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Lab: Tue 9:30 – 12:20  
Office Hours: Mon & Wed 1:00 – 1:50; Thr 10:00 – 12:00  
and other times by appointment

The laboratory is the heart of the Instrumental Analysis course. Here you will learn how the instrumentation may be utilized to solve chemical problems.

EQUIPMENT

Safety glasses  
Laboratory Notebook  
Breakage Card  
Formatted 1.44 MB (IBM) floppy disk for data acquisition  
Laboratory manual

LABORATORY GRADE

Lab Reports 30%  
Independent Project 10%

LABORATORY NOTEBOOKS AND REPORTS

See sheet for preparation of lab reports. Write down everything during the lab while the details are fresh in your memory. You will be working in groups during the semester. However, each of you is responsible for maintaining your own laboratory notebook. While discussion of data and calculations of results with your lab partners is encouraged, each person should write their own discussion section of the lab report. Reports with identical discussions and conclusions will not be accepted. You may hand in up to 2 reports per week, but no more. The reports are due in class on the Friday of the week following the completion of the laboratory experiment. **No Late Lab Reports will be excepted!!**. **All lab reports you wish to be considered for credit are due by the end of class, Friday, December 6, 2002.**
INDEPENDENT PROJECT

After completion of the final “prepared” laboratory experiment, the lab will consist of you and your partner(s) proposing, designing, researching, performing, and writing up an extended project. You should start thinking of your project topic early in the semester so we can talk about its feasibility and equipment requirements. **Two months before** the date of the beginning of the project, you will have to provide a detailed preliminary proposal, including chemicals, procedures and instruments needed to complete the project. You should start thinking of your project topic early in the semester so we can talk about its feasibility and equipment requirements. The purpose of this project is to get you away from the “handout” type of lab and get you to perform a “real” lab where you must design the experiment and decide the scope of the problem. There are many issues involved in an analytical analysis in addition to merely running an instrument. At the end of the project, you will write up a scientific paper on your project. As we get closer to the date of beginning the projects, there will be more information provided that covers the schedule and expectations.

Please note that in order to pass the course, you must receive a passing grade in both the lecture and the laboratory parts of the course.
One of the main activities that you will perform as a scientist will be to write up your experimental results for other scientists either in peer-review journals or for corporate reports. It is essential that you learn to write clear and concise scientific reports. Therefore, there will be a strong emphasis in this course to get you accustomed to thinking and writing like a scientist.

**Purpose of the Laboratory Notebook**

The laboratory notebook is *your* record of what *you* did during an experiment. It is your account of the purpose, method, procedure, and all data you acquired. Laboratory notebooks cannot be too detailed and are more than likely not detailed enough. In reality, no experiment is just done once, but repeated many times over the course of weeks or months or even years. The laboratory notebook is there to go back to when you do repeat something so you know how you did it the previous time. If your initial record of the experiment is not complete, it will be hard to repeat the experiment and impossible to compare the results. Whether you enter industrial, government, or academic research, a well-kept laboratory notebook is very important.

**Writing in the Laboratory Notebook**

Each person has his or her own style of keeping a laboratory notebook. It is your responsibility to maintain your laboratory notebook and it will not be reviewed or graded by the instructor. A few general guidelines for keeping a notebook:

a) Don’t hesitate to put down too much detail. Even things that seem obvious can be forgotten later if they are not written down.

b) Try to use complete sentences. Invariably, a cryptic note in the margin of the notebook cannot be deciphered later and important information will be lost.

c) Write everything in the notebook! Don’t write a weight measurement on a paper towel and stick that in your notebook to transfer later. Record observations and data immediately. Having tables made before hand for measurements will save time and insure that you did not forget an important measurement.

d) Errors you make in the notebook should be crossed out but still legible in case what you crossed out was actually correct. Don’t erase, white out, or tear pages out of your notebook. Keep your notebook as an honest account of the experiment; mistakes do occur.

e) Number the pages in the notebook sequentially and save room at the beginning for a table of contents.
Format for the Laboratory Notebook

Laboratory notebooks will not be checked or collected during the Instrumental Analysis course. It is assumed that you will use a notebook and that from your experience in other courses, you know the importance of the laboratory notebook and how to keep one. If you fail to keep a laboratory notebook, it will be evident in the quality and accuracy of your final laboratory reports. Using your laboratory notebook effectively should be habit by now.

Format for the Final Laboratory Report (for the group labs)

Title Section

Your name and that of your lab partners, date the experiment was performed, date you are submitting the report and the title of the experiment

Introductory Section

Purpose/Theory: Write a paragraph in your own words about why you performed the experiment and what it demonstrated.

Results and Observations

Data: A record of all raw or primary data collected in the experiment, i.e., mass, length, volume, intensity, time, etc. Include associated units. Often it is convenient to record this information in the form of a table or chart. Include any spectra, graphs, charts or other data that were produced during the experiment. Since you will be generating many spectra during this course, make sure you label each one clearly and record the conditions under which that spectrum was taken. Present tables, graphs and charts of final analyzed data (if appropriate).

Apparatus: Include drawings of any special equipment or setups that are used in the experiment. When working with instruments, it is important to record the dial and switch settings (i.e., chart speed, attenuation, etc.) used in the experiment for later reference and in case you want to repeat measurements.

Observations: Write a narrative description of what you did and what you saw, especially if there were accidents or mistakes. Include physical changes like color, physical state, reactivity, etc.

Results and Discussion

Calculations: Perform a sample analysis of each type of data that was collected during the experiment. These sample calculations can be written by hand. Include the proper error analysis.

Discussion: Include an interpretation of what you think the data means. Use this space to consider what you did and saw during the experiment. Do not simply restate the data. Use this section to understand the data. If the data fit your hypotheses, explain how; if not, discuss why. Include possible sources of error. Speculation is appropriate in this section. Questions are given at the end of the lab to help guide your discussion of the data. Make sure you address these questions in your
discussion. This serves as a minimum that should be discussed. Be sure that you understand the questions and answers, as these are usually important points of the experiment. This section contributes the most to the grade received on the laboratory report.

Format of the Independent Project Report

The format of the report on the independent project will take the form of a manuscript from the journal Analytical Chemistry. More details will be available on this report as the project gets started. You will have an opportunity to submit the article for an initial review and have a chance to make corrections.
Introduction

This experiment will use atomic absorption spectroscopy to analyze a food product for the element iron using the Beer’s Law Method. The measured iron content will be compared with the amount of iron reported by the manufacturer.

Preparation of Samples for Beer’s Law Plot:

1. Prepare 1 Liter of 8 M HCl.

2. Prepare 1 Liter of Stock Iron Solution having a concentration of 1000 µg Fe / mL.
   
   a) Wipe several strands of Fe wire and rinse them with hexane to remove the protective oil.

   b) Dissolve 1.000 g Fe wire in a beaker by adding 50 mL 1:1 HNO₃:H₂O (USE HOOD)

   c) Quantitatively transfer this solution to a 1 L volumetric flask and bring to volume with DI water.

3. Prepare 250 mL of Diluted Iron Solution have a concentration of 100 µg Fe / mL. Use a 250 mL volumetric flask and DI water.

4. Beer’s Law Standard Solutions:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diluted Iron</th>
<th>8M HCl</th>
<th>Final Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.00 mL</td>
<td>125 mL</td>
<td>250.00 mL</td>
</tr>
<tr>
<td>1 µg Fe / mL</td>
<td>1.00 mL</td>
<td>50 mL</td>
<td>100.00 mL</td>
</tr>
<tr>
<td>3 µg Fe / mL</td>
<td>3.00 mL</td>
<td>50 mL</td>
<td>100.00 mL</td>
</tr>
<tr>
<td>5 µg Fe / mL</td>
<td>25.00 mL</td>
<td>250 mL</td>
<td>500.00 mL</td>
</tr>
<tr>
<td>10 µg Fe / mL</td>
<td>10.00 mL</td>
<td>50 mL</td>
<td>100.00 mL</td>
</tr>
<tr>
<td>15 µg Fe / mL</td>
<td>15.00 mL</td>
<td>50 mL</td>
<td>100.00 mL</td>
</tr>
<tr>
<td>20 µg Fe / mL</td>
<td>20.00 mL</td>
<td>50 mL</td>
<td>100.00 mL</td>
</tr>
</tbody>
</table>
**Preparation of Sample**

1. Pulverize approximately 6 grams of Cereal and place an accurately weighed, nearest 0.1 mg, sample of about 4 grams into a 125 mL Erlenmeyer Flask containing around 50 mL of DI water.

2. Stir with a magnetic stirrer for approximately 15 minutes to allow the iron to release from the cereal and stick to the magnet.

3. Remove the magnet and gently rinse with DI water, being careful not to knock off any of the iron.

4. Place the magnet into a 100 mL beaker containing 50 mL of 8 M HCl and gently heat until all the iron dissolves (USE HOOD). Do not boil.

5. Allow the solution to cool.

6. Quantitatively filter solution through Watman #1 Paper and transfer to a 100 mL volumetric flask and dilute to volume with DI water.

**Experimental Procedure:**

1. See your instructor for an overview of the BUCK 200A Atomic Absorption Spectrometer.

2. Use Section Five of the BUCK 200 A Instruction Manual to prepare the AA for use.

3. Perform the following steps:
   
   5.1 – Set up gas lines  
   5.1.1 – Lamp alignment  
   5.1.3 – Burner alignment. Use 5 μg Fe / mL Fe solution  
   5.2.7 - Nebulizer optimization and Fuel/air ratio optimization  
   5.2.5 – Auto zero mode  
   5.2.1 - Absorbance mode – Perform Beer’s Law Experiment
Include in the Report:

1. Sketch the main components of an atomic absorption spectrophotometer.

2. Why is iron wire a good primary standard for this analysis?

3. What is the U.S. RDA of iron?

4. Outline of method used to prepare food sample including all observations

5. Prepare a Beer’s Law plot of your standards and determine the best fit line or curve for the data and plot it. Determine the amount of Fe in your food sample from your calibration curve.

6. Report the amount of Fe in your food sample and compare to the value reported by the manufacture.
Introduction

UV-VIS absorption spectrophotometry can be applied both quantitatively (such as Beer’s Law analysis) and qualitatively (compound identification and purity). This lab will explore the use of the UV-VIS spectrophotometer to analyze various UV-VIS absorption organic and inorganic species. Your goal should be to gain intuition into what types of chemical systems benefit most from UV-VIS spectrophotometry and those that do not. You will also gain experience into what the various scan parameters do and why and when you should change them.

Wavelength Accuracy

This procedure does NOT need to be performed every time you use the spectrophotometer. However, it is important to realize that just because the readout from the instrument indicated that 500.00-nm light is being measured, this does not absolutely guarantee that this is the case. If wavelength accuracy is of primary concern, then you should verify the spectrophotometer’s calibration by measurement of the absorption lines of holmium glass or emission lines of the deuterium (D$_2$) lamp. The following procedure will allow you to check the wavelength accuracy of the spectrophotometer.

*Holmium Glass Method.* This method allows you to verify the wavelength calibration at three different wavelengths, 460.0 nm, 360.9 nm and 279.4 nm. The performance is considered satisfactory if the wavelength errors of the holmium glass absorption lines are within $+/-1$ 0.3 nm

a) With the Holmium Glass Filter in the sample compartment, measure the 460.0 nm absorption from between 455.0 to 465.0 nm at a 0.5 nm bandwidth, 10 nm/min scan speed, and a data interval of 0.1 nm.

b) Determine the wavelength of maximum absorbance using the Peak Pick Table.
Effects of Bandwidth

1. Place one drop of benzene in the bottom of a clean dry cell and seal the cell. Do not get benzene on the walls of the cell. Put cell in the sample compartment; no reference is necessary.

2. Obtain absorbance spectra of the benzene vapor from 220 to 280 nm (30 nm/min, 0.1 nm data interval) at the following bandwidth settings: (Plot on same graph to see differences/similarities).
   a) 4 nm  
   b) 2 nm  
   c) 1.0 nm  
   d) 0.5 nm  
   e) 0.2 nm  
   f) 0.2 nm

Cuvettes and Solvents

1. Obtain spectrum of empty quartz cuvette across the maximum wavelength range of the instrument.

2. Obtain spectrum of empty glass cuvette across the maximum wavelength range of the instrument.

3. Using the quartz cuvette, obtain spectra of the following solvents:
   a) water  
   b) methanol  
   c) hexane

Electronic Transitions of Organic Molecules

1. Obtain UV-VIS spectra of the following compounds using the solvent as the reference. You must prepare the sample in order that the absorbance is “on scale” (less than about 1.5). You should choose the appropriate wavelength range (which will necessitate a quick survey run on the samples before the final spectra is obtained) in order to get all of the important spectral features. Use a bandwidth of 1.0 nm unless otherwise noted. Also record the peak positions.

   - benzene in cyclohexane (at 0.5 nm and 1 nm bandwidth)  
   - naphthalene in cyclohexane  
   - anthracene in cyclohexane

Note: Many of these compounds are toxic and/or carcinogens. Do not leave bottles open or samples sitting around. Prepare solutions in the hood. Clean up spills. Dispose of organic wastes in proper containers.
Include in the Report:

1. Sketch the basic components (block diagram) of a UV-VIS scanning spectrometer.

2. For what types of molecules is UV-VIS spectroscopy most useful? For which is it not? As a consequence, what are the best solvents for UV-VIS spectroscopy?

3. What is meant by bandwidth? As you narrow the bandwidth of the UV-VIS spectrometer, what might you expect to happen to the spectrum?

4. Labeled copies of all the spectra and peak data.

5. Discuss in what situations would an accurate calibration of the UV-VIS be most important?

6. Discuss how changing the bandwidth changes the spectrum of the benzene vapor.

7. Compare the different optical material used to make cuvettes. What wavelength ranges is each material useful for?

8. Compare the spectra of the different solvents. State which of these solvents could be used in UV-VIS absorption spectroscopy.

9. Discuss the difference and similarities between the following spectra.
   a) benzene in the vapor phase vs. benzene in cyclohexane (at 0.5 nm bandwidth)
   b) benzene vs. naphthalene vs. anthracene (all in cyclohexane)

Do you find any correlation between molecular structure and the spectra?

10. How would you quantitatively describe the concentration of the organic samples you prepared? Suggest a procedure for preparing these concentrations quantitatively.
Introduction

Fluorescence spectroscopy is one of the most sensitive analytical techniques. This experiment will expose you to the functioning of our Fluorescence Spectrophotometer. You will be quantitatively examining the fluorescence of acetylsalicylic acid and salicylic acid, compounds found in a commercial aspirin tablet.

Salicylic Acid: MW 138.12 g/mol
Acetylsalicylic Acid: MW 180.17 g/mol

Preparation of Solutions

All solutions should be prepared with 1% acetic acid in chloroform. If this stock solvent is not available, consult your instructor. NOTE: Chloroform is toxic and should be used only in a fume hood. Dispose of waste properly.

Using 25 mL volumetric flasks, prepare the following:

a) 25 mL of 2x10^{-5} M Acetylsalicylic Acid solution

b) 25 mL of 2x10^{-5} M Salicylic Acid Solution

Spectrofluorometer Setup

1. Consult instructor about the operation of the spectrometer.

2. Set the parameters to these initial settings.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Slit</td>
<td>10 nm</td>
</tr>
<tr>
<td>Emission Slit</td>
<td>5 nm</td>
</tr>
<tr>
<td>Scan Speed</td>
<td>120 nm/min</td>
</tr>
</tbody>
</table>

Excitation and Emission Spectra for acetylsalicylic acid

(Print a copy of all spectra)

1. Fill a quartz cuvette at least ¾ full with your acetylsalicylic acid sample.

2. Set the Emission Wavelength to 0 nm (All wavelengths pass).

3. Scan the excitation range from 200-500 nm.
4. Set the excitation wavelength to the appropriate setting as determined from the previous spectrum.

5. Scan the emission from 300 – 600 nm.

**Excitation and Emission Spectra for salicylic acid**

1. Fill a quartz cuvette at least ¾ full with your salicylic acid sample.

2. Set the Emission Wavelength to 0 nm (All wavelengths pass).

3. Scan the excitation range from 200-500 nm.

4. Set the excitation wavelength to the appropriate setting as determined from the previous spectrum.

5. Scan the emission from 300 – 600 nm.

**Selection of Analysis Wavelengths**

Based on the excitation and emission scans for acetylsalicylic acid and salicylic acid, determine the optimum excitation and emission wavelength for each. For example, the wavelength used for acetylsalicylic acid should produce a maximum response for acetylsalicylic acid and a minimum signal for salicylic acid.

**Effects of Slit Width**

For this part of the experiment, you need to obtain 3 spectra. Each spectrum will be an emission scan from 350-600 nm, using your salicylic acid sample and the maximum excitation wavelength for this sample. The variable will be the size of either the Excitation Slit width or the Emission Slit width.

<table>
<thead>
<tr>
<th>Spectrum A</th>
<th>Excitation Slit Width: 2.5 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emission Slit Width: 2.5 nm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spectrum B</th>
<th>Excitation Slit Width: 10.0 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emission Slit Width: 2.5 nm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spectrum C</th>
<th>Excitation Slit Width: 2.5 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emission Slit Width: 10.0 nm</td>
</tr>
</tbody>
</table>
Include in the Report:

1. Sketch the basic components (block diagram) of a spectrofluorometer.

2. What are the differences between this instrument and the UV-VIS spectrometer?

3. Instrumentally, what is the difference between an excitation spectrum and an emission spectrum?

4. Which types of molecules will have the highest fluorescence intensity?

5. Hand in printouts of all spectra, with appropriate labels, including instrumental settings.

6. Based on your spectra, discuss which excitation and emission wavelengths are most suitable for the analysis of acetylsalicylic acid and salicylic acid in an aspirin tablet.

7. Discuss the effect of changing the excitation and the emission slit width. What changes and why?
CHL311 Instrumental Analysis Laboratory
Fourier Transform Infrared Absorption Laboratory

Qualitative Analysis of Aspirin Tablet

Introduction

Infrared (IR) radiation with wavelengths of 700 nm to 50,000 nm is found in the electromagnetic spectrum between the visible and microwave regions. It can be applied to the analysis of organic molecules by causing molecular rotation and/or molecular vibrations (stretching or bending of bonds) in the molecules. This experiment will measure the absorption of infrared light by salicylic acid and acetylsalicylic acid. A commercial aspirin tablet containing both of these molecules will be analyzed and the spectra will be compared.

Materials

- Polystyrene Calibration Film
- KBr (Potassium Bromide)
- Salicylic Acid
- Acetylsalicylic Acid
- Commercial Aspirin Tablet

Procedure

1. Preparation of KBr Pellets

The sample container material must be transparent to infrared radiation in order to measure the absorption of the sample. Halide salts (including KBr) have excellent transparency in the IR. Liquid samples are typically placed between two large, polished crystals of KBr (known as salt plates) that are placed in the spectrometer. Solid samples are measured by grinding the solid sample into a fine powder and mixing a small amount of sample powder with powdered KBr. Halide salts have the property of “cold flow” in which they have glass-like transparent or translucent properties when sufficient pressure is applied to the finely powered materials.

   a) Blank KBr Pellet

   i. Grind 200 to 300 mg of KBr into a fine powder using a mortar and pestle.

   ii. Following the “Operating Instructions for Evacuable 13 mm KBr Die”, create a KBr pellet. Do not apply vacuum. After making each KBr pellet, rinse the metal pellets (D) with acetone into a waste beaker IN THE HOOD.
b) Salicylic Acid in KBr Pellet
   i. Grind 200 to 300 mg of KBr into a fine powder using a mortar and pestle.
   ii. Grind 1 to 2 mg sample of Salicylic Acid into a fine powder.
   iii. Add the Salicylic Acid to the ground KBr and mix well by grinding.
   iv. Press this mixture into a KBr pellet.

c) Acetylsalicylic Acid in KBr Pellet
   i. Grind 200 to 300 mg of KBr into a fine powder using a mortar and pestle.
   ii. Grind 1 to 2 mg sample of Acetylsalicylic Acid into a fine powder.
   iii. Add the Acetylsalicylic Acid to the ground KBr and mix well by grinding.
   iv. Press this mixture into a KBr pellet.

d) Commercial Aspirin Tablet in KBr Pellet
   i. Grind 200 to 300 mg of KBr into a fine powder using a mortar and pestle.
   ii. Grind 1 commercial aspirin tablet into a fine powder.
   iii. Add 1 to 2 mg of the powdered aspirin tablet to the ground KBr and mix well by grinding.
   iv. Press this mixture into a KBr pellet.
2. Obtain IR Spectra

Consult your instructor as to the operation of the Perkin-Elmer 1600 Series FTIR. Obtain all spectra using the following parameters unless otherwise noted. Save all scans to a 3.5” floppy disk.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>2.0 cm⁻¹</td>
</tr>
<tr>
<td>Apodization</td>
<td>Strong</td>
</tr>
<tr>
<td>Range</td>
<td>4400 cm⁻¹ to 450 cm⁻¹</td>
</tr>
<tr>
<td>Mode</td>
<td>Ratio</td>
</tr>
<tr>
<td>Number of Scans</td>
<td>4</td>
</tr>
</tbody>
</table>

a) IR Background Spectrum

Obtain a background IR spectrum without a sample in the instrument.

b) Polystyrene Calibration Film Spectrum

i. Place the polystyrene calibration film in the sample holder and obtain the spectrum at a resolution of 16.0 cm⁻¹.

ii. Change the resolution back to 2.0 cm⁻¹ and obtain another spectrum of the polystyrene calibration film.

c) Blank KBr pellet Spectrum

Place the blank KBr pellet in the sample holder and obtain the spectrum.

d) Salicylic Acid Spectrum

Place the salicylic acid/KBr pellet in the sample holder and obtain the spectrum.

e) Acetylsalicylic Acid Spectrum

Place the acetylsalicylic acid/KBr pellet in the sample holder and obtain the spectrum.

f) Commercial Aspirin Spectrum

Place the aspirin /KBr pellet in the sample holder and obtain the spectrum.

g) Dispose of rinse acetone properly.
Include in the Report:

1. Sketch the main components of an FTIR infrared absorption spectrophotometer.

2. Hand in copies of all spectra acquired during this experiment.

3. Explain why the IR Background spectrum has peaks even though the sample chamber was empty. (See Figure 16-9, pg. 397 of text)

4. Compare the polystyrene calibration film spectra obtained at a resolution of 16.0 cm\(^{-1}\) and 2.0 cm\(^{-1}\). What are the differences?

5. Compare the polystyrene calibration film spectrum obtained at a resolution of 2.0 cm\(^{-1}\) with the spectrum appearing in the text in Figure 16-1, page 382.

6. Examine the salicylic acid spectrum. Using the structure of salicylic acid below and Table 17-2, pg. 410 in the text, identify the major peaks in the spectrum.

7. Examine the acetylsalicylic acid spectrum. Using the structure of acetylsalicylic acid below and Table 17-2, pg. 410 in the text, identify the major peaks in the spectrum.

8. Examine the commercial aspirin spectrum and determine spectral contributions from salicylic acid and acetylsalicylic acid.
CHL311 Instrumental Analysis Laboratory

Qualitative Cyclic Voltammetry

Introduction

Cyclic Voltammetry (CV) is a versatile electrochemical method used to observe the reduction/oxidation (redox) behavior of chemicals in solution. CV consists of cycling the potential of an electrode in an unstirred solution and measuring the resultant current. A cyclic voltamogram is obtained by measuring current at the “working” electrode versus the applied potential of this electrode with respect to a reference electrode. This experiment will examine the redox couple of hexacyano-iron:

$$\text{Fe}^{\text{III}}(\text{CN})_{6}^{3-} \rightarrow \text{Fe}^{\text{II}}(\text{CN})_{6}^{4-}$$

When the potential becomes sufficiently negative to reduce $\text{Fe}^{\text{III}}(\text{CN})_{6}^{3-}$, “cathodic current” (electrons leaving the electrode) will be measured at the working electrode. Since the solution is not stirred, the current will peak and then decrease due to the decrease of $\text{Fe}^{\text{III}}(\text{CN})_{6}^{3-}$ in the vicinity of the electrode surface. When the potential is slewed back toward the positive potential, the $\text{Fe}^{\text{II}}(\text{CN})_{6}^{4-}$ that had accumulated at the electrode surface will be oxidized back to $\text{Fe}^{\text{III}}(\text{CN})_{6}^{3-}$ by the flow of an “anodic current” (electrons entering the electrode). By this procedure, CV is capable of rapidly generating new oxidation states of species during the forward scan and probing them on the reverse scan.

Some of the important parameters in a CV measurement are the anodic and cathodic peak current ($i_{pa}$ and $i_{pc}$) and the anodic and cathodic peak potentials ($E_{pa}$ and $E_{pc}$). A redox couple where both species rapidly exchange electrons with the electrode is called an “electrochemically reversible” couple. The stoichiometric number of electrons ($n$) transferred in the electrode reaction for a reversible couple can be determined from the separation between the peak potentials:

$$\Delta E_v = E_{pa} - E_{pc} \approx 0.059/n$$

Thus, a one-electron process should have a $\Delta E_v$ of approximately 0.059 V. Slow electron transfer will lead to increased peak separation and electrochemical irreversibility. A second condition of electrochemical reversibility is that $i_{pa} = i_{pc}$. 
The peak current for a reversible system is described by the following equation:

\[ i_p = 2.69 \times 10^5 \; n^{3/2} \; A \; D^{1/2} \; C^{1/2} \]

where  
- \( i_p \) = peak current (in Amps)  
- \( n \) = electron stoichiometry  
- \( A \) = electrode area (the electrode is 1.6 mm in diameter)  
- \( D \) = diffusion coefficient (cm\(^2\)/s)  
- \( C \) = concentration (mol/ml)  
- \( v \) = scan rate (V/s)

**Sample Preparation**

1. Prepare 100 mL of 1.0 M KNO\(_3\)
2. Prepare 50 mL of 2 mM K\(_3\)Fe(CN)\(_6\) in 1 M KNO\(_3\)

**CV Sample Analysis**

1. See your instructor on operation of the Bioanalytical Systems (BAS) cyclic voltammetry apparatus.
2. Fill the cell with 1.0 M KNO\(_3\). Deoxygenate the sample and stir.
3. Assemble the CV instrument with a platinum working electrode (1.6 mm diameter), an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode. The working electrode may need polished before use. The solution should be stirred between experiments and should not be stirred during experiments.
4. Set the following scan parameters:
   - Initial E: 750 mV  
   - High E: 750 mV  
   - Low E: 0 mV  
   - Scan Rate: 20 mV/s  
   - Sweep segments: 2  
   - Scan Sensitivity: 10 \( \mu \)A/V
5. After deoxygenating, turn off N\(_2\) flow and make certain that all bubbles are removed from the electrode surfaces.
6. Obtain a scan of the electrolyte solution (KNO\(_3\)). Save the data under an appropriate filename and print the data.
7. Clean the cell and rinse the electrodes. **(Do not lose the small stir bar)**
8. Fill the cell with the 2 mM K$_3$Fe(CN)$_6$ solution.

9. Following the same deoxygenation procedure, obtain a CV of this solution. Save the data under an appropriate filename and print the data.

10. Change the scan rate and obtain CVs at the following scan rates (in mV/s):

    20
    50
    75
    100
    125
    150
    175
    200

    Save the data under an appropriate filenames and print all data.

11. Clean the cell and rinse the electrodes.
Report:

1. Sketch the main components of the Cyclic Voltammetry Apparatus.

2. Include all CV data plots in the report.

KIO₃ Solution

3. Why does the CV of the KIO₃ contain no peaks?

2 mM K₃Fe(CN)₆ Solution

4. Construct a table of the data $E_p$ (both anodic and cathodic) and $i$ (both anodic and cathodic) as a function of scan rate.

5. Plot the anodic current and cathodic current versus the square root of scan rate ($\nu^{\frac{1}{2}}$).

6. Based on the separation of $E_{pc}$ and $E_{pa}$ and the relative magnitudes of $i_c$ and $i_a$, determine whether this system is electrochemically reversible.

7. Using the slope of the plot obtained in step 5, calculate the diffusion coefficient for the system.

8. Why is it important not to stir the sample during a CV experiment but to stir between experiments?

9. Why is the current measured in a CV “limited” as the voltage is scanned?
CHL311 Instrumental Analysis Laboratory

Qualitative GC-MS Laboratory

Introduction

Gas Chromatography – Mass Spectrometry is one of the most useful techniques available for compound identification and analysis of complex mixtures. Therefore, GCMS is widely used in organic, biochemistry, environmental, analytical, and pharmaceutical chemistry. The mass selective detector has replaced most other detectors for common GC work. In this lab, you will learn the operation of the HP GCD instrument and analyze a sample of gasoline and its components.

GC/MS Analysis of Gasoline

The government has required gasolines to be formulated to reduce emissions. New fuel detergents and oxygenates are added. Regulations have also mandated the amount of aromatic components that can be present in fuel. While fuels with high aromatic content generally have higher octane ratings and lower vapor pressures than aliphatic hydrocarbon fuels, they have been linked to higher particulate emissions leading to increased pollution and costly engine repair. In this experiment, the main components of gasoline will be examined.

Method:

Note: Gasoline is toxic and contains many highly volatile and flammable components. Handle only in the hood and do not breath any vapors or expose vapors to flame.

Take a 10 µL sample of gasoline and dilute 1:1000 with methylene chloride. Inject 1 µL of the diluted sample into the GCMS with the following parameters:

- Solvent Delay: 3 minutes
- Initial Temperature: 45 °C (hold for 1 minute)
- Rate: 15 °C/min
- Final Temperature: 210 °C (hold for 10 minutes)
- Flow Rate: 1.0 mL/min

Using the library functions of the HP GCMS, identify as many of the peaks as you can. Run a chromatogram of the clean solvent for a background.
Report:

1. Sketch the main components of the GCMS.
2. Hand in copies of chromatograms and mass spectra.
3. What are the main aromatic components of gasoline?
4. What other non-aromatic components are present?
5. What types of detectors are available for a GC?
6. What is the advantage of using MS over other types of detectors?
CHL311 Instrumental Analysis Laboratory

Qualitative HPLC Laboratory

Introduction

Caffeine is one of the most widely consumed drugs in the world. Its stimulant effects are well known and no doubt contribute to its popularity. Caffeine finds its way into many of our everyday food and pharmaceutical products. From coffee, tea, and cola to some analgesics and decongestants, caffeine is added for some obvious and not so obvious reasons. This lab will introduce you to High Performance Liquid Chromatography (HPLC).

Equilibration of Column

This analysis will employ a LiChrosorb RP8, reverse phase column that is stored in methanol (MeOH). The mobile phase for the experiment is a 30/70 water/methanol mixture. The first step is to change the solvent in the column from methanol (reservoir A) to the mobile phase (reservoir B) using a flow rate of 0.8 mL/min. The column should be equilibrated for 15 minutes.

Preparation of Caffeine Samples:

While the column is equilibrating, prepare one, 25-mL, caffeine standard having a concentration of 50 ppm. Use a 30/70 - water/methanol mixture as the solvent.

Recall 1 ppm \(\approx 1 \mu g/mL\)
Caffeine: FW 194.19 g/mol

Prepare one 25-mL sample of Diet Coke® using the following procedure:

1. Filter 5 mL of degassed Diet Coke® through a 0.45 \(\mu\)m disposable filter cartridge.

2. Place 2.5 mL of the filtered Diet Coke® sample into a 25-mL volumetric flask.

3. Bring the 25-mL volumetric flask to volume with 30/70 – water/methanol mixture.

Chromatography
1. The HPLC time program and parameter program should be set up to pump 100% mobile phase (reservoir B) at 0.8 mL/min throughout the entire experiment. Use a chart speed of 2 cm/min.

2. Load 20 µL of the caffeine standard into the injection loop. Start the recorder. Inject the sample and mark the beginning of the chromatogram on the chart paper using the MARK button on the detector.

3. When the sample has eluted, check the chromatogram to make sure that the height of each peak is sufficiently large on the chart paper (greater than 40, but less than 100 on the chart paper scale). If necessary, repeat the injection until a satisfactory chromatogram is achieved by varying the AUFS (arbitrary units/full scale) setting on the detector. The AUFS setting may be adjusted during the course of the experiment, but should not exceed a value of 1.024. Note the AUFS setting for all chromatograms.

4. Obtain a total of three (3) chromatograms of the 20-µL caffeine sample.

5. Adjust the sample volume to 10 µL and obtain three (3) additional chromatograms.

6. Load 20 µL of the diluted/filtered Diet Coke® into the injection loop. Start the recorder. Inject the sample and mark the beginning of the chromatogram on the chart paper using the MARK button on the detector. You may need to adjust the sample volume to obtain a quality chromatogram.

7. Obtain a total of three (3) chromatograms of the Diet Coke® sample.
Report:

1. Sketch the main components of the HPLC.

Caffeine Standard

2. Label each chromatogram. Determine the retention times of each caffeine peak. Submit labeled chromatograms with report (photocopies are acceptable). How do the retention times vary with the amount of caffeine sample injected?

3. Report your experimental details: how the sample was made, injection volumes, etc.

4. How repeatable are the peak heights? How do the peak heights vary with the amount of caffeine in the sample?

5. One method of determining the area under the peak on a chromatogram when using a strip-chart recorder is called triangulation. In the triangulation process, the peak is estimated to be a triangle and the area is calculated as (1/2) x (Peak Height) x (Peak Base Width). Refer to Figure 26-6 in the course textbook for an illustration of how to determine peak height, peak base width, and retention time of a symmetric chromatographic peak. Calculate the area of each of the caffeine peaks using this method. How repeatable are the peak areas? How do the peak areas vary with the amount of caffeine sample injected? Can you suggest a more accurate method for determining the area under the peaks?

Diet Coke® sample

6. Label each chromatogram. Determine the retention times of each peak observed in the chromatogram. Using the retention time of the caffeine standard, identify the caffeine peak in the Diet Coke® sample.