion/Questions/Definitions	2	
References	0.5	
Total grade	15	

* >40% Deviation 0, 40-30% 0.5, 30-20% 1, <20% 2

Each group will produce **one report** and the report writing should be a shared responsibility.

RESEARCH PAPER

You will need to choose a “Modern Analytical Technique” research topic from either the list provided by the instructor or the one you are interested in. However, an outline must be submitted for instructor’s approval before you start. If another student before you has already picked up a similar topic, you **must** select another one. So start early. You should research the topic in depth including an extensive literature search from various chemistry journals. A list of common chemistry journals is included in this manual. Your assignment includes a **15-minute oral presentation** and a **formal review paper** on your research topic.

Your presentation and your paper should cover the following items:

- I. History and development of the technique.
- II. Instrumentation required (including schematics and operating principles).
- III. Applications of the technique (industrial, environmental, research, etc.)
- IV. Advantages and disadvantages of this technique over other similar methods (include a list of competitive techniques).
- V. The “future” of the technique: according to the experts in the field (i.e. how many recent publications or reported applications)
- VI. The “future” potential of the technique in your informed opinion as follows:
 - a) Do you think the technique will increase, decrease or remain about the same in popularity after the year 2001? Explain your answer.
 - b) What major trends in instrumentation are designed to improve the performance of the technique such as resolution, sensitivity, analysis time and cost?
 - c) If faced with a restricted budget, will you use an alternative method to obtain similar results?
 - d) If money is not your problem, will this technique be your method of choice?

There is no preset minimum length to this paper, however it will probably difficult to cover all of these points in less than about 10 typewritten pages. You have most of the semester to work on this paper. **START NOW!** I will post “topic selection” and “presentation schedule” signup lists on my office door for everybody’s convenience.

All presentations will be generated using Microsoft Power-Point, which is widely available in most FIU computer labs. You should familiarize with the software early in the semester in case you need to attend one of the training sessions offered by FIU computer services. You will also have access to a scanner during the lab period to reproduce any graphs that you may need. Your presentation must be ready to load in the computer the day before your presentation by 12:00 noon so plan accordingly.

Common Analytical Chemistry Journals and Their Citation Ranking
(From *Anal. Chem.* 1989, 61, 526A)

1	Analytical Chemistry	5.55
2	CRC Critical Reviews of Analytical Chemistry	4.73
3	Journal of Chromatography	4.14
4	Journal of Electroanalytical Chemistry	4.01
5	Journal of Chromatographic Science	3.93
6	Chromatographia	3.35
7	Analytica Chimica Acta	3.01
8	Journal of Liquid Chromatography	2.93
9	Separation and Purification Methods	2.62
10	International Journal of Environmental Analysis	2.47
11	The Analyst	2.34
12	J. of Assoc. of Official Analytical Chemists	2.17
13	Separation Science and Technology	2.12
14	Talanta	2.07

Newer journals or those with average citation rates less than two

American Environmental Laboratory
Analysis
Analytical Instrumentation
Analytical Letters
Analytical Proceedings
Chemtracts - Analytical and Physical Chemistry
Environmental Science & Technology
High Resolution Chromatography and Chromatogr. Comm.
Journal of Analytical Applied Pyrolysis
Journal of Applied Spectroscopy
Journal of Forensic Sciences
Journal of Labeled Compounds
Journal of Mass Spectrometry and Ion Processes
Journal of Microcolumn Separations
Journal of Planar Chromatography - Modern TLC
Journal of Radioanalytical and Nuclear Chemistry
LC·GC
Mikrochimica Acta
Organic Mass Spectrometry
Radiochimica Acta
Thermochemica Acta
Trends in Analytical Chemistry

Hardcopy or On-line versions of most of the journal listed above are available at FIU. Others are available via interlibrary loan.

THE INTERNATIONAL SYSTEM OF MEASUREMENT (SI UNITS)

Seven fundamental units required to describe what we know as the Universe:

LENGTH - Meter (m); MASS - Kilogram (kg); TIME - Second (s); TEMPERATURE -Kelvin (K);
LUMINOUS INTENSITY - Candle (cd); ELECTRIC CHARGE - Coulomb (C);
MOLECULAR QUANTITY - Mole (mol)

Prefix	Abbreviation	Connotation	Some conversion factors
Tetra-	T	$10^{12} \times$	1 mile = 1.61 kilometers (k)
Giga	G	$10^9 \times$	1 yard = 0.914 meter (m)
Mega	M	$10^6 \times$	1 inch = 2.54 centimeters (cm)
Kilo	k	$10^3 \times$	1 lb = 0.454 kilograms (kg)
Hecto-	h	$100 \times$ (or $10^2 \times$)	1 quart = 0.946 liters
Deka-	da	$10 \times$ (or $10^1 \times$)	1 atm = 760 mm
Deci-	d	$0.1 \times$ (or $10^{-1} \times$)	1 atm = 29.9 inches mercury
Centi-	c	$0.01 \times$ (or $10^{-2} \times$)	1 atm = 14.7 PSI
Milli-	m	$10^{-3} \times$	1 ounce = 28.4 grams
Micro-	μ	$10^{-6} \times$	1 fluid ounce = 29.6 milliliters
Nano-	n	$10^{-9} \times$	
Pico-	p	$10^{-12} \times$	
Femto -	f	$10^{-15} \times$	
Atto-	a	$10^{-18} \times$	
Zepto-	z	$10^{-21} \times$	

Milliliter = ml = $1 \text{ cm}^3 \sim 1 \text{ gram (g)}$
(VOLUME) (LENGTH) (WEIGHT)

Celsius degree = $^{\circ}\text{C}$ Water freezes at 0°C and boils at 100°C

Mole = number of atoms in 12 g of carbon-12 isotope.

SYSTEMS FOR EXPRESSING CONCENTRATIONS OF SOLUTIONS

Name of System	Symbol	Definition
Formal	F	gram-formula-weights solute/liter solution
Molal	m	moles solute/kilogram solvent
Molar	M	moles solute/liter solution
Mole Fraction	X	moles solute/moles solute + moles solvent
Normal	N	equivalents solute/liter solution
Volume percent	vol %	100 X liters solute/liters solution
Weight percent	wt %	100 X grams solute/grams solution
Parts per million	ppm	milligrams solute/kilograms solution or mg/liter
Parts per billion	ppb	micrograms solute/kilograms solution or μg /liter
Parts per trillion	ppt	nanograms solute/kilograms solution or ng/liter

LABORATORY SCHEDULE

Spring 2007

	Date	Schedule A	Schedule B
Week 1	1/11	Lab introduction, group assignment, equipment checkout	
Week 2	1/18	Ultraviolet Absorption Spectroscopy "Quantitative Analysis of Aspirin Tablets"	Graphite Atomic Absorption Spectroscopy "Determination of Selenium in aqueous Sample"
Week 3	1/25	Graphite Atomic Absorption Spectroscopy "Determination of Selenium in Aqueous Sample"	Ultraviolet Absorption Spectroscopy "Quantitative Analysis of Aspirin Tablets"
Week 4	2/01	Fluorescence Spectroscopy "Determination of Fluorescein in Antifreeze"	Visible Absorption Spectroscopy "Simultaneous Determination of Chromium and Cobalt"
Week 5	2/8	Visible Absorption Spectroscopy "Simultaneous Determination of Chromium and Cobalt"	Fluorescence Spectroscopy "Determination of Fluorescein in Antifreeze"
Week 6	2/15	Gas-Liquid Chromatography "Analysis of Hydrocarbons in Common Fuels"----- 1	Gas Chromatography-Mass Spectrometry "Analysis of an unknown mixture by full scan GC/MS"
Week 7	2/22	Gas-Liquid Chromatography "Analysis of Hydrocarbons in Common Fuels"----- 2	High-performance Liquid Chromatography "Determination of Caffeine in Beverages"
Week 8	3/01	Gas Chromatography-Mass Spectrometry "Analysis of an unknown mixture by full scan GC/MS"	Gas-Liquid Chromatography "Analysis of Hydrocarbons in Common Fuels"----- 1
Week 9	3/8	High-performance Liquid Chromatography "Determination of Caffeine in Beverages"	Gas-Liquid Chromatography "Analysis of Hydrocarbons in Common Fuels"----- 2
Week 10	3/15	Lab make up	Lab make up
Week 11	3/22	Spring Break	Spring Break
Week 12	3/29	Research Paper preparation	Research Paper preparation
Week 13	4/05	Research Paper presentation	Research Paper presentation
Week 14	4/12	Research Paper presentation	Research Paper presentation
Week 15	4/19	Research Paper preparation Research paper due (5:00 pm CP 315)	Research Paper preparation Research paper due (5:00 pm CP 315)

Determination Of Selenium In Aqueous Sample Selenium Supplement Using Graphite Furnace Atomic Absorption Spectrometry

Introduction:

In many cases, the use of graphite furnace as an atomizer improves significantly the sensitivity of the atomic absorption spectrometric technique for trace element analysis. Graphite furnace AAS will be used to determine selenium in aqueous samples in this experiment. The experimental procedure will be based on the EPA Method 7740 for selenium analysis in wastes, soils and ground water. A copy of this method can be downloaded from EPA website <http://www.epa.gov/SW-846/7xxx.htm>.

Chemicals:

concentrated HNO₃

Se standard stock solution (1000 mg/L)

Ni(NO₃)₂·6H₂O

Unknown sample

Procedure:

Preparation of reagents:

- Nickel solution (5%) Dissolve the appropriate amount of ACS reagent grade Ni(NO₃)₂·6H₂O in DI water and dilute to 50 mL.
- Selenium sub-stock standard. Prepare 1 ppm selenium sub-stock standard by taking appropriate amount of selenium stock (1000 ppm), and dilute to 500 mL using DI water.
- Selenium working standards. Prepare dilutions of the stock solution to be used as calibration standards at the time of the analysis. Accurately prepare five (5) 100 mL standard solutions varying in concentrations (0, 5, 10, 20, and 40 µg/L) using the 1ppm sub-stock solution. To do so, withdraw appropriate aliquots of the sub-stock solution; add 0.5 mL concentrated HNO₃, and 2 mL of the 5% nickel nitrate solution. Dilute to 100 mL with DI water.

Sample preparation:

- Weigh one Selenium dietary supplement tablet in a 250-mL glass beaker; add 100 mL DI water, and then add enough concentrated HNO₃ to result in an acid concentration of 1% (v/v).

- Heat for 1 hr at 95 °C or until the volume is slightly less than 85 mL.
- Cool and transfer to a volumetric flask, and bring back to 100 mL with DI water.
- Dilute properly before analysis using AA. Note the concentrations of Ni and HNO₃ in the final solution should be identical to those in Se working solution.
- Prepare another diluted sample without adding the 5% nickel nitrate solution.

Unknown analysis:

- Dilution and matrix matching are needed for Unknown analysis. Dilute properly (1:1 is suggested) before analysis using AA. Note the concentrations of Ni and HNO₃ in the final unknown solution should be identical to those in Se working solution.
- Prepare another sample without adding the 5% nickel nitrate solution.

Analysis:

- Turn on the instrument (Perkin Elmer AAnalyst 600) and computer under the Instructor's direction. Understand the purpose of the major parts of the instrument.
- Familiarize with the procedure for setting up method for your analysis under the instructor's instruction.
- Make a calibration curve by running the selenium-working solutions prepared above.
- Run both samples and blanks (with and without adding 1% nickel nitrate solution) at least three times.

Data Analysis and Questions:

- Construct and evaluate a calibration curve by plotting absorbance vs concentration.
- Calculate the concentrations of Se and L-selenomethionine in the Se supplements and compare your results with that of manufacturer and discuss. Calculate Se concentration in the Unknown sample.
- Why is nickel nitrate solution added into both standard and sample solutions? What causes the difference observed for the samples with and without addition of 1% nickel nitrate solution?
- Why is the selection of temperature and times for the dry and char (ash) cycles important?
- Define:
 - Zeeman Effect
 - Inductively Coupled Plasma (ICP)
 - L'vov Platform

Quantitative Analysis of Aspirin Tablets by Double-Beam Ultraviolet Absorption Spectrophotometry

Introduction:

The absorption of electromagnetic radiation by molecules forms the basis for a multitude of instrumental analytical techniques. This experiment deals with the absorption of photons with wavelengths in the ultraviolet range. The concentration of analyte can be determined from the photon absorption using the well known Beer-Lambert Law. The amount of aspirin (acetylsalicylic acid) in commercial analgesics can be determined once it has been hydrolyzed to salicylic acid and diluted to a concentration where Beer's Law is obeyed.

Chemicals: 0.1 N NaOH
 salicylic acid
 commercial aspirin tablets
 unknown solution

Procedure:

Warm up the Shimadzu UV-2101 PC double-beam scanning spectrophotometer (see the operating manual for the instruments) and read the instructions for operation of the instruments thoroughly before beginning the experiment.

STANDARDS: Accurately weigh (to 0.1 mg) about 0.1g of pure salicylic acid (weight by difference using a weighing bottle) and quantitatively transfer to a 100 ml volumetric flask. After thorough mixing dilute to the mark with a NaOH solution so that the final concentration is 0.1 N NaOH. Calculate the salicylic acid concentration (mg/L) in this Stock solution. Prepare five dilutions of your stock solution using 1,2,3,4, and 5 mls diluted to 100ml with 0.1 N NaOH. Calculate the salicylic acid concentration (mg/L) in your stock solution and each standard.

CALIBRATION CURVE: Using 3ml dilution of salicylic acid, measure the UV absorption spectra with the Shimadzu UV-2101PC Spectrophotometer and determine the wavelength of maximum absorbance. Measure the absorbance for each of the standards at the wavelength of maximum absorbance. Plot a calibration curve for your salicylic acid standards (absorbance versus concentration).

ASPIRIN ANALYSIS: Allow one commercial aspirin tablet to dissolve in 0.1 N NaOH solution contained in a 250 ml volumetric flask. Bring the volumetric flask to volume and mix thoroughly. Dilute this solution 1 to 50 with 0.1 N NaOH and measure the absorbance

of this solution at the same wavelength as your standards. Use the calibration curve to find the concentration of salicylic acid present in the solutions using the scanning UV-VIS spectrometer. Compare these results to those obtained with the B&L Spectronic 20. Calculate the weight of acetylsalicylic acid (ASA) in the aspirin tablet applying the appropriate stoichiometric factors and considering the dilutions involved. **Compare your result to the content specified on the label and compute the percent recovery.**

UNKNOWN ANALYSIS: Determine the concentration of salicylic acid in the unknown solution, after appropriate dilutions if needed, using the calibration curves from the scanning UV -VIS.

In your report you should address the following questions:

-Why do we use a 0.1 N NaOH solution for our solvent in this experiment, why using salicylic acid?

-Give possible explanations for the recovery you found when analyzing the aspirin tablet. Are your results within expected variations allowed for over-the-counter analgesics?

-What compounds present in some analgesics might interfere with aspirin determination by this method?

-Define:

Chromophore
Light Scattering
Bathochromic shift

Simultaneous Determination Of Chromium And Cobalt By Visible Absorption Spectrophotometry

Introduction:

Quantitative analysis using UV-Vis spectroscopy is based on the Beer-Lambert Law:

$$A_{\lambda} = \epsilon_{\lambda} b C \quad (1)$$

A_{λ} = absorbance at wavelength λ = $-\log$ transmittance

ϵ_{λ} = Molar extinction coefficient

b = path length of beam through cell (thickness of cell) in cm.

C = concentration in moles/liter.

Under suitable conditions the Beer-Lambert law can be used for simultaneous determinations involving two or more absorbing substances. Provided that the components do not react or affect the light absorbing properties of one another in any other way, the absorption of light by several components is additive. Then at wavelength λ :

$$A_{\lambda} = A_{1\lambda} + A_{2\lambda} + A_{3\lambda} + \dots \quad (2)$$

$$A_{\lambda} = \epsilon_{1\lambda} b C_1 + \epsilon_{2\lambda} b C_2 + \epsilon_{3\lambda} b C_3 + \dots \quad (3)$$

Where the subscripts 1, 2, and 3 refer to the absorbing components. Since the cell thickness is the same the equation reduces to,

$$A_{\lambda} = k_{1\lambda} C_1 + k_{2\lambda} C_2 + k_{3\lambda} C_3 + \dots \quad (4)$$

where:

$$k_{1\lambda} = \epsilon_{1\lambda} b \text{ etc.} \quad (5)$$

The purpose of this experiment is the simultaneous determination of the concentration of $[\text{Cr}(\text{H}_2\text{O})_6]^{+3}$ (hexaaquochromium(III)) and $[\text{Co}(\text{H}_2\text{O})_6]^{+2}$ (hexaaquocobalt(II)). For this two component mixture our equation reduces to:

$$A_{\lambda 1} = k_{\text{Cr } \lambda 1} C_{\text{Cr}} + k_{\text{Co } \lambda 1} C_{\text{Co}} \quad (6)$$

$$A_{\lambda 2} = k_{\text{Cr } \lambda 2} C_{\text{Cr}} + k_{\text{Co } \lambda 2} C_{\text{Co}} \quad (7)$$

$k_{\text{Cr } \lambda 1}$, $k_{\text{Cr } \lambda 2}$, $k_{\text{Co } \lambda 1}$ and $k_{\text{Co } \lambda 2}$ are calculated by measuring the absorbance of a pure solution of each of the complexes of interest at the two wavelengths you want to study ($\lambda 1$ and $\lambda 2$). The two remaining unknowns, C_{Co} and C_{Cr} , are determined by obtaining the

absorption spectra of the mixture at the two different wavelengths. Since we already know the values for k_{Cr} and k_{Co} at these wavelengths we have two equations with two unknowns that can be solved simultaneously.

In this experiment selection of the wavelength is quite important. The ideal situation is to use a wavelength where one of the components does not absorb at all and the other one absorbs substantially. This would reduce our simultaneous equation problem to one of substitution. Since normally the situation is not so nice then a good choice is one wavelength where most of the absorption is by one species (Co(II)) and in the other wavelength most of the absorption is by the other species (Cr(III)).

Chemicals: $Cr(NO_3)_3 \cdot 9H_2O$
 $Co(NO_3)_2 \cdot 6H_2O$
unknown mixture

Procedure:

Warm up the double-beam scanning spectrophotometer (see the operating manual for the instrument) and read the instructions for operation of the instrument thoroughly before beginning the experiment.

A. Verification of the additivity of the absorbances of $[Cr(H_2O)_6]^{+3}$ and $[Co(H_2O)_6]^{+2}$.

1. Using $Cr(NO_3)_3 \cdot 9H_2O$, prepare 50 ml each of solutions which are about 0.01, 0.02, 0.03 and 0.04 M in Cr(III).

2. Using $Co(NO_3)_2 \cdot 6H_2O$, prepare 50 ml each of solutions which are about 0.04, 0.08, 0.12 and 0.16M in Co(II).

3. Prepare 50 ml of a Cr(III)/Co(II) mixture which is about 0.02M in Cr(III) and 0.08 M in Co(II). Actual concentrations should match the 0.02M Cr(III) and the 0.08 M Co(II) solutions prepared in parts 1 and 2.

4. Determine the absorption spectra from 650 to 350 nm of the 0.02M Cr(III) solution, the 0.08 M Co(II) solution and the mixture. Record all three spectra, along with the baseline, on the same chart paper. Choose the two wavelengths to be used for the remainder of the experiment.

B. Determination of k's from Beer's Law and determination of absorbance for an unknown solution.

1. Measure the absorbance of each of the pure Cr(III) and pure Co(II) solutions at both wavelengths. Make sure that the same cell is used as the sample cell for all measurements.

2. Measure the absorbance of the Cr(III)/Co(II) unknown at both wavelengths.

Treatment of Results:

1. At 50 nm intervals on the spectral curves determine the sum of the absorbances for Cr(III) and Co(II). Compare the absorbances of the mixture. A small correction for the baseline may be needed.
2. From the data for the pure Cr(III) and Co(II) solutions determine the values for k_{Cr} and k_{Co} for both wavelengths.
3. Determine the concentrations of Cr(III) and Co(II) in the unknown.

Define:

Isobestic Point
 $n \rightarrow \pi^*$ transition
Photoelectric effect

Determination of Fluorescein in Antifreeze by Fluorescence Spectroscopy

Introduction:

Fluorescence spectroscopy may be used as both a qualitative and quantitative analytical tool. The observable excitation and emission spectra are characteristic of a given molecule and may be used to identify the fluorescing molecule. Emission spectra may also be used to quantitatively analyze fluorescent species. The intensity of radiated emission, keeping excitation and emission wavelengths constant, is proportional to the number of molecules in the excited state relaxing back to the ground state. This in turn is proportional to concentration of molecules in solution,

$$I_f = kc$$

Where I_f is the intensity of the emission radiation, k is a proportionality constant for that particular cell and fluorescing species and c is the concentration of the analyte in solution.

Fluorescein has a high quantum yield (ca. 0.85) such that low concentrations yield intense fluorescence. This fluorescence has a slight pH dependence within the range from pH 5 to 11. Use of a buffer, such as 0.05 M disodium hydrogen phosphate (pH 9), insures constant emission efficiency.

In this experiment you will become familiar with various aspects of fluorescence spectroscopy. You will also determine the concentration of fluorescein in commercial antifreeze and an unknown solution.

Chemicals: fluorescein, disodium salt (uranine)
0.1 N NaOH
HCl
NaBr
Prestone antifreeze
unknown solution

Procedure:

Warm up the Jobin Yvon Horiba FluoroMax-3, FLUORESCENCE SPECTROMETER (see the operating manual for the instrument) and read the instructions for operation of the instrument thoroughly before beginning the experiment.

Prepare a set of 4 solutions (50 ml each) varying in concentration from 1×10^{-6} to 0.5×10^{-7} M (1×10^{-6} , 0.5×10^{-6} , 1×10^{-7} , and 0.5×10^{-7} M recommended) fluorescein in 0.1 M NaOH (perform your dilutions with 0.1 M NaOH or to add enough NaOH at the end to make the solution 0.1 M). Concentrated solutions are stable in the refrigerator. Dilutions must be made and used in the same day, within 10 h. All solutions must be kept in the dark because they are photosensitive.

Using the 1×10^{-7} M dilution, obtain the excitation spectrum of fluorescein from 300 - 700 nm (select 510 nm as emission wavelength). Print out the spectrum.

Using the same solution, obtain the emission spectrum of fluorescein from 300 - 700nm (select 325 nm as excitation wavelength). Print out the spectrum.

Calibration Curve: Obtain intensity measurements on each solution, including a blank, after setting both excitation and emission monochromators to their respective maxima.

ANTIFREEZE AND UNKNOWN ANALYSIS: Obtain intensity measurements on a sample of commercial antifreeze and an unknown solution, making appropriate dilutions to insure you are within your working curve. Determine the concentration, including confidence limits, of fluorescein in (1) the antifreeze and (2) your unknown solution using the working curve constructed from the standard solutions.

pH DEPENDANCE: Prepare a solution with a concentration of 1×10^{-6} fluorescein but which is 0.1 M in hydrochloric acid. Obtain the excitation and emission spectra of this solution. Compare the spectra obtained for this solution to that obtained for the same solution concentration that was 0.1 M in NaOH. Explain the results in your report.

EFFECT OF HEAVY ATOMS: Prepare a solution with a concentration of 1×10^{-6} fluorescein but which is both 0.1 M NaOH and 0.1 M NaBr. Compare the observed intensity to that of the identical solution without Br⁻. Explain the results in your report.

In your report you should address the following questions:

-Is your calibration curve linear?. If not, explain why.

-What is the effect of pH on fluorescence intensity and wavelength maxima for fluorescein?

-What is the effect of heavy atoms on fluorescence intensity and wavelength maxima for fluorescein?

-What is the purpose of fluorescein in antifreeze?

-Explain the emission and excitation spectra. Why is the emission wavelength of fluorescein longer than its excitation wavelength?

-Define:

Resonance fluorescence
Intersystem Crossing
Stokes Shift

Resolution and Analysis of Hydrocarbons in Fuel Mixtures by Gas-Liquid Chromatography

Introduction:

Gas-liquid chromatography (GLC) is the most widely used technique for the separation, qualitative analysis, and quantitative determination of volatile organic compounds. Separation is achieved by selective partition of analytes between a mobile phase (helium) and a liquid stationary phase (column coating) contained in an open tubular capillary column. Qualitative identification of components in a mixture by GC is usually based on retention time, Rt , which is the time required to elute half the solute from the column, or the relative retention time, RRt , defined by,

$$RRt = \frac{Rt}{Rs}$$

where, Rs is the retention time for an reference standard compound added to the sample.

In order to successfully separate a mixture, the peaks for the individual components must be resolved from each other. The extent of separation of two adjacent, approximately gaussian peaks, can be expressed by the resolution, S , of the chromatographic system by,

$$S = \frac{Rt_2 - Rt_1}{0.5(W_1 + W_2)}$$

where, Rt_1 and Rt_2 are the retention times, and W_1 and W_2 are the peak widths (the magnitudes of the base of the triangles) of the first and last of adjacently eluting peak, respectively.

A pair of peaks are considered fully resolved when $S = 1.5$, but for practical purposes, $S = 1$ (ca. 98% separation), is usually more than adequate.

The qualitative identification of unknown peaks in a chromatogram can be simplified by using the fact that for compounds in the same family (e.g. n-paraffins or n-alkanes), a plot of the log of the adjusted retention ($\log RRt$) time versus number of carbon atoms (homologues series plot) should yield a straight line under isothermal conditions.

The area under the peaks is directly related to the analyte concentration. Therefore, quantitation of the separated components is achieved by integrating the areas of each peak. Assuming a gaussian distribution, for some applications, the peak height can also be related to the concentration of the eluting compound.

Chemicals: Methylene Chloride
Unleaded gasoline
Diesel fuel
Lighting Fluid
Unknown sample

Procedure:

- (1) Set the flow rate of the chromatograph as specified by the instructor or as determined by a van Deemter plot for one of the hydrocarbons. Familiarize yourself with the GC and its operation.
- (2) Obtain individual chromatograms of the diluted fuel samples.
- (3) Obtain a chromatogram of your unknown sample.

In your report you should address the following:

- Construct plots of log relative retention time (RRt) versus carbon number and retention time (Rt) versus carbon number from the chromatograms of the homologue series you identified in diesel fuel. Which is the best method and why?
 - From the relative retention times of the components in the unknown sample identify as many peaks as possible referring to the log RRt plots from the known fuel chromatograms.
 - Calculate the resolution of three pairs of adjacent peaks ranging from barely resolved to totally resolved.
 - Identify your unknown. Note that your unknown may be a mixture of up to 3 different fuels.
 - Go and do some research and determine the "octane rating" of the unleaded gasoline (state it on the final report!).
 - How do unleaded gasoline, diesel fuel, and lighting fluid oil differ from each other?
- Define:

Signal to noise ratio of 10
Distribution constant (Kc)
Kovats Index (I)

Analysis of an Unknown Mixture by Full Scan GC/MS

Introduction:

Mass Spectrometry is one of the fastest growing instrumental techniques. At the simplest level a mass spectrometer is simply a very sensitive balance that makes it possible to "weigh" molecules. In addition to determining the mass, the fragmentation patterns are used to determine the structure of an unknown compound.

Mass spectrometers actually measure the mass to charge ratio (m/z) of an ion. During ionization many molecules undergo fragmentation. The mass spectrum is produced the different probabilities for the formation of each fragment ion. This provides a structural fingerprint of the molecule.

In this experiment you will use a Hewlett Packard 5970/1 single quadrupole mass selective detector with a Chemstation data system to identify several unknown organic compounds.

The GC/MS instrument represents a device that separates chemical mixtures (the GC component) and a very sensitive detector (the MS component) with a data collector (the Chemstation computer). Once the sample solution is introduced into the GC inlet it is vaporized immediately because of the high temperature (250 degrees C) and swept onto the column by the carrier gas (usually Helium).

The sample flows through the column experiencing the normal separation processes. As the various sample components emerge from the column opening, they flow into the capillary column interface. This device is the connection between the GC column and the MS. Some interfaces are separators and concentrate the sample via removal of the helium carrier.

The sample then enters the ionization chamber. The most frequently used is electron impact (EI). Another occasionally used alternative is chemical ionization (CI). For electron impact ionization a collimated beam of electrons impact the sample molecules causing the loss of an electron from the molecule. A molecule with one electron missing is represented by M^+ and is called the molecular ion (or parent ion). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Chemical ionization begins with ionization of methane (or other gas), creating a radical which in turn will impact the sample molecule to produce $M+H^+$ molecular ions.

Some of the molecular ions fragment into smaller daughter ions and neutral fragments. Both positive and negative ions are formed but only positively charged species will be detected.

Less fragmentation occurs with CI than with EI, hence CI yields less information about the detailed structure of a molecule, but does yield the molecular ion; sometimes the molecular ion can not be detected by the EI method, hence the two methods complement one another.

Once ionized, a small positive potential is used to repel the positive ions out of the ionization chamber.

The next component is a mass analyzer (filter), which separates the positively charged particles according to their mass. Several types of separating techniques exist; quadrupole filters, ion traps, magnetic deflection, time-of-flight, radio frequency, cyclotron resonance and focusing to name a few. The most common are quadrupoles and ion traps.

After the ions are separated according to their masses, they enter a detector and then on to an amplifier to boost the signal.

The detector sends information to the computer, which acts as a "clearing house". It records all the data produced, converts the electrical impulses into visual displays and hard copy displays. The computer also drives the mass spectrometer.

Identification of a compound based on its mass spectrum relies on the fact that every compound has a unique fragmentation pattern. Even isomers can be differentiated by an experienced operator. Generally, more information is

generated than could possibly be used. A library of known mass spectra, which may be several thousand compounds in size, is stored on the computer and may be searched using computer algorithms to identify the unknown. It is important to incorporate all other available structural information (chemical, spectral, sample history) into the interpretation wherever appropriate.

The ultimate goal is accurate identification of a compound, which can be facilitated by the utilization of the GC/MS.

Experimental:

1. Review parts of the instrument

- a. Inlet
- b. Source region
- c. Mass analyzer
- d. Vacuum system
- e. Electronics cabinet
- f. Data system

2. Setting up the instrument for operation

- a. Setting the ionization mode
- b. Setting the Electron Multiplier (EM) voltage
- c. Turn on the electron beam filament

3. Calibration of the mass spectrometer.

The mass axis of the spectrometer is calibrated with perfluoro-tributyl-amine, (n-C₄F₉)₃N, abbreviated PFTBA as follows.

- a. Open the calibration valve to let PFTBA into the instrument.
- b. Start the calibration software.
- c. Observe the PFTBA signal to check instrument performance.
- d. Run the manual or automatic calibration sequence.
- e. Close the calibration window, saving calibration if necessary.

4. Setting up the experiment

- a. Open the data acquisition program
- b. Open a method and Check/Set the following parameters c
- c. Select the scan type
- d. Set the GC temperatures
- e. Save method to mass spectrometer
- f. Start run (At the same time you inject or load your sample).
- g. Open the data analysis program to observe data collection during sample run.

5. Data to collect. For this experiment you will identify a set of unknown compounds. After the instrument is setup you will inject your unknown mixture and determine the chemical composition of the unknowns by doing a library search and interpreting the mass fragmentogram an internal standard will be added to your sample so you can obtain quantitative results.

Laboratory Write-up:

Your Laboratory write-up should include the following information

1. Clearly labeled and annotated graphs that show the following:
 - a. Total ion chromatograms for your sample run.
 - b. The mass spectrum of each unknown.
 - c. A library search report.

For each of these unknowns:

- i. Report the results of the library search
- ii. Determine which search hit matches your unknown
- iii. Interpret the fragmentation patterns observed.
- iv. Look up unknowns in the Merck index and report their potential toxicity.
- v. Locate a MSDS of the unknowns and include it with your report (I suggest using internet for this).
- vi. If you have an unknown with several possible structures.

First identify the possible structures.

Then print out the mass spectra for these compounds using the GC/MS library.

Compare these spectra with your unknown to narrow the possible structures.

2. Assuming the first unknown peak in your sample is your internal standard and its concentration is 100 ng/ μ l calculate the concentration of all other unknown peaks. Assume that you extracted 50 g of a sediment sample, the volume of your extract is 1 ml and the response factor for all analytes is 1. Calculate the concentrations of the unknowns in sediment. Do your own research for this before asking me.

Please address the following questions:

1. What type of ionization did you use to analyze your sample? How does it work?
2. Name three other ionization techniques used for mass spectrometry analysis. Briefly describe their operating principle.
3. What type of mass analyzer did you use? What are its limitations?
4. Define:
 - NCI (Negative chemical ionization)
 - Selected ion Monitoring (SIM)
 - Precursor Ion

Determination Of Caffeine In Beverages By High-performance Liquid Chromatography (HPLC)**Introduction**

Reversed-phase HPLC will be used to determine the concentration of caffeine in coffee, tea, Coca-Cola and Pepsi-Cola products. The traditional method for the determination of caffeine is via solvent extraction followed by spectrophotometric quantitation. HPLC allows for the rapid separation and quantitation of caffeine from the many other substances found in these beverages including tannic acid, caffeic acid and sucrose. Retention time, t_R is used as a qualitative measure and peak area as a quantitative measure for external standard determination.

Instrumentation: HPLC System with Reversed-phase (i.e. C18) column and UV detector

Chemicals: Caffeine
1 L 50:50 Methanol:Water solution (volume %)
Freshly Brewed Coffee (bring your own from home or cafeteria)
Bottled Unsweetened Tea (bring your own)
Diet Coke (we will provide)
Diet Pepsi (we will provide)
Unknown

Procedure: Make sure you understand the operation of the HPLC before proceeding.

1. Prepare 1 L of a 50:50 Methanol:Water solution (volume %) adjusted to approximately pH 3.5 to be used as your HPLC mobile phase.
2. Accurately prepare five 50 ml standard solutions varying in concentration from 0.5 to 10 ppm using your prepared mobile phase for dilution.
3. Brew selected coffee in coffee maker. Add 0.5 ml of coffee into a 50 ml volumetric flask and dilute to the mark with your prepared HPLC mobile phase. Add 0.5 ml of the bottled tea into another 50 ml volumetric flask and dilute to the mark with your prepared HPLC mobile phase. Finally, dilute 0.5 ml each of previously degassed Coca-Cola and Pepsi-Cola into two separate 50 ml volumetric flasks using your prepared HPLC mobile phase.
4. Turn on the HPLC, detector and computer and allow the prepared mobile phase to pass through the column for at least 10 minutes. Make sure the baseline is stable before injecting your standards and unknowns. Set wavelength to 276 nm and flow rate to 1 ml/min.
5. Introduce at least 20 μ l of the least concentrated standard through the 20 μ l rotary injection valve while in the LOAD position.
6. Turn the valve to the INJECT position and simultaneously begin the computer integration.

7. Allow the peak due to the caffeine to be recorded and repeat this procedure for your other standards as well as your unknowns.

In your report you should address the following:

1. In tabular form record the identity of each sample including the concentrations of your standards, retention times, adjusted retention times, peak areas and peak heights.
3. Explain the rationale for using a reversed-phase C18 column for the determination of caffeine.
4. What was the purpose of adjusting the mobile phase pH to 3.5?
5. Why was 276 nm chosen for the UV detector?
6. Could an ion-exchange column be used for the determination of caffeine? Explain.
7. Compare the caffeine concentration of each sample studied and compare to expected values (you will need to call the bottling company to find out, use their 1-800 number on the can/bottle).
8. Why diet Pepsi and Cola were selected for this experiment?
9. Define:

Peak-width at Half height (w_h)
Dead-Volume
Ion-exclusion Chromatography

OPERATING INSTRUCTIONS FOR INSTRUMENTS

***** VERY IMPORTANT! *****

The instruments you are using for this laboratory are delicate and expensive pieces of equipment. Make sure you have read the operating instructions for the instruments **before** starting any experiments. If you are unsure of what you are doing please ask your instructor. Be careful to keep the instrument clean and dry. Avoid any spills on or near the instrument. Prep all samples in the Analytical Chemistry laboratory and bring them to the instrument for analysis. **DO NOT** prep samples next to the instruments. **If an accident occurs or a problem with an instrument develops notify your instructor immediately.**

Abbreviated Instructions for PerkinElmer AAnalyst600

Atomic Absorption Spectrometer

Getting Started

- Turn on Argon Gas
- Turn on Spectrometer
- Open Software WinLab32 For AA
 - This will start the initialization procedure for the machine, which takes approximately three minutes to complete. If an error message appears on the CRT screen during this time, notify your instructor.

Selection of Operating Conditions

- Method
 - From File, open an existing Method (for example, CHM4130L-Se)
 - Set up Calibration curve
 - Set up Furnace program
 - Check all other parameters and change if necessary
 - Save Method using a unique name for your experiment (e.g. 4130-05)

- Sample Information
 - From File, open an existing Sample Information file (e.g., CHM4130L-Se)
 - Set up sample log-table, sequence.
 - Check all other parameters and change if necessary
 - Save Sample information file using a unique name for your experiment (e.g. 4130-05)

-
- Workspace
 - From File, open an existing Workspace file (e.g., CHM4130L-Se). This will allow you to see on the CRT the Calibration curve, Results, and Peak display.
 - Check all other parameters and change if necessary

-
- Furnace checking
 - Click Furnace icon on the top of screen
 - Select Open/Close to change graphite sample cup
 - Select Align Tip to adjust tip position
 - Select Condition tube if necessary
 - Check all other parameters and change if necessary

-
- Lamp Selection
 - Click Lamp icon on the top of screen
 - Select right elemental Lamp
 - The Lamp should be turned and pass the test, if not notify the Instructor.
 - Close the Lamp window before analyzing samples

-
-
- Pop up parameters (a quick configuration, similar to previous parameter selection)
-

Analyzing Samples

- Load your samples on the auto-sampler according to the Sample Information file
-
- Using Auto Run
 - Select Auto run on the top of the screen
 - Select Set Up
 - Check Method file
 - Load Sample Information file
 - Enter Results Data Set Name (To save your data!!!!) using a unique file name for your experiment
 - Check “Save Data” Square
 - Click Analyze
 - Select Analyze All, Calibration Standards, or Sample only
 - Check other options
 -
- Run samples
 - Remove the blank from front compartment (leave the blank in the back compartment)
 - Place sample in cuvette (see Miscellaneous below for cuvette notes)
 - Place sample cuvette into front compartment
 - Close lid
 - Click Start

Shut down Procedure

Turn the power OFF. Turn argon gas off.

Abbreviated Instructions for Shimadzu UV-2101PC

UV-VIS SPECTROPHOTOMETER

Getting Started

- UV switch ON
 - Switch at bottom front right of instrument
 - Will see a light from the back, left side
 - Let lamp(s) warm up for about 30 min. (no more than 1 hr.)
- Cuvettes
 - Blank goes in back compartment
 - Sample goes in front
- Open UVPC software
 - This will start the initialization procedure for the machine, which takes approximately three minutes to complete. If an error message appears on the CRT screen during this time, turn the machine OFF for a few seconds, then back on ON. If the error message appears again, notify your instructor.

Selection of Operating Conditions

- Acquire mode
 - Choose Spectrum
 - Quantitative is another option
- Configurations - from Parameters toolbar choose:
 - Measuring Mode: Abs
 - Recording range: Abs scale (0 - 2.5 or other appropriate range)
 - Wavelength range: 250 - 600nm
 - Scan speed: med (or fast) only
 - Slit width: set at 1
 - Sampling interval: auto
 - Click OK
- Pop up parameters (a quick configuration, similar to previous parameter selection)
-

Acquire Sample Spectra

- Run a baseline scan before analyzing the sample
 - Place a solvent blank into each of two compartments
 - Click Auto Zero
 - Click Baseline
 - Note that the baseline should be run using the same parameter settings as to be used for the measurement of a spectrum
- Run samples
 - Remove the blank from front compartment (leave the blank in the back compartment)
 - Place sample in cuvette (see Miscellaneous below for cuvette notes)
 - Place sample cuvette into front compartment
 - Close lid
 - Click Start

Interpreting your Data

- After the analysis
 - Name your spectrum file using a unique file name
 - Manipulate (to play with peaks)
 - Peak pick
 - Click mouse on desired peak (computer will label and number the peaks)
- Save your data
 - Create a unique folder
 - Place data file in the folder
- Print and Save spectra as they are obtained

Miscellaneous Notes

- To erase channels: Make sure files are saved first, then Click File, Channels, Erase
- When doing scan, to obtain the Abs at a particular wavelength: Data print, then Parameters and enter desired wavelength
- Acquire mode (spectrum for single run/sample, quantitative for standards)
- Leave blank in back compartment when analyzing sample
- Before analyzing, absorbance reading at lower right of screen should be about zero
- Condition sample cuvette (rinse with solution to be analyzed)
- Wipe cuvettes with a Kimwipe before each analysis to get rid of any smudge marks
- Make sure there are no bubbles or particles in solution

Shut down Procedure

Turn the power OFF. Remove and clean the sample and reference cuvettes.

Abbreviated Instructions for Jobin Yvon Horiba FluoroMax-3

FLUORESCENCE SPECTROMETER

Getting Started

- Turn on instrument
 - Switch on the Instrument at back right of instrument
 - Will see a light from the top left side
 - Let lamp(s) warm up for about 10 min.
 - Turn on Computer
- Open software
 - Go to Program
 - Find Data Max-32
 - Instrumental Control Center
 - Click Run Experiment
 - Allow Instrument Initiates

Selection of Operating Conditions

- For Excitation Acquisition Mode (excitation wavelength scan while fixing emission wavelength)
 - Click Collect
 - Select Experiment
 - Select Experimental file from Experiment... window
 - Select 4130Lexc.exp
 - Check all parameters (Scan range: 300-700 nm; Emission: 510 nm; Integration time: 0.05 sec; Increment: 1; Signal: S; Slit: 3.0)
 - Select Data File to save your data before run!
 - Save Experimental file
 - Click Run
 -
- For Emission Acquisition Mode (emission scan while fixing excitation wavelength, acquisition of fluorescence spectrum)
 - Click Collect
 - Select Experiment
 - Select Experimental file from Experiment... window
 - Select 4130LFL.exp
 - Check all parameters (Scan range: 300-700 nm; Excitation: 325 nm; Integration time: 0.05 sec; Increment: 0.5; Signal: S; Slit: 3.0)
 - Select Data File to save your data before run!
 - Save Experimental file
 - Click Run
 -
- Record and print results
 - After the spectrum is done, left click the peak of interest
 - Go to Peaks
 - Select Insert Peak
 - Record the peak wavelength and intensity
 - If you want or print the peak records, then
 - Go to Peaks
 - Select Print Peak Report

-
- Print spectrum
 - Go to File
 - Click Print

Shut down Procedure

Turn the power OFF. Remove and clean the sample and cuvettes.

Abbreviated Instructions for the Perkin Elmer LS 50 LUMINESCENCE SPECTROMETER

Start-On: Switch the instrument (at the left side), the computer, printer and screen on. At the C> ; type FLDM. (The program will be loaded)

Loading the sample: Insert your sample (in a fluorescence cuvette) in the rack's number 1 position and close the lit.

Running the spectrum: With the mouse pointer highlight the word **INSTRUMENT**. This will display the menu. Within the menu highlight the word **SCAN** and click the left button of the mouse to activate the routine. When the program is displayed, select the desire experiment by clicking the pointer in the corresponding box.

EMISSION: Select the excitation wavelength by clicking the mouse on the corresponding box. **IMPORTANT** : Don't press the return key until you finish to set up all the parameters. The return key activates the scan mode.

Emission parameters:

Emission range: 200 - 800 nm

Ex. Slit range: 2.5 - 10

Em. Slit range: 2.5 - 10

Scan Speed range: 240 - 1000 (500 is a good value)

To run the scan: click at **OK** or press return. The spectrum will be display in the screen.

EXCITATION: Select the emission wavelength (same as fluorescence wavelength).

Excitation parameters:

Excitation range: 200 - 900 nm

Ex. Slit range: 2.5 - 10

Em. Slit range: 2.5 - 10

Scan Speed range: 240 - 1000 (500 is a good value)

If peak saturation problems arise, change the emission filters. To do that, select the **EMISSION OPTION** subroutine, inside the **INSTRUMENT** menu. Once here, select **THE ATTEN** parameter and exit by clicking at **OK**. Run your sample again.

Manipulation of Spectral Data: Once the spectrum is obtained, the **VIEW** menu is available.

To change the axes scale: It can be done automatically or manually. The automatic subroutine has the advantage of giving the actual peak intensity as its maximum value. For automatic rescaling, select **AUTO** inside the view menu. Once the scale has been changed, select **SCALE AXES** inside the **VIEW** menu. The peak intensity value will show as the y-axis.

For manual scaling, select **SCALE AXES** inside the **VIEW** menu.

To Print: Select **PRINT WINDOW** inside the **FILE** menu. This will give you a copy of the spectrum shown in the screen.

Shut down Procedure: Select **QUIT** inside the **FILE** menu. Switch everything off and remove your sample. Remember to leave the cuvette clean.

Your Name _____ (will **not** be disclosed to speaker)

~~✂~~ ✂ will be cut off before returning to speaker ~~✂~~ ✂ will be cut off before returning to speaker ~~✂~~ ✂

CHM-4993L Instrumental Analysis Symposium Series 2002

SPEAKER EVALUATION FORM

Name of Speaker: _____

Paper Topic: _____

1. **Topic Choice and Relevance** : Excellent Very Good Good Average Poor

(Did the topic inspire your attention? Was it adequate for a "Mass Spectrometry Class"?)

2. **Speaker preparation**: Excellent Very Good Good Average Poor

(Enough knowledge about subject? Speaker thoroughly investigated the topic?)

3. **Scientific merit of topic** : Excellent Very Good Good Average Poor

(Is this a useful technique/application? What are the limitations?)

4. **Presentation quality** : Excellent Very Good Good Average Poor

(Good clear presentation? Timing ok? Too many numbers in a table?)

5. **Answering questions** : Excellent Very Good Good Average Poor

(Did the speaker answer questions to your satisfaction?)

6. **Overall rating** : Excellent Very Good Good Average Poor

(Compared with other seminars you have attended)

7. **Your comments to the speaker**: Review from your colleagues is the most effective way to improve so, "*please*" give the speaker some feedback!

Each of the answers will have a number of points associated with it Excellent = 4 points, Poor = 0 points. Student evaluations will count for your final grade; after all "*they are your audience too*".