CHEM 4553 Instrumental Analysis
Fall 2004

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REFERENCE BOOKS

There are many good ones, a few are listed below:

a) *Principles of Instrumental Analysis* (Fifth Edition)
Skoog, Holler and Nieman

b) *Instrumental Methods of Analysis* (Seventh Edition)
Willard, Merritt, Dean and Settle

c) *Undergraduate Instrumental Analysis* (Fourth Edition)
Robinson

d) *Contemporary Instrumental Analysis*
Rubinson and Rubinson

HELPFUL WRITING BOOKS

Elements of Style, Strunk and White
ACS Style Guide, Dodd, Editor

CLASS FORMAT

This is an instrumentally intensive course designed to give students hands-on experience with chemical instrumentation. The course will also focus on preparing the student to do chemical research in various laboratory settings.

EVALUATION

Laboratory Notebook	25%
Project Report	30%
Proposal	25%
Written Final	20%

Scale	Letter Grade
90-100	A
80-89	B
70-79	C
60-69	D
<60	F

Graduate and undergraduate students will be graded on the same scale.

Remember, you will use very expensive and sophisticated instruments in this laboratory. You must always treat the instruments properly. ***Abuse of equipment will result in failure of the course.***

ATTENDANCE

DURING CORE EXPERIMENTS. Roll will be taken at 1:40 pm each day. If you are not here at 1:40 pm, you will be marked absent and receive a zero for the laboratory notebook report. If absent, you are forbidden to copy your partner's data and turn in a report. Doing so will result in disciplinary action.

DURING PROJECT EXPERIMENTS. You are expected to be in the laboratory at the scheduled time. If you have to go off campus, you must check in with your Teaching Assistant or me before leaving.

CORE LABORATORY EXPERIMENTS

Both core and project experiments will be carried out during the semester. The core experiments will provide experience with some of the typical instruments used by professional chemists. The core experiments at the beginning of the semester will help you to establish basic skills necessary for the project experiments. During the core laboratories, you will learn also how to properly maintain a notebook.

Below is a brief summary of experiments that will be performed.

Experiment 1

Gas Chromatography/Mass Spectrometry of a Complex Mixture

Experiment 2

Structure Elucidation using Fourier Transform Infrared Spectroscopy

Experiment 3

Flame Atomic Absorption of Metal Ions in Food

Experiment 4

Fluorescence Determination of Pharmaceutical Components in a Mixture

Experiment 5

High Performance Liquid Chromatographic Separation of Hydrocarbons

PROJECT EXPERIMENTS

The major emphasis of this course is to get you to become an independent thinker so that you may assess a chemical problem, propose a hypothesis to solve the problem, consult the literature to learn how to carry out experiments that will allow you to prove or disprove your hypothesis, perform experiments carefully, successfully, and safely, and evaluate the results of experiments.

In order to facilitate such training, you will be able to choose the type of project that you would pursue for the project experiments. The list of topics for projects below was derived from reading the newspaper, Chemical and Engineering News, and with discussions with colleagues. You probably have had many questions regarding some chemical topic that is of an analytical nature during your life. This is an opportunity to pursue such a question.

In addition to the topics below, we are open to suggestions from you. However, your ideas must not be too grandiose due to the limited resources that we have available in the lab and the time which you, as a student, can expend during a given semester.

Your group will come to me and discuss the topics that you are considering for your project. Once your topic is approved, you will write a five (5) page proposal (not including bibliography) that will outline your proposed project experiments. ***These proposals will be due in early October***, giving you about six weeks to construct the proposal. It is of utmost importance that you get going on this proposal from day one of the course, for the proposal will account for 25% of your grade !

Possible Topics for 4553 Analytical Projects

1. Drugs and US Currency
2. Plasticizers, Antibacterials, and Babies
3. Analysis of Water and TV Specials
4. Herbicides, PCBs, and Bodies of Water near the Capitol
5. Pesticides, "Veggie-wash" and Produce
6. Metals, Pots, Pans and Crystalware
7. Odor removers and You
8. Fabrics, Plastics and Forensics
9. Rainwater and your Hair; No need to remove the Gray!
10. Cracking catalysts and Failure
11. Artwork, Pottery and Non-destructive Analyses
12. Herbal remedies and You
13. Heterocyclic Amines and Hamburgers

To give you some feeling for how the course will work, I outline below the general flow of the laboratory:

Flow of the Course

1. Introduction with Syllabus, Group Assignments
2. A. Core Experiments
   - i. FAAS
   - ii. IR
   - iii. GC/MS
   - iv. HPLC
   - v. Fluorescence

   B. Library Research

   C. Writing of Proposal
3. Discussion of Independent proposal with "Agency Chairman"
4. Implementation of Proposed Plan
5. A. Written Research Report

   B. Oral Research Presentation in Class

LABORATORY PARTNERS

Each experiment will be done in pairs. Your laboratory partner will be assigned to you the first day of class. Keep in mind, you will perform the experiment and collect data in pairs. BUT, YOU MUST WRITE UP YOUR LABORATORY REPORT INDIVIDUALLY. If you do not write your lab reports individually, I will report you to

the Dean of Students Office and your case will be dealt with according to the LSU Code of Student Conduct.

PRE-ARRIVAL AND DEPARTURE PREPARATION

Each laboratory group will be prepared to do the experiment upon arrival. You will review the material contained in the 4553 Laboratory Manual, appropriate reference books and primary literature sources that you obtain yourself, and the specific instructions on operation procedures for the instrument to be used *before the scheduled time* of the laboratory. This will be reviewed during a "check-in" time. In addition, your notebook and your work area must be inspected before departure during a "check-out time." Failure to participate in the check-in and check-out procedures will result in a grade of zero for the particular core laboratory write-up, or loss of credit on your project report.

THE LABORATORY NOTEBOOK:

1. All experimental procedures and data must be neatly and concisely recorded into a scientific notebook. I suggest the National Brand 43-581 (or equivalent) that is sold at The LSU Union Bookstore - it is a thin blue notebook. You must use a black, permanent ink, ball-point pen. Do not record data on loose pieces of paper. <u>You must have a Table of Contents in your notebook; allow 4 pages for this at the beginning of the notebook.</u>

If you wish, one partner may make the measurements and the other record the data into their notebook in tabular form. After the experiment is completed, the other partner must transcribe the data into his/her notebook. This does not mean that one person does all the work and the other merely copies the data into the notebook. You both will be expected to be adept at experimental procedures.

2. Spectra, chromatograms, and other data obtained from printouts from equipment or computers must be affixed in the notebook using glue. Keep in mind, the ability to write a good report will depend upon how well you organize data in the notebook. The teaching assistants and professor will check your notebook to make sure data are being entered properly and that your notebook is what we expect for professional work. ***Failure to maintain a proper notebook will result in serious consequences.***

3. Unknowns will be given to you for some core experiments. Make sure when you are given an unknown that you record the identifying number in your notebook and clearly state the unknown number on the front page of the laboratory report. ***Failure to do so in either case will result in a grade of zero for the laboratory report.***

4. You must have written in your laboratory notebook, at the beginning of the laboratory period, a procedure for the laboratory experiment of the day that entails how you will do the experiment. You will also have data tables set up for recording purposes. The procedure should be detailed enough to allow you to carry out the procedure without referring to the 4553 Laboratory Manual or other literature. <u>In addition, you must obtain information from a materials safety data sheet (MSDS) for the chemicals that you will use in the experiment from sources such as the internet or the</u>

chemical library. Any special handling techniques, precautions, incompatibilities, or waste disposal issues must be noted in the procedure. Any additional questions you have regarding the procedure must be directed to the teaching assistants before the laboratory period. You must also have written out in your notebook procedure the chemical structures of all chemicals to be used in the experiment, noting their chemical characteristics that you might need for the experiment.

The teaching assistants will check everyone's notebooks at the beginning of the class period for the procedure. **If it is not present or is incomplete, you will receive a grade of zero for the laboratory report and will be sent away.** We will call this checking of notebooks the "check-in."

5. At the end of the laboratory period, you and your partner must find the teaching assistant and have them sign and date your notebook at the point you stopped for that period. Data acquired manually must be recorded in the notebook. This will ensure your keeping up the notebook on a daily basis and should help you obtain a good grade for the notebook portion of your course grade. In addition, your work area will be checked to make sure that you have it returned to its original state. **If you do not have your notebook "checked out", you will receive a zero for the laboratory report.**

SAFETY

Wear safety glasses at all times. You are not permitted to wear contact lenses in this laboratory. If you have eyeglasses, you may wear them instead of safety glasses if they conform to good laboratory practice protocols. If you are found without protective eyewear three times during the semester, you will receive a grade of F for the entire course. **No open-toe shoes, shorts, or tank tops are allowed.** Also, no eating, drinking, or smoking are allowed in the laboratory at any time - this includes chewing gum and the use of smokeless or regular tobacco products. Never work in the laboratory unless a TA or professor is present. You may not "come and go" either - no smoking breaks are allowed! Violation of any of these rules will result in serious consequences that will be decided on a case-by-case basis.

LIBRARY RESOURCES

Due to the heavy requirement for library work associated with this laboratory, you must be familiar with the Sadtler index, Chemical Abstracts, and other reference material. There are a number of journals which publish general reviews periodically pertaining to specific topics in chemistry. A good example is the Fundamental Reviews in Analytical Chemistry which is published every two years. Keep in mind that the library staff can be of great assistance in helping you find what you need.

LABORATORY NOTEBOOKS FOR CHEM 4553

The following sections contain the breakdown of points (for the Core labs) in the laboratory write-up in the notebook and the format of the write-up. Also included are important points and concepts that should be included in each section of the write-up. You must adhere to the following format. Any deviation from this format will result in loss of points for the write-up preparer. **All sections must be included in the write-up or serious consequences will result.**

FORMAT

General Writing Aspects

You are expected to write in a fashion that demonstrates your scientific and literary skills. You will be graded accordingly. Thus, it will behoove you to consult *The ACS Style Guide, The Elements of Style,* and a dictionary as needed. Proper referencing in ACS format is required.

1. General Information (Mandatory)

Title of experiment
Date experiment started (As noted by teaching assistant)
Date experiment completed (As noted by teaching assistant)
Date write-up completed
Unknown Identification Number

2. Purpose/Introduction/Theory (25 points)

The student should state the specific experimental objective (purpose) in performing this experiment - what is to be accomplished or determined. The theory should also be developed in this section of the laboratory along with the appropriate background and equations. *For the theory, remember to include all relevant equations and formulae.* Many times, in a particular laboratory, there will be specific points that should be covered in this section of the report. Make sure that you address each of these in your write-up. Also, include footnote references for all of the reference material that you used to write this section of the write-up and the remainder of the write-up.

Keep in mind that this is an instrumental analysis course. As such, one section of the introduction should deal with the *particular* instrument (type, model) that will be used for that experiment. You should include a block diagram of the instrument that will be used, highlighting the important components. *Also, you must explain the function of each of these components.* This is to demonstrate that you understand how the instrument works. Again, the use of one of the reference books will help you accomplish this.

3. Experimental Procedure (10 points)

This section should contain a summary of procedures, descriptions of experimental approach, sample preparation (dilutions, pH, etc.) and experimental conditions. You must include the instrument type and the settings/conditions that were set on the instrument to perform the measurements. Check out ahead of time (previous lab period) the equipment that you will use for the write-up at hand.

4. Data and Results (30 points)

In this section, all data must be presented neatly and organized in tabular form. Data should be easy to find and organized in such a manner that the reader can follow what was done in order to obtain this data. Make sure that each column of a table is clearly identified. Also, make sure that all units are included.

Graphs and spectra should also be included in this section as well. All graphs and spectra should be titled so that it can be determined what each represents. For the graphs, all axes should be clearly labeled with names and units. Remember, all graphs should be created by a computer and not done by hand. ALL figures must be identified with numbers or letters as referred to in the text.

Also, in this section you must give one sample calculation from start to finish for your analysis. Be sure to indicate all of the units that were used in the calculation along the way. All of your final numerical answers should be reported as the mean with confidence intervals at the 95% level. If you feel that some of your data was bad, you must fully describe what happened during the course of obtaining the data and why this makes the data suspicious. Then, and only then, may you perform the appropriate statistical tests to evaluate if it is appropriate to remove suspicious data. You must then report your values as described immediately above.

5. Discussion and Analysis of Results and Questions (35 points)

Discuss the meaning of your data and what the data implies, drawing any conclusions that can be based on a critical evaluation of the experimental results. Give as much evidence as possible for any conclusions drawn. You should also make a statement on the overall quality of the results obtained and briefly discuss the precision of the experimental value and the most probable sources of errors. Also, suggest steps that can be taken to improve the reliability of the data and results. Answer the objective to the extent possible and summarize important conclusions previously discussed.

And finally, clearly give the unknown number and present your result(s) for the unknown. Explain any problems that you may have encountered when analyzing the unknown. Also, there will be specific questions for various laboratories and in this section of the report, those questions must be answered fully.

6. Bibliography

You must use a bibliographic format that is generally accepted by the chemical community in the USA - that would be the format generally accepted by the American Chemical Society. We will use that format which is used in the journal *Analytical Chemistry*, which is further outlined in The ACS Style Guide. Refer to references using their assigned number.

You are required to have **at least three references** - one can be a secondary literature reference (book) while the other two must be from the primary literature (journal article). **These references must be referred to in the text of the report as needed.**

STUDENT GROUPS:

A	Shana Williams	Blake Babcock
B	Chris LeBlanc	Nathan Henderson
C	Kim Rogers	Gina Odom

LAB SCHEDULE BY GROUP AND DAY OF SEMESTER

Date ***Group***	**8/25 W**	**8/30 M**	**9/1 W**	**9/8 W**	**9/13 M**	**9/15 W**	**9/20 M**	**9/22 W**	**9/27 M**	**9/29 W**	**10/4 M**	**10/6 W**
A	**Check In/ Safety**	**Library Training**	**#2** due 9/3 return 9/7 re-do due 9/9 return 9/10	**off**	**#3** due 9/15 return 9/17	**off**	**#4** due 9/22 return 9/24	**off**	**#5** due 9/29 return 10/1	**off**	**#1** due 10/11 return 10/13	
B	**Check In/ Safety**	**Library Training**	**#3** due 9/3 return 9/7 re-do due 9/9 return 9/10	**off**	**#4** due 9/15 return 9/17	**off**	**#2** due 9/22 return 9/24	**off**	**#1** due 9/29 return 10/1	**off**	**#5** due 10/11 return 10/13	
C	**Check In/ Safety**	**Library Training**	**#4** due 9/3 return 9/7 re-do due 9/9 return 9/10	**off**	**#2** due 9/15 return 9/17	**off**	**#3** due 9/22 return 9/24	**off**	**off**	**#1** due 10/1 return 10/4	**off**	**#5** due 10/11 return 10/13

Experiment 1

Gas Chromatography/Mass Spectrometry of a Complex Mixture

Experiment 2

Structure Elucidation Using Fourier Transform Infrared Spectroscopy

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Experiment 5

High Performance Liquid Chromatographic Separation of Hydrocarbons

Important Dates

Literature Search Training, Room 230B, Middleton, Mon., Aug. 30, 1:30 - 3:00.
Proposal target date: October 6 at 5 pm (as PDF)
Final project written report: December 1
Final project oral presentations: November 29 and December 1, 210 Choppin
Final: December 7, 210 Choppin 1:30-4:00 pm

Basic Format for Chem 4553 Proposals

1. Project Summary (Abstract) - This should tell the reader in no more than eight sentences what is the problem, approach to solving said problem, and the significance of the problem/approach.

2. Project Description

Introduction

- problem and the approach (hypothesis and expts to prove) should be outlined
    - pertinence of problem
    - background of problem
    - previous approaches (other or similar hypotheses/expts to prove)

Experimental Approach

- high points for proof of hypothesis
- experimental details for the "high points"
- predictions/support from literature
- data analysis and reporting

Applications and Future Work

- outcome of work and impact on the general public/fundamental research
- general applicability of technique(s) to other problems

References Cited (Analytical Chemistry Format)

3. Project Needs

- Chemicals, supplies, equipment
- Timeline and milestones of accomplishments

EXPERIMENT 1
Gas Chromatography/Mass Spectrometry (GC/MS) of a Complex Mixture

(1) Introduction

GC/MS represents one of many hyphenated instrumental techniques in Analytical Chemistry. Hyphenated instruments involve the coupling of two independent and complementary instruments together. GC/MS allows one to analyze, both qualitatively and quantitatively, components of a mixture. The components of the mixture are separated by GC into the individual components and then swept into the MS in order to obtain an EI (electron ionization) mass spectrum of each component. From the EI spectrum, the components can then be identified. The reason for separating the components prior to the MS acquisition, is that mass spectrometers are not capable of handling mixtures, whereas GC can.

(2) Background and Reference Material

You are to read about GC/MS by finding secondary literature references. A good start can be afforded by looking at the suggested textbooks for this laboratory course. For the interpretation of the fragmentation patterns, I would consult Prof. Fred McLafferty's book on EI-MS.

(3) Basic Procedure

In this experiment, the student will be given a mixture of components (a liquid fuel sample) that will be separated using capillary GC. The separation parameters for this sample on the GC have already been worked out for you. All that will be required is to inject the sample into the GC and start the data acquisition. (See instructions for operation of the Varian GC/MS immediately following this description). After acquisition of the total ion current chromatogram, the students will then identify each component in the standard mixture from the library of EI spectra on the computer used to collect the data. In addition, you are to take four of the spectra of individual chromatographic peaks and present a description in your report of the fragmentation pattern, making sure to identify the major fragments. The data to be reported is the number of components and the identity of each component based on the fragmentation pattern!! REMEMBER, YOU MUST DILUTE YOR SAMPLES TO A CONCENTRATION LEVEL OF ROUGHLY 1 mg mL^{-1}!!!!!!

The operation procedures for the GC/MS will be provided to you by direct interaction with the teaching assistants or me - you will be trained to use the GC/MS by one of us. It is of utmost importance that you write down, in your laboratory notebook, the steps to using the instrument.

(4) Experimental Notes

Make sure that you describe in detail the mass spectrometer instrumentation used in this experiment. Specifically, one should discuss the mass filter used, the ionization source, the interface between the GC and the MS, and the ion separator and detector, and GC operational

parameters, such as column type, operating temperature, carrier gas and other relevant information. Also, make sure you clearly identify each component in the unknown and include reference mass spectra for each component.

(5) Questions

1. Is there a mass spectral technique that one could use if it were desired to obtain analyte molecular weight information with little fragmentation? Please discuss this technique and the differences with respect to electron ionization.

2. Through inspection of the EI mass spectra, how could one determine the number of carbon atoms in the organic sample? How could one determine if Chlorine were present in the sample compound?

3. What is the difference between the average mass and exact mass of a compound? Give an example using a compound that will readily exemplify this difference.

4. Why is it easier to interface a GC to the MS and it is typically more difficult to interface a liquid chromatograph to the MS?

5. In the discussion section of your report, find two research articles that have used GC/MS for some analytical analysis. Make sure you comment on these articles in this section of the report and include a full reference citation in the bibliography section.

6. Excluding a quadrupole mass analyzer, what mass filter would be best suited for interfacing to CE, LC, or GC?

Brief Guide to the Varian GC/MS Operation for Chemistry 4553

Setup and Acquisition

1. Determine if the GCMS instrument is in use by checking the *real-time display* on the computer screen or the instrument acquisition message at the top of the system acquisition screen. If in use this message will indicate ACQUISITION or NOT READY. If the system is ready for use, this message will display READY.

2. Before an injection is made, determine if the correct method is loaded for acquisition. The correct method to use is *4553-1mario.mth* and is displayed in the *method box*. If this method is not correct, ask a Teaching Assistant to change the method.

3. The sample to be injected must be diluted to an appropriate concentration in an organic solvent to be used correctly with this instrument. **Aqueous samples cannot be used with this technique.** Concentrations typically used for GC/MS should be in the part-per-thousand to part-per-million range. Concentrations too high will overload the ion trap and cause the instrument acquisition to be shut down and will cause overloading of the column (capacity exceeded, resulting in broad peaks).

4. To inject the sample, first thoroughly rinse a 10 μL syringe using methylene chloride to remove any contaminants. Dose the syringe with the sample by rinsing the syringe with several microliters of sample. When ready, withdraw 1-2 μL of sample into the syringe and inject into the injection port of the instrument. The run will start automatically. After the injection is complete, thoroughly rinse the syringe with methylene chloride.

5. Allow the sample run to finish, this will take 30 min using the 4553-1mario.mth method.

Data Analysis

6. When the acquisition is complete, click on the data file operations button at the top of the screen and select *view/edit MS chromatogram*. Note the file number associated with the sample for future reference. This will open the last chromatogram acquired.

7. To scale or zoom on peaks in the chromatogram, depress and hold down the left mouse button and create a box around the desired area. When finished, release the mouse button. To rescale the chromatogram, left click on the *rescale button* at the top of the chromatogram window. Ask the TA for additional help if problems arise.

8. To view the mass spectrum associated with a peak, left click anywhere on a peak of interest. This will then display two windows, one of the chromatogram and the second containing the mass spectrum. The mass spectrum will automatically change each time a new peak is selected in the chromatograrn. Ask the TA for additional help if problems arise.

9. To print a chromatogram and the mass spectrum, go to File and click on *print chromatogram*. A preview screen will be opened to allow the user to view what will be printed. If all is acceptable, click on the *print* icon, then close the window to go back to the data analysis window.

10. When finished with all data analysis, close the data analysis window to return to the acquisition window.

EXPERIMENT 2
Structure Elucidation Using Fourier Transform Infrared Spectroscopy

(1) Introduction

In this experiment, the student will be accustomed with the use of Fourier Transform Infrared Spectroscopy (FTIR) for the determination of organic structure. In addition, the student will learn the instrumental aspects of FTIR as well as the basic concepts associated with FTIR. The major function of FTIR is to give information on the presence of functional groups in organic compounds, such as alcohols, carbonyls and/or amines. Each functional group has characteristic frequencies in which they vibrate giving a signature in the FTIR spectrum. From the FTIR spectrum, it becomes difficult to determine the complete structure of an organic unknown, as such it is typically used in conjunction with NMR and mass spectrometry for structure elucidation.

In the present experiment, the student will acquire IR spectra for several standard organic compounds, both liquids and solids (these are unknowns and will be provided at the beginning of the class period). From the spectra, the student will make some tentative assignments of bands in the IR with the known functional groups contained within the reference compound. Once this is completed, the student will also go to the library to look up IR spectra of these compounds in the Sadtler index (Chemistry Library).

(2) Background and Reference Material

A theoretical and instrumental description of FTIR and IR can be found in the reference books presented in the opening section of this laboratory manual. In addition, a number of Organic Chemistry books contain some basic information on IR spectroscopy. For a listing of characteristic frequencies of common functional groups associated with organic compound, one can consult Spectrometric Identification of Organic Compounds by Silverstein and Bassler.

(3) Basic Experimental Procedure

Consult the instructions (found at the end of this section) for proper use of the Mattson IR spectrometers. The unknown compounds for which to obtain an IR spectrum will be provided to you by the Teaching Assistants; make sure to record the unknown numbers in the front of your write-up and use them in your discussion of what you think the compounds are. For the liquid samples, use a glass stirring rod to wipe a thin layer of liquid between the NaCl plates. NOTE: CHECK THE INSTRUCTIONS BELOW FOR SAFE HANDLING OF NaCl PLATES! If the absorbance of the bands is too intense (bottoms out on the screen), then make the layer of sample thinner on the NaCl plates. Between samples or after use of the plates, make sure that you rinse the plates with copious amounts of a volatile solvent like dichloromethane. Return the plates to the dessicator when done. For the solid sample, you will be required to make a KBr pellet. In this case, place about 50-100 mg of dry KBr in the mortar that has been dried in the oven. To this add about 1% (by weight) of the solid organic sample. Then, use the pestle to carefully and thoroughly mix the two solids together. After this is complete, add a few milligrams of the

mixture to the stainless steel press. Once this is added to the press, apply sufficient pressure to create a nearly clear window of sample in the KBr pellet. If the pellet is cloudy, the background absorbance reading will be high. In this case, add less sample to the press.

(4) Experimental Notes

Make sure that you get sufficient signal (absorbance) in order to see all of the relevant bands in the IR spectrum. Also, if you see bands that are flat on top, then you probably loaded too much sample in the press or on the NaCl plates. If this is the case, reduce the amount of sample. Make sure that you identify the major bands in the reference spectra. For the unknown, make sure that you clearly mark the unknown number on the laboratory report and also, in the spectrum, label the major bands which aided in the identification process. Failure to do so, will result in the loss of points.

(5) Questions

1. Explain the difference between UV/vis absorptions and IR absorptions. What are the selection rules for each? Why are UV/vis absorption bands broader than IR bands?

2. You will notice that in the FTIR instrument, there is a red beam of light that travels through the sample compartment. This is a He-Ne laser. What is the purpose of this red laser beam?

3. Explain the differences between scanning or grating IR and an FTIR instrument. What are the advantages one typically gets from doing FTIR instead of grating IR?

4. What type of detector did you use in this experiment? How does it work?

5. What is the beam splitter made of in the IR you used here? What materials would be good for the 700-200 wavenumber region?

(6) Handling of NaCl Plates

1. Don't contaminate them with water.
2. DO NOT TOUCH THE OPTICAL SURFACES WITH YOUR FINGERS!
3. Use the same set of plates for your background and sample.
4. After use, place the plates back in the dessicator.

Mattson FTIR operation

Double-Click "Start-IR" Icon macro ==> then [F1]
Follow Instructions to scan background with nothing in the sample area ==> [ENTER]
Then scan sample ==> [ENTER]
MAKE SURE Lid is shut tight*

Name & Save sample spectra to C:/ft-ir/Chem2002-#/Data/Group#, and save to a:/ floppy drive, [F2] on bottom menu,
Be sure to save as last prompt name on drive a:/ (use file name again, instead of tmp.ras)

SCAN next SAMPLE, put in sample cell holder, under SCAN menu, select [scan next sample]<==> [Alt-S]

OR

DATA analysis

Hit [SIMPLE MENU] button on the bottom menu bar

Go to DATA analysis

Load the sample from a:/ drive or C:/ft-ir/Chem2002-#/Data/Group#, use top menu bar, under <file>, <load>

<Under math tab> you can do auto baseline correct

You can change form absorbance to transmittance

Check out other options

Use the notation menu (table of icons, top right) to label peaks and determine their wavenumber (frequency)

Click on to label peaks

Click on to notate peaks

Click on to edit notation

Under <Tools> <Correlation Chart> use the following to look for R-groups ==> [F10]

<SAVE into File> ==> [F6]

PRINT ==> [F7] ==> < Alt-P > ==> Click "Done"

SCAN next SAMPLE, put in sample cell holder, under SCAN menu<select scan next sample>==> <Alt-S>

EXPERIMENT 3
Flame Atomic Absorption Spectrometry of Metal Ions in Food

(1) Introduction

Atomic absorption spectrometry is a popular technique for the determination of metals in many types of samples. It is commonly used for the analysis of food. In this experiment, the food is first digested in acid to release the metals into a soluble form for determination.

(2) Background and Reference Material

Any good instrumental text will be a good source for this laboratory. In addition, recent reviews in *Analytical Chemistry* concerning atomic spectroscopy and food analysis will be very helpful.

(3) Experimental Procedures

A. Preparation of the Sample to be Analyzed. Place a 2 g food sample (apple is best, we can provide) in a 250-mL Erlenmeyer flask; add 25 mL 8 *M* HCl. Boil slowly on a hot plate for 5 min. Cool and add 10 rnL deionized water. Filter through a Whatman No. 1 filter paper. Transfer the filtrate to a 50-mL volumetric flask, dilute to the mark with deionized water, and use this solution directly for determination of copper and iron. For a calcium determination, pipet 10 mL of this solution to another 50-mL volumetric flask, add 20 mL 8 *M* HCI and 10 mL of the lanthanum solution, and dilute to the mark. The lanthanum prevents the calcium from forming complexes with phosphates; such complexes hinder atomization in the flame.

B. Determination of Calcium. Use the instruction manual for the instrument or talk to the Teaching Assistant to set the parameters for the determination of calcium. Prepare a Ca^{2+} standard by pipetting 10 mL of the stock standard (500 μg/mL) you have prepared into a 100-rnL volumetric flask and diluting to the mark. This diluted solution should contain ~50 μg/mL Ca, and is used to make the standards for the calibration curve. Prepare these standards by pipetting 2-, 5-, 10-, and 20-mL aliquots of the 50 μg/mL solution into successive 100-mL volumetric flasks. Add 50 mL 8 *M* HCI and 20 mL of the lanthanum solution to each flask and fill to the mark with deionized water. These diluted solutions must be prepared fresh each day. Aspirate the diluted standards into the flame and record the absorbance. Record four readings (four trials of aspiration of sample) for each concentration so that you can carry out statistics on the data.

Aspirate the sample solution that was prepared in Procedure A. If the absorbance is within the range of the standards of the calibration curve, record the value. If the absorbance is higher than that of the most concentrated standard, dilute the sample ten times and repeat the analysis. To make a tenfold dilution, pipet 10 mL into a 100-mL volumetric flask, add 45 mL 8 *M* HCI, and 18 mL of the lanthanum solution, and dilute with deionized water to the mark. Record four absorbance readings (four trials of aspiration of sample) for the sample so that you can carry out statistics on the data.

C. Determination of Iron. Use the instruction manual for the instrument to set the parameters for the determination of iron. Pipet 10 mL of the stock iron solution (1000 μg/mL Fe)

into a 100- mL volumetric flask and dilute with deionized water to the mark. Pipet 1, 3, 5, and 10 mL of this diluted standard (100 μg/mL) successively into 100-mL volumetric flasks. Add 50 mL of 8 *M* HCl to each and dilute with deionized water. Aspirate each of the calibration standards (1, 3, 5, and 10 μg/mL Fe) and record the absorbance. Record four readings (four trials of aspiration of sample) for each concentration so that you can carry out statistics on the data.

Aspirate the sample prepared in Procedure A into the AA spectrophotometer. If the absorbance is higher than that of the highest standard, make the appropriate dilution. For example, try a tenfold dilution by pipetting 10 mL of the sample in a 100-mL volumetric flask and adding 45 mL 8 M HCl; dilute to the mark. Record four readings (four trials of aspiration of sample) for the sample so that you can carry out statistics on the data.

D. Determination of Copper. After setting the instrument parameters for copper, make appropriate dilutions to give calibration solutions of 1, 3, 5, and TO gg/mL Cu by use of the same procedure as for iron. Aspirate the diluted standards and record the absorbance. Record four readings (four trials of aspiration of sample) for each concentration so that you can carry out statistics on the data. Aspirate the sample as prepared in Procedure A in the spectrophotometer. Adjust the concentration as for iron in the event the absorbance is too high for the standards. Record four readings (four trials of aspiration of sample) for the sample so that you can carry out statistics on the data.

(4) Experimental Notes

Plot calibration curves for the calcium, iron, and copper solutions (absorbance vs. concentration). Make sure to include statistics on the data points (confidence intervals). Determine the amount of each of these elements in your sample. Compare your values, as % by weight, with those on the food package.

(5) Questions

1. How does lanthanum aid in the determination of calcium?
2. Why are the diluted calibration solutions stable for only 1 or 2 days?

Operation of Perkin Elmer Model 3100 Spectrophotometer in AAS Mode

The following conditions are to be considered:
Lamp current:..See PE Manual for conditions
Integration:..0.3 sec
Replicates:...4
Fuel: ....Acetylene (12-15 psi) @ tank
Oxidant: ....Compressed Air (60 psi) @ tank
Slit width:..See PE Manual for conditions for each lamp
Wavelengths:..See PE Manual for conditions
....See PE Manual for conditions

There are two AA instruments available in the lab. It is your responsibility to check if the correct lamp is inserted. If the lamp needs changing or adjustment, ask your TA to assist you. The lamps should have inscriptions with the proper information. The lamp position is located on the upper right side of the AA.

TO SET UP THE INSTRUMENT:

1) Turn on the POWER SWITCH on the lower right side of the AA. The instrument should be allowed to warm for at least 15 to 20 minutes.

2) Press the **PARAM key** on the front panel. An automatic self-testing program will run at this time. Wait for few seconds until the screen displays **Lamp Current (0-50 mA)**. This current is a measure of the lamp intensity. Next input **10** and press **ENTER. Never exceed this value!!!**

3) The following screen should appear and request for **Int. Time (0.1-60 sec)**. This parameter will define the time taken by the machine to take a single reading. Input **0.3** and press the **ENTER** key.

4) For the **Replicates (1-99)** screen, input **4** and press **ENTER.** This command allows the instrument to take 3 absorbance readings for the same sample, and the display will be the average.

5) **Ignore** the subsequent screens. Press **ENTER** until **Lamp Current** is displayed.

6) Adjust the wavelength to the desired value. The wavelength is shown on the upper left part of the AA instrument. It can be changed by using the dial on the left side.

7) Press the **ENERGY** key on the front panel, and notice the output. It should display number of counts and relative energy output. This is a measure of the intensity of light reaching the detector and should be maximized. If the lamp has been previously aligned, there should be no need to adjust this output. A relative energy output of 60 units or more is sufficiently good. If that is not the case, you may need slight wavelength adjustments or TA assistance.

IGNITING THE AA FLAME:

***** **NEVER LEAVE FLAME UNATTENDED !!!!!!!!!!!!!!**

1) An acetylene-air flame is used to atomize the sample. To ignite the flame, open first the compressed air, the bigger cylinder's main valve, the outer valve on the tank. Next adjust the regulator valve to **60 psi**. The regulator has two pressure gauges: one is internal tank pressure and one is the flow pressure to the rubber hose. The brass handle allows gas flow adjustment from the cylinder to the instrument. Repeat the same procedure for the acetylene (smaller cylinder tank), adjusting the regulator valve between **12 to 15 psi**. DO NOT EXCEED THIS RANGE. ACETYLENE IS AN EXPLOSIVE GAS! If the acetylene tank pressure drops below 75 psi (main valve), have the tank replaced or call for assistance.

Turn the knob in the lower left part of the front panel from **OFF** to **AIR**. This will allow the acetylene/air mixture to flow into the burner. Notice the two flow rate indicators (flow levels in glass tubes), --- one for oxidant --- and --- one for fuel. Using the corresponding knobs beneath these indicators, adjust the relative flow rates so that a 2 to 1 mixture of oxidant to fuel will be obtained (oxidizing flame). Proper settings on the glass tubes are 4 units for oxidant and 2 for fuel. (The AA instrument has a safety mechanism: if the flame is not ignited within 10 sec after flow has been established (i.e. switch in AIR position), flow will be automatically interrupted. To reestablish flow, turn the switch back to **OFF** position and then back to **AIR**.)

4) After adjusting air to fuel flow rates, press the IGNITE button; hold it down for few seconds. This should ignite the flame. However, if it does not work, try again. To do this turn the switch to **OFF** and back to **AIR** and try again! Call for assistance, if the flame is not being ignited after the third try.

Reading the AA SAMPLES

1) Make sure that the tube through which the sample is aspirated into the flame is always immersed in the sample, or in distilled water if no sample is being measured. The aspiration rate should have been adjusted for you.

2) To **zero** the instrument, use distilled water as a blank or the matrix used in the preparation of the standards. Press **DATA** key on the front panel. Dip the aspirating tube into blank and press **A/Z** key (upper left).This will define the zero absorbance for the sample. The final display should be **Abs: 0.000** and the standard deviation (**sd**) and relative standard deviation (**rsd**) for the triplicate readings. Remember, we set the sampling rate to be three.

3) To measure a sample, dip the aspirating tube into the new solution, and wait for about 10 sec and then press **READ** (lower left). A screen displaying Absorbance, Standard deviation, and Relative standard deviation will appear. Write this information down. Repeat the same procedure a total of 3 times for each sample.

4) **It is necessary to zero the instrument between samples!**

SHUTTING-DOWN PROCEDURE

10) To turn off the flame, close first the acetylene cylinder valve and let the flame extinguish by itself. Then close the compressed air tank valve. Switch the knob on panel from **AIR** back to **OFF**. Then, close both the acetylene and compressed air knobs on the instrument. Finally, bleed the gas from the regulator. Press the lever on the regular the gauges should read 0 (zero) psi.

Shut the main power switch OFF **(right side of AA).**

Operation of Atomic Emission Mode of PE AA/AES Instruments for Chemistry 4553 When Using Calcium as the Analyte

1. Turn on instrument by following directions for AAS instrument start-up; let warm up about 15 min.

2. On the touch pad, depress [EM] => emission mode.

3. Ignite flame.

4. Press [PARAM] => get screen {lamp current (0-50 mA)}; change this value to 0.0 and depress [ENTER].

5. Screen will now display {INT. Time (.1-60 sec)}; change to 0.3. followed by [ENTER].

6. Screen will now display {replicates (1-99)}; change to 4, followed by [ENTER].

7. Depress enter until {lamp current (0-50 ms)}screen reappears.

8. Set wavelength control dial on left side of instrument to 422.7 nm for Ca and move slit width to 0.2 nm; move slit height to high position.

10. Depress [Energy] and then aspirate the highest concentration standard; depress [ENTER] followed by [Gain].

11. Slowly rotate wavelength ≈ ±1 nm to obtain maximum energy counts, and then depress [Gain].

12. Depress [Cont] or [data].

13. Aspirate blank, then press [A/Z].

14. To read standards and samples, aspirate, then press [Read]. Make sure to rinse between readings with high-purity (DI) water.

EXPERIMENT 4
Fluorescence Determination of Pharmaceutical Components in a Mixture

(1) Introduction

Acetylsalicylic acid (aspirin), often referred to as ASA, hydrolyzes to salicylic acid (SA), which is present to some extent in most aspirin. This method demonstrates how both of these substances can be accurately determined by fluorometry. This experiment is adapted, in part, from G. H. Schenk, F. H. Boyer, C. 1. Miles, and D. R. Wirz, Anal. Chem., 44, 1593 (1972) and D.T. Sawyer, W.R. Heieneman, and J.M. Beebe, *Experiments for Instrumental Methods*; Wiley, 1984.

(2) Background and Reference Material

Any of the reference books listed in this manual can be used to read about the instrumentation and general concepts associated with fluorescence spectroscopy. Make sure that in the Introduction section of the laboratory report that you discuss the block diagram of the instrument. Also discuss why it is that the emission spectrum of a compound, in general, is "a mirror image" of the excitation spectrum.

(3) Experimental Procedures

You will first obtain excitation and emission spectra of dilute solutions of pure acetylsalicylic acid and salicylic acid in the chloroform/acetic acid solvent. From these spectra, you will choose the optimal wavelength of excitation and emission (observation) for determination of the two components in a mixture (aspirin tablets).

You will then obtain a standard calibration curve for ASA as follows. Prepare a 4.0-μg/rnL solution of pure ASA in the acetic acid/chloroform mixture; make enough of this solution so that you can prepare the calibration curves needed to complete the experiment. Pipet 5, 10, 15, and 20 mL of the 4.0-μg/rnL solution of ASA into 25-mL volumetric flasks, successively. Fill each to the mark with a 1% v/v acetic acid-chloroform solution. Using the chosen excitation wavelength, obtain % fluorescence measurements for each of the five solutions. Use the 1% acetic acid-chloroform solution as a blank. Construct an intensity versus concentration plot for the ASA.

Now, you will obtain data for construction of a calibration curve for SA. Prepare a 7.5-μg/rnL solution of pure SA in the acetic acid/chloroform solvent make enough of this solution so that you can prepare the calibration curves needed to complete the experiment. Pipet 5, 10, 15, and 20 mL of the 7.5-μg/mL SA solution into 25-mL volumetric flasks, successively. Fill each to the mark with the 1% acetic acid-chloroform solution. Using the chosen excitation and emission wavelengths, obtain % fluorescence measurements for each of the five solutions. Construct an intensity versus concentration plot for the SA.

The next stage of the experiment is determination of the amounts of each component in commercially available aspirin tablets. You may bring your own or we will supply some for

you. Grind three to five aspirin tablets to a powder using a mortar and pestle. Weigh 400 mg of the prepared powder (or the equivalent of one tablet) and transfer to a 100-mL volumetric flask; dissolve in 1% v/v acetic acid-chloroform and dilute to the mark with this solvent. Filter this solution rapidly through Whatman No. 1 filter paper. Determine the % fluorescence of the salicylic acid by use of the same instrument settings as for the standard curve. Save the filtered solution for the next step (determination of ASA). To determine the acetylsalicylic acid content, dilute the above filtered solution 1: 1000 with 1% acetic acid-chloroform. Obtain the % fluorescence with the appropriate instrument parameters. Readings should be made within 1 h from the time of the dissolution of the aspirin.

(4) Experimental Notes

Remember to run a linear least squares analysis on the calibration plot and report the relevant statistics. If you can find some "old" aspirin tablets laying around your abode, bring them and a sample of "new" aspirin tablets. Report the amount of ASA and SA in units of milligrams per aspirin tablet, and compare the observed amounts to that stated by the manufacturer. Make sure that when you write up the laboratory report, you state the name of the products you tested.

(5) Questions

1. Are your standard curves linear? If not, where do they begin to deviate and why?
2. What is the significance of the presence of salicylic acid in your tablets? Is it undesirable?
3. Why do you think the fluorescence intensity associated with the ASA and SA changes upon standing of the prepared solutions?
4. What other analytical method could you use to verify your analyses of the tablets? Describe (briefly) how you would do it and what challenges you might encounter.
5. Why did you use several aspirin tablets for your sample?

EXPERIMENT 5
High Performance Liquid Chromatographic Separation of Hydrocarbons

(1) Introduction

Although it may seem that GC would be more appropriate at first glance, HPLC can achieve separations of mixtures of semi-volatile organics that would prove problematic to GC instrumentation. In this case, the stationary phase is a solid (adsorbent), and the mobile phase is a liquid. There are many types of LC stationary phases, but the major ones are normal and reverse phase.

In normal phase chromatography, the stationary phase is polar and the mobile phase is non-polar. A typical stationary phase used here is silica and a mobile phase consisting of hexane or another alkane. In reverse phase chromatography, the stationary phase is non-polar and the mobile phase is polar. Most often, stationary phases in reverse phase chromatography are silica particles (5-10 micrometers in diameter) that are coated with an alkane and the mobile phase consists of a mixed aqueous/organic solvent, such as methanol. In the present experiment, we will be using a C18 alkane stationary phase (octadecylsilane-silica) with a water/acetonitrile mobile phase.

As with GC, the analytes are separated based on their partitioning between the mobile and stationary phases. In GC, the elution order of the analytes is determined primarily by their boiling points, while in LC, the elution order is determined by the relative hydrophobicity of the compounds. For example, in the series benzene, naphthalene and anthracene, the elution order would be anthracene, naphthalene and benzene, if one were performing a normal phase separation.

Just as in GC, one must be able to detect the compounds as they move through the LC system. There are many different types of LC detectors, such as UV/vis, fluorescence, electrochemical, and Raman, all of which have particular advantages and disadvantages. In this experiment we will be using a UV/Vis detector and monitoring the absorbance of the analyte (caffeine) as it migrates through the LC system. For the UV/Vis detector, it operates the same as the conventional UV/Vis spectrometer and in addition, it obeys the same laws as conventional UV/Vis spectroscopy. Therefore, the use of the Beer-Lambert Law holds in this case as well, and a calibration plot can be constructed (absorbance versus concentration) from which the concentration of an unknown can be determined.

(2) Background and Reference Material

Any of the reference books listed in this manual can be used to read about the instrumentation and general concepts associated with LC. Make sure that in the Introduction section of the laboratory report that you include the following points:

1. The band broadening mechanisms in LC.
2. Components of the LC instrumentation (pump, detector, injector).
3. Plate theory of chromatography (N, resolution, how calculated).

As with the other laboratory reports, a detailed discussion of the instrumentation is critical. Make sure that you show a block diagram of the required instrumentation. In addition, make sure that you discuss in great detail the detector used in this experiment and the general law which relates the observed analytical signal to the concentration.

(3) Experimental Procedures

In this experiment, the first step will be to optimize the gradient elution conditions so as to obtain the best possible separation of the mixture of aromatic hydrocarbons. You will vary the gradient elution profile (the mixture of acetonitrile and water supplied to the column) using the Prostar software (Varian software). The second step will be to determine the identity and concentration of three analytes in your mixture; you will be given three compounds that are known to be in the mixture you have already separated previously. You will identify the compounds in the mixture by comparing the retention times of the analytes. The concentration of the unknowns in the mixture will be obtained through ratioing the areas of the chromatographic bands (see Prostar software instructions for details on how to integrate the bands) or by use of the standard addition method. **You should run the LC mobile phase at a flow rate of no more than 1.5 mL/min. You will operate the UV lamp detector as 254 nanometers.**

The following specific actions should be carried out. Do not write these "cookbook" instructions in your notebook:

1. Obtain a solution containing five unknown compounds from the TA.

2. Obtain a HPLC chromatogram using a 30/70 Acetonitrile/Water mobile phase (this is isocratic elution) at a flow rate of 1.5 mL/min for 60 minutes.

3. Perform gradient elution runs in order to optimize the separation of the unknowns.

4. After the conditions are optimized, obtain, from the Teaching Assistants, two known compounds that are present in the unknown solution. Prepare standard solutions of these compounds and obtain a chromatogram using the optimized conditions for each of these standards. You may want to think about this and try to do a standard addition for determining the concentrations of your unknowns.

5. Identify the peaks corresponding to your samples on the chromatogram containing all five unknowns.

6. Calculate the concentration of the two components in the unknown solution.

CREATING A METHOD:

1. Click on the Create and edit icon (second icon from the top left corner)

2. Click Cancel on Select Configuration pop-up screen.

3. Open an existing method:
   File
   Open
   CHEM 4553
   Spring 2002

4. Click on Pump and CIM program

5. Edit program to the left
   - Do not change step 2
   - You may add steps
   - Change %B to desired concentration and %A will automatically be adjusted
   - Do not use a flow rate greater than 1.5 mL/min

6. Save the method as a different filename under your folder.

(4) Experimental Notes

In this experiment, you will be gaining an understanding of the way in which a separation is optimized using gradient elution. You may change the flow rate and the composition of the mobile phase to separate the very similar aromatic hydrocarbons.

In addition to the identity and quantity of the unknowns in the mixture, you should calculate the separation efficiency (number of theoretical plates) and the resolution of the separation for your mixture.

(5) Questions

1. Why did the compounds you have as your three "unknowns" behave the way they did when you varied the chromatographic conditions? Think about their characteristics and that of the solvent (mobile phase), as well as the flow rate, etc.
2. Go to the chemistry library and find two research articles where scientists have reported on the quantification and identification of aromatic hydrocarbons. Make a full citation of the references in your bibliography. Discuss here what those two studies did and why their method was inferior or superior to that here.
3. Do you expect to have any changes in the separation efficiency of the chromatograms if you were to change the detector wavelength to 270 nm? Why or why not?
4. Discuss the differences in the elution order that one would expect for your three compounds if you had used normal phase chromatography. Why is this the case?