UNIVERSITY OF MASSACHUSETTS LOWELL
DEPARTMENT OF CHEMISTRY

Analytical Chemistry Laboratory II
Instrumental Analysis 84.316
Spring Semester

Instructor: Dr. David K. Ryan

Introduction

General information

Each week, before the experiment starts, we will meet in the analytical laboratory, Olney 309. We will prepare all the solutions needed in this room, and then the experiment may be conducted where the instrument locates and will be performed in groups. The experiments are designed to take no longer than 4 hours each, if you are prepared.

Laboratory Safety Rules

Even though every wet chemical analytical experiment to be carried out in the lab has been tested many times before and every procedure has been known in details, laboratory safety CANNOT be overlooked. Accidents do happen from time to time. In order to protect you, your classmates, and the university properties, the following rules will be enforced AT ALL TIMES.

1. Be acquainted with the location and use of facilities and familiar with safety precautions and procedures.
2. Students must wear adequate eye protection at all times. Contact lenses are never permitted to be worn in the laboratory as vapors can collect under the lenses.
3. Familiar with the method of operations and all potential hazards involved before engaging in any lab work. Know the properties, such as flammability, reactivity, corrosiveness, toxicity, etc., of the chemicals you are using.
4. Responsible behavior is required at all times.
5. No eating, drinking, or smoking in the laboratory.
6. Proper clothing (coverage from the shoulders to the knees) must be worn in the laboratory. No sandals or open-toed shoes are permitted.
7. Do not fill pipettes by mouth suction.
8. Do not use glassware that has been chipped or broken.
9. Dispose chemicals according to an approved procedure. Do not dump them down the sink.
10. Report all accidents including minor injuries to your TA.
11. Clean everything you used and wash your hands thoroughly before leaving the laboratory.

Lab reports

Reports are due after one week of completion of the experiment and should be handed in at the beginning of the following lab period. Though experiments are to be performed by groups, lab reports and unknowns are to be done on an individual basis. Lab reports should be prepared using a word processor using 8.5" x 11" paper and pages should be numbered. The format should be similar to a journal article (see *Analytical Chemistry*) with some differences in emphasis due to the nature of the course. More instrumental details than one normally finds in journal articles should be included in lad reports. The general format includes following sections:

- **Title Page** including the title of the experiment, your name, and the date on which the experiment were performed.

- **Abstract** summarizing the work done and reporting major results, including numerical results, instrumental technique used, instrument used, and the result of the analysis of the unknown. This is not an introduction or purpose. The abstract is very important and should only be written after your results have been evaluated completely.
- **Introduction** describing the basis for the experiment. In general, present the theory behind the technique utilized. Keep your theory pertinent to the actual measurements taken, use your own words, and use reference where appropriate. Also a block diagram of the instrument to be used should be presented. Cite your source of this diagram correctly and a description of its components and how it works should be provided.
- **Experiment** brief outline of procedure, noting where different than the lab manual: Write in past tense in complete sentences. Follow examples shown in the journal *Analytical Chemistry*. You do not need a great deal of information here; especially avoid presenting step-by-step instructions or directions. Describe equipment used including manufacturer and model, preparation of solutions, etc. Less than one page should almost always be sufficient.
- **Results** including tables of data, graphs or figures, and data analysis. A description of the data presented in tables, figures and calculations should be included to increase clarity of reading.
    - Tables should be numbered consecutively and consist of a row and column format, with a title at the top of the tables. Tables should be designed for ease and clarity of reading.
    - Figures should be numbered consecutively with a title at the bottom of the figure. The title should not just be axes labeled on the figure. The X- and Y-axes should be labeled, including units. All lines should be determined by linear regression. Data points should be made with distinct symbols.
    - Data analysis should include the determination of the concentration of the unknowns and the equation for any linear regression curves that are obtained.
    - Any spectra or chromatogram obtained directly from the instrument (or a copy of that) should be attached at the end of the report and refereed to in your result section. Chromatogram should be taped to 8.5 by 11 paper if not already in that format. On the spectrum or chromatograms, mark all pertinent information: sample identity and concentration if known, wavelength (for fluorescence and UV-Visible), absorbance (for UV-visible), chromatography method condition, etc.
- **Discussion** containing the following:
    - Any observations you make during the experiment,
    - A discussion relating your results to the theory,
    - A brief summary of any sources of error associated with results, and
    - Answers to the numbered questions in the lab manual.
- **Conclusion** including some brief remarks on the experimental results.
- **References** should be in the style of the current chemical literature. Each reference is numbered according to when it is first used and thereafter is referred to by that number. The references are listed in order at the end of the report.

Grading

Absences from the lab will result in no points for the scheduled experiment. There will be no make-up labs. 5-point penalty will be exercised for each day late. Each lab report counts 10 % toward the total, and the total counts 80 % of your final grade, your lab notebook counts the other 20 %. Grading of the lab report is as following:

Abstract	10
Introduction and theory	20
Experiment	15
Result	25
Data analysis 10	
Graphs and tables 10	
Discussion and questions	25
References	5

Lab notebook

The importance of a good lab notebook cannot be overemphasized. Your lab notebook should include enough information that another experimenter familiar with the field can readily discern what was done and, if necessary, repeat the work. Even if an observation seems trivial, record it. Later experiments may reveal that this observation was significant.

All entries into your notebook should be in pen. Pencil is unacceptable. The pages should be numbered consecutively and several pages at the front should be left blank for on-going table of contents. With each experiment start a new page in your lab notebook and sure to clearly identify each experiment with a title and date (each subsequent page should also list the date of the experiment). Each instrument or piece of equipment used in the experiment used in the experiment should also be documented in your lab notebook. In addition, the structure of any important compounds used should be identified, and any reaction should be shown as well.

Do not write over numerical entries. Cross out neatly and write correct version nearby. An example of each type of calculation should be shown, and the final answer should always be rounded to the appropriate number of significant figures. No results should be rejected unless there is a valid reason for doing so. The reason should be documented.

Include a brief result and discussion section at the end of each experiment. All spectra and chromatograms should also be kept in your lab notebook.

Video

An instructional video may be played before each experiment period. In each video, a brief introduction to the instrument is given, followed by a step by step instrumental operation and ends with a discussion on the experiment that is performed for that week. Please do not be late to your lab period as you may miss the video.

List of Experiments

Determination of Caffeine and Acetylsalicylic Acid in an Analgesic Tablet by Ultraviolet Spectrophotometry

Reference

Skoog, Holler & Nieman. Principles of Instrumental Analysis, 5th Ed., 1998.

Introduction

In this experiment, you will determine the amount of caffeine and acetylsalicylic acid in an analgesic tablet by UV spectrophotometry. Many molecules absorb ultraviolet or visible light. When an atom or molecule absorbs energy, electrons are promoted from their ground state to an excited state.

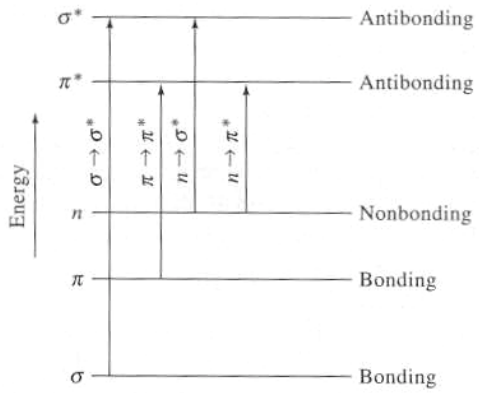


Figure 1 Molecular energy levels (Skoog et al, 1998)

Absorption spectrometry involves measuring the fraction of light of a given wavelength that passes through a sample. When a monochromatic light beam passes through a layer of solution with a thickness b and a concentration c of an absorbing species, as the consequence of interactions between the photons and absorbing particles, the power of the beam is attenuated from P_o to P. The absorbance A of a solution is defined by the equation:

$$A = \log (P / P_o) = abc$$

This is Beer's law; where a is a proportionality constant called *absorptivity* and b is the path length of the light beam through the absorbing medium. When the c is expressed in M (moles per liter), and b in cm, a is called the *molar absorptivity* and is given the special symbol ε, with the units of L cm^{-1} mol^{-1}. Thus,

$$A = \varepsilon bc$$

Absorbance is an additive parameter. That is, the total absorbance, A_t of a sample containing more than one component that can absorb the same wavelength is the sum of the individual absorbance, A_i:

$$A_t = \Sigma A_i$$

The additive characteristic of *A* enables absorption measurement to be applicable to multi-component analysis without separation of the components using simultaneous equations to determine the concentrations. In this case it is necessary that the absorptivity of the substances be different at various wavelengths but not necessary zero for one of the components.

In this experiment, there are two components caffeine and acetylsalicylic acid) in the analgesic tablet. You will first determine the molar absorptivity ε of each component by constructing a calibration curve (absorbance vs. concentration) with standard solutions. Then by measuring absorbance of the tablet solution at maximum absorption wavelength of both components, you will be able to figure out the amount of each component in the tablet.

CHEM2000 UV-vis Spectrophotometer

The CHEM2000 UV-vis Spectrophotometer Systems utilize the optical bench of Ocean Optic's second-generation miniature fiber optic spectrometer, the S2000, by mounting it onto an A/D converter and turning the system into a PC plug-in spectrometer. The CHEM2000 UV-vis consists of four basic elements: the PC2000 UV-vis PC Plug-in Fiber Optic Spectrometer (200-850 nm), a miniature deuterium tungsten light source with integrated cuvette holder, a 300-∝m solarization resistant optical fiber, and OOIChem operating software.

The light source supplies light to the sample. The light transmitted through the sample is collected and sent to the spectrometer via the fiber. The spectrometer measures the amount of light at each wavelength in the sampled spectrum. The A/D converter, on which the spectrometer is mounted, transforms the analog data from the spectrometer into digital information that is passed to a computer. Finally, the software performs basic acquisition and display functions on your data.

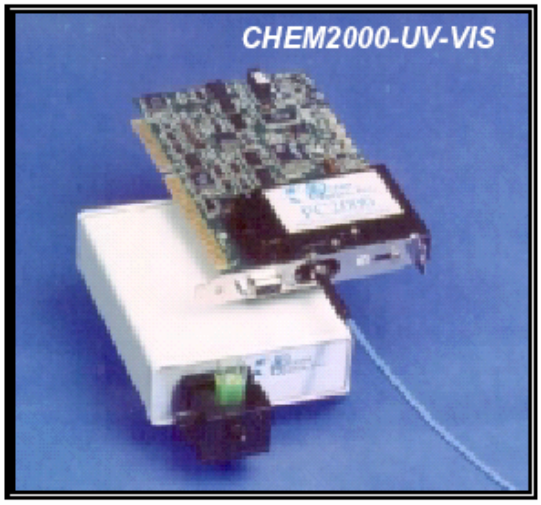


Figure 2 CHEM2000-UV-VIS Spectrophotometer

Equipment Needed

CHEM2000-UV-VIS Spectrophotometer

Volumetric flask, 50 mL
Pipets: 3 mL or 5 mL

Reagent Needed

Methanol, reagent grade
Caffeine
Acetylsalicylic acid
Analgesic tablet

Preparation of solutions

1. *Caffeine stock solution* – Dissolve 0.024 g of caffeine in 50 mL volumetric flask with methanol and dilute to the mark.
2. *Acetylsalicylic acid* (ASA) stock solution – Dissolve 0.024 g of ASA in 50 mL volumetric flask with methanol and dilute to the mark.
3. *Analgesic sample solution* – Dissolve 0.06~0.09 g of an analgesic tablet or the content of an analgesic capsule with 20 mL of methanol in 50 mL volumetric flask with methanol and dilute to the mark. Make three sample solutions out of three tablets.

Procedure

1. Prepare working standards and sample from the stock solutions in the following manner:

Standard no.	Caffeine stock added (mL)	ASA stock added (mL)	Total volume (mL) Diluted w/ methanol
ASA1	-	0.5	50
ASA2	-	1.0	50
ASA3	-	1.5	50
ASA4	-	2.0	50
ASA5	-	2.5	50
Caffeine1	0.5	-	50
Caffeine2	1.0	-	50
Caffeine3	1.5	-	50
Caffeine4	2.0	-	50
Caffeine5	2.5		50
Test	0.5	1.0	50
Sample1	2.5 mL of sample solution 1		50
Sample2	2.5 mL of sample solution 2		50
Sample3	2.5 mL of sample solution 3		50

2. Run the spectra for ASA5 and Caffeine5; determine the wavelength of maximum absorbance (λ_{max}) for each chemical.
3. For each solution in the table, measure the absorbance at the two selected wavelengths.

Data Treatment

1. Tabulate the absorbance at the two selected wavelengths and concentration for the ASA and caffeine standard solutions.

2. Plot absorbance vs. concentration (M) for both caffeine and ASA at the two selected wavelengths. Determine molar absorptivity for each component at those two wavelengths from the calibration curve.
3. Calculate the expected absorbance for test at each wavelength and compare to that of the experimental values.
4. Determine the weight percent of each component in the original analgesic tablets and report the standard deviation.
5. Compare your result with the amount labeled on the bottle and report the precision of your results.

Questions

1. Would benzene be a satisfactory solvent for the analysis?
2. If a pharmaceutical mixture has three components with different maximum absorbance, comment the possibility of determining all three components simultaneously.
3. Would it be more desirable to use spectra grade methanol for the blank solution? Justify your answer.

Operation of Ocean Optics Chem2000-UV-VIS

1. Turn the light source power on. The switch is located at the back oh the cuvette holder. Allow half an hour for the lamp to warm up before the experiment starts.
2. Launch OOIBase32.
3. Make sure you are in scope mode, by either clicking the **Scope Mode** icon on the toolbar, or selecting **Spectrum | Scope Mode** from the menu. Make sure the signal is on scale. Maintain the intensity of the reference signal at about 3500 counts by adjusting the integration time. Take a reference spectrum by first making sure nothing is blocking the light patch going to your sample. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Store Reference** icon on the toolbar or selecting **Spectrum | Store Reference** from the menu.
4. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your sample, but do not turn off the light source. Take the dark reading by clicking the **Store Dark** icon on the toolbar or selecting **Spectrum | Store Dark** from the menu.
5. Begin an absorbance measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then choose the **absorbance mode** or select **Spectrum | Absorbance Mode** from the menu. By moving the cursor along the wavelength axis, you can take the absorbance reading at any wavelength. To save spectrum, click the **Save** icon on the toolbar or select **File | Save | Processed** in the menu.
6. When finished, exit OOIBase32, turn the light source power off. Clean the cuvette with solvent and tidy up the space around before leaving.

Note

If at any time any sampling variable changes—including integration time, averaging, boxcar smoothing, distance from light source to sample, etc.—you must store a new reference and dark spectrum.

Spectrophotometric Determination of Formula and Stability Constant of Complex Ions

Reference

Sawyer, Heineman & Beebe, *Chemistry Experiments for Instrumental Methods,* 1984.

Introduction

Spectrophotometry is a valuable tool for elucidating the composition of complex ions in solution and for determining their formation constants. The power of the technique lies in the fact that quantitative absorption measurement can be performed without disturbing the equilibria under consideration. The three most common techniques employed for complex-ion studies are (1) the methods of continuous variations, (2) the mole-ratio method, and (3) the slop-ratio method. In this experiment, you will determine the formula and stability constant of Fe (II)-2,2-bipyridyl complex.

Mole-Ratio Method

In the mole-ratio method, the absorbance of a series of solution is measured in which the analytical concentration of one reactant (usually the metal-ion is held constant while that of ligand is varied. A plot of absorbance versus mole ratio of ligand to metal ion concentration gives a curve like the one shown in Figure 1 if only the complex absorbs. The straight-line portions are extrapolated to where they cross. The ratio at this point is the ratio of ligand to metal ion in the complex. The difference between the extrapolated values and actual values of the absorbance can be used for stability constant calculations.

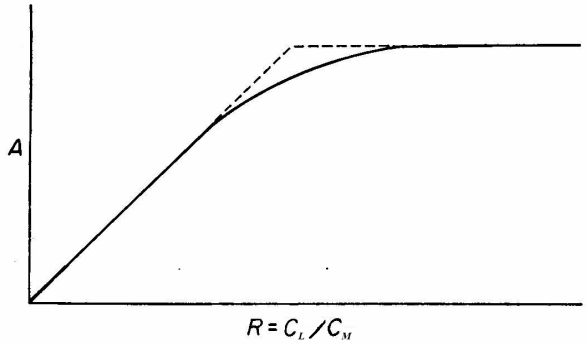


Figure 1 Mole – ratio Plot (Sawyer et al, 1984)

Determining the Stability Constant of a Complex

For the reaction:

$$xM + yL \leftrightarrow M_xL_y$$

we can write

$$C_M = [M] + x[M_xL_y]$$
$$C_L = [L] + y[M_xL_y]$$

where C_M and C_L are the molar concentration of M and L before reaction occurs.

The extrapolated values ($A_{expt.}$) near the "equivalence point" on the mole-ration plot correspond to the total absorbance of the complex if the complex formation was complete. Actually, the complex is slightly dissociated in this region, and the actual absorbance reading (A) is somewhat lower. When only the complex absorbs, and when the ligand is of limiting concentration,

$$A = \varepsilon_c \left[M_x L_y\right]$$

$$A_{\exp t.} = \varepsilon_c \left(\frac{C_L}{y}\right)$$

$$\frac{A}{A_{\exp t.}} = \frac{\left[M_x L_y\right]}{C_L / y}$$

where ε_c is the molar absorptivity of the complex. Then,

$$\left[M_x L_y\right] = \left(\frac{A}{A_{\exp t.}}\right)\left(\frac{C_L}{y}\right)$$

$$\left[M\right] = C_M - x\left[M_x L_y\right] = C_M - x\left(\frac{A}{A_{\exp t.}}\right)\left(\frac{C_L}{y}\right)$$

$$\left[L\right] = C_L - y\left[M_x L_y\right] = C_L - y\left(\frac{A}{A_{\exp t.}}\right)\left(\frac{C_L}{y}\right)$$

$$K = \frac{\left[M_x L_y\right]}{\left[M\right]\left[L\right]} = \frac{\left(A / A_{\exp t.}\right)\left(C_L / y\right)}{\left[C_M - x\left(A / A_{\exp t.}\right)\left(C_L / y\right)\right]\ \left[C_L - y\left(A / A_{\exp t.}\right)\left(C_L / y\right)\right]}$$

where K is the stability constant.

Equipment Needed

UV-VIS Spectrophotometer
Cuvettes, two
Graduated pipets
10 mL volumetric flasks
100 mL volumetric flasks

Reagent Needed

Ferrous ammonium sulfate
2-2"-bipyridyl

Preparation of solutions

1. 1.16×10^{-3} M ferrous ammonium sulfate [$(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$]---- dissolve 0.0455 g in 100 mL volumetric flasks with distilled water and dilute to mark
2. 1.16×10^{-3} M 2-2"-bipyridyl---- dissolve 0.0183 g in 100 mL volumetric flasks with distilled water and dilute to mark

Procedure

1. Pipet 1 mL aliquots of the Fe(II) solution into each of ten 10 mL volumetric flasks.
2. To the flasks, add successively 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 mL aliquots of bipyridyl solution. Fill each flask to the mark with distilled water.
3. Measure the absorbance of these 10 solutions at 522 nm (or) as close to this wavelength as possible on the spectrophotometer used). Use distilled water as the blank.

Data Treatment

1. Plot the absorbance versus the mole ratio of 2-2"- bipyridyl to Fe(II) concentration for the 10 solutions measured.
2. Determine the formula and stability constant for the Fe (II)-2-2"- bipyridyl from this graph.

Questions

1. Briefly describe how to determine the formula of Fe(II)-2-2"- bipyridyl with the method of continuous variations.

Determination of the Active Ingredient in a Pharmaceutical Preparation by Fluorometry

Reference

Skoog, Holler & Nieman. Principles of Instrumental Analysis, 5th Ed., 1998.

Introduction

In this experiment, you will determine the amount of aspirin in an analgesic tablet by fluorescence spectrometry.

Fluorescence is the emission of light by a molecule which has absorbed radiant energy. When an atom or molecule absorbs energy, electrons are promoted from their ground state to different vibrational energy levels of an excited state. The excited species can quickly give up its excess energy and relax to its ground state through nonradiative relaxation and radiative relaxation.

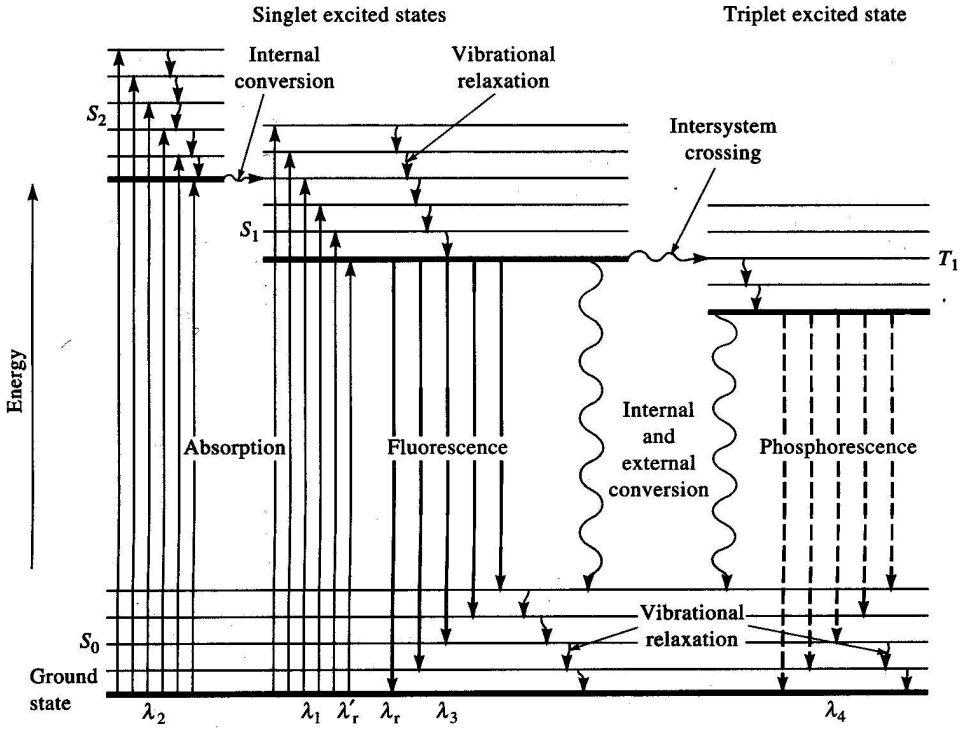


Figure 1 Energy diagram for photoluminescent molecules (Skoog et al, 1998)

There are two types of nonradiative relaxation, vibrational relaxation and internal conversion. Electrons in the excited state will drop to the lowest vibrational energy level via vibrational relaxation. Vibrational relaxation takes place during collisions between excited molecules and molecules of the solvent. Nonradiative relaxation between the lowest vibrational level of an excited electronic state and the upper vibrational level of another electronic state can also occur. This type of relation is called internal conversion. The mechanisms by which this

type of relaxation occurs are not fully understood. The net effect of nonradiative relaxation is a slight increase in the temperature of the medium.

Molecules can relax from the lowest vibrational energy level of the excited state to any of the vibrational energy levels of the ground state through fluorescence, in which the excess energy is given up as emission of light at a longer wavelength than absorbed. The other radiative relaxation process, phosphorescence, is not discussed here.

When Absorption $A < 0.05$, the relationship between fluorescence intensity and sample concentration can be expressed as:

$$F = 2.3\ K'\varepsilon bc\ P_o$$

where K' is a constant depends on the quantum efficiency of the fluorescence, εbc is the absorbance A, P_o is the power of the beam incident on the solution, F is the fluorescence intensity. At constant P_o,

$$F = Kc$$

where K is a new constant that is equal to $2.3\ K'\varepsilon b\ P_o$

A plot of the fluorescence intensity vs. concentration is used as calibration curve for quantitative analysis, and ideally should be linear if A is smaller than 0.05. Extremely dilute samples can be analyzed because of the high sensitivity of the method.

Equipment Needed

Fluorometer
Cuvette, one
60 mL separation funnel
Volumetric flasks, 50 mL, 100 mL
Beaker, 300-400 mL, 40 mL
Pipets, 1, 2, 5, 10 mL
Graduate cylinder, 10 mL

Reagent Needed

Na_2CO_3
NaOH
Salicylic acid
Chloroform
Aspirin tablet

Preparation of solutions

1. 1 % Na_2CO_3 - Dissolve 2.5 g of Na_2CO_3 in 250 mL H_2O.
2. 0.5 M NaOH - Dissolve 2 g of NaOH in water and dilute to 100 mL.
3. 100 ppm salicylic acid - dissolve 0.01 g of salicylic acid in 100 mL volumetric flask and dilute to mark with water.

4. Salicylic acid standard solutions, 1 ppm, 2 ppm, 5 ppm, 10 ppm - Transfer 1, 2, 5, 10 mL of 100 ppm Salicylic acid solution to four 100 mL volumetric flasks separately. Dilute all standards to mark with distilled water which has been adjusted to pH 11 with NaOH.
5. Aspirin tablet sample solution
   - Weigh three aspirin tablets and dissolve 0.1 g of each tablet in 20 mL of chloroform.
   - Using 60 mL separation funnel, extract each solution two times with 10 mL 1 % Na_2CO_3 and once with water. Collect the aqueous phase into 50 mL volumetric flasks. (Make sure that no organic phase is present in your aqueous phase collection.)
   - Add 20 mL of 0.5 M NaOH into the collected aliquots and dilute to the mark with water.
   - Transfer 0.4 mL of each solution into 100 mL volumetric flasks and dilute to the mark with a 1 % Na_2CO_3 solution.

Procedure:

1. Using 10 ppm standard to find the maximum wavelength for excitation and emission by scanning the excitation wavelength from 250 to 350 nm while holding the emission wavelength at 400 nm, then set the excitation wavelength at the maximum and scan the emission wavelength from 350 to 500 nm.
2. Set the excitation wavelength at the maximum as in step 1 and scan a blank of distilled water for zero emission from 350 nm.
3. Repeat step 2 to continue scanning emission standard solutions and aspirin tablet solutions. Record fluorescence intensity at maximum emission wavelength.

Data Treatment

1. In tabular form record the salicylic acid standards concentration and fluorescence intensity at maximum emission.
2. Plot a calibration curve of fluorescence intensity at maximum emission versus concentration of salicylic acid. Determine the concentration of salicylic acid obtained due to the hydrolysis of the aspirin tablet. Report the amount of aspirin in the tablet in mg/tablet, consider the dilution factor. Compare the results with the bottle- labeled.

Questions

1. Why the linear relationship between fluorescence intensity and concentration is lost when $A > 0.05$?
2. The radiation from fluorescence is measured 90^o from the excitation radiation. Explain the reason.

Operation of the Fluorometer

1. Turn on lamp power supply by depressing the **POWER ON** switch. Then ignite the lamp by depressing the **LAMP ON** switch; hold this switch until the lamp stays on. Then turn on the instrument.
2. Set scan rate to 50 nm/min; intensity range 0.1, time constant 3 sec.
3. Open the photomultiplier shutter by rotating the shutter knob counterclockwise to the stop poison at which the black dot points towards the monochromator.
4. Place the cuvette containing the sample in the cell holder and cover the sample chamber.

5. Select scan mode by depressing **EXC** or **EM.** Set emission and excitation wavelength at desired value by rotating the monochromator dials, and fix them by pressing the manual set knob.
6. Depress **SCAN ▲** or **SCAN ▼**, simultaneously start data acquisition on the computer with Lab Calc.
7. Repeat steps 4 to 6 for further samples; rinse the cuvette with solvent between samples.
8. At the end of the laboratory, close the photomultiplier shutter; set the instrument at Standby Mode by depressing the **STOP** key between **SCAN ▲** and **SCAN ▼**. Then turn off the lamp and power supply by depressing the **POWER ON** switch, wait a few minutes for the lamp to cool, and then turn off the instrument.

Collecting Data with Lab Calc

1. Turn on computer/monitor.
2. At DOS prompt C:\> type **lc** (lab calc), <enter>
3. After calibration is finished, press any key to continue.
4. Press **F2 = Menu**
5. Using arrow keys to highlight **Collect**, <enter>
6. Put down a run name, memo (if desired) and duration (in minutes, run time of data acquisition).
7. Using arrow keys to move down to **Start Run**, <enter>, simultaneously start Scan on the fluorometer.
8. Press **ESC**, the spectrum should be visible.
9. Press **F2** = **Menu**, select **Environment**, use arrow key to select **Limits** to scale X, Y axes.

FTIR: Comparison of Sample Preparation Techniques and Interpretation of Spectra of an Unknown

Reference

Skoog, Holler & Nieman. Principles of Instrumental Analysis, 5th Ed., 1998.

Introduction

In this experiment, you will be familiarized with the operation of FT-IR spectrometer and solid sample handling techniques, learn how to interpret IR spectra.

Infrared (IR) spectroscopy provides information concerning the vibrational modes within a molecule (see Figure 1). It is most commonly employed as a qualitative technique. In this technique, the Infrared radiation is absorbed and converted by a sample molecule into energy of molecular vibration. Infrared region is generally considered to be about from 10,000 cm^{-1} to 100 cm^{-1}; however, most instruments are limited to the mid-IR range between 4000-400 cm^{-1} where most application occurs. The IR spectra exhibit narrow, closely spaced absorption peaks resulting from transitions among the various vibrational quantum levels. The frequency of absorption depends on the masses of the atoms in the molecule, the force constants of the bonds, and the geometric structure of the molecule determine. Individual peaks can often be associated with specific chemical groups such as carbonyl, hydroxyl, cyano, and nitro groups. IR spectra then can be used to give information on the functional groups in a molecule, as well as the molecular structure as a whole.

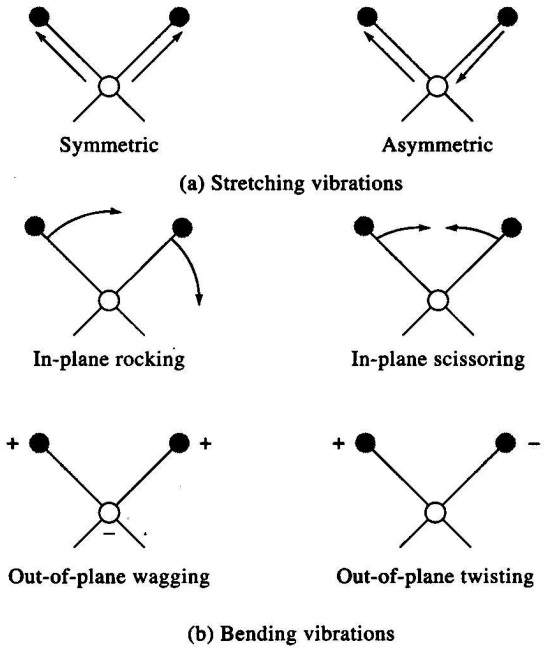


Figure 1 Types of molecular vibrations

You will use the Fourier transform (FT) IR spectrometer to collect the data in this experiment. The FT-IR instrument contains no dispersing element, and all wavelengths are

detected and measured simultaneously. In order to separate wavelengths, it is necessary to modulate the source signal in such a way that it can subsequently be decoded by a Fourier transformation, a mathematical operation that requires a high-speed computer. An interferometer is utilized to modulate the relatively high IR frequencies to a lower frequency signal that can be monitored by typical IR detectors.

Sample Preparation Techniques for IR

Infrared spectra can be obtained from samples in the solid, liquid, solution or gas phases.

A. Solid Samples

Three of the traditional methods to sampling solid materials are listed below:

1. Mull Technique - Suspending the finely ground sample in a mineral oil such as Nujol. The disadvantage of this method is the interfering C–H bond absorption frequencies.
2. Pellet Method - Suspending the solid in KBr pellet prepared under great pressure. This method is rapid and easy. The main disadvantage are the uncertainty in the appearance of the spectrum caused by the process of producing the pellet and the difficulty in avoiding some moisture pickup, which gives rise to interfering O-H bond frequencies.
3. Solution Methods - Solids are commonly run as solutions of about 5 % in some suitable solvent. There is no non-absorbing solvent in the IR region. The best solvents for IR use are non-polar, non-hydrogen liquids, such as CS_2, or CCl_4. A more polar solvent such as $CHCl_3$ is useful except that the C-H stretch region may be distorted.

B. Liquid Samples

Traditionally, liquid samples can be run either as the pure liquid if a cell of suitable thickness is available (approximately 0.02 mm) or as a solution in a longer cell (approximately 0.50 mm).

C. ATR micro-sampler

In this lab you will be introduced to a more modern sampling technique employing attenuated total reflectance (ATR) as shown in Figure 2. Small drops of liquid and minute smears of pastes can be placed directly on the ATR crystal. Coating on wire, paint chips, fabrics, powders, fibers, and miniature hard samples can be pressed against the crystal using the built-in pressure applicator. Softer samples can be compressed using either the built-in pressure applicator or one of the optional calibrated presses. The ATR micro-sampler makes sampling simple and straightforward.

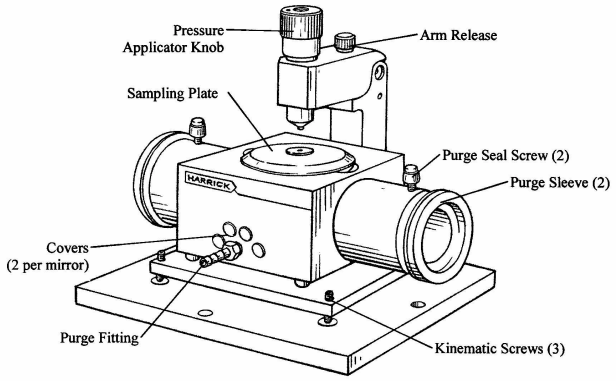


Figure 2 ATR micro-sampler, Bruker Tensor

Infrared radiation entering a prism made from a high refractive index infrared transmitting material can be totally reflected internally. This internal reflection creates an effect called the evanescent wave that extends beyond the surface of the crystal into the sample that is in contact with the crystal (Figure 3.). In regions of the infrared spectrum where the sample absorbs energy, the evanescent wave will interact with the sample and a spectrum can be obtained. This is the basis of sampling by ATR.

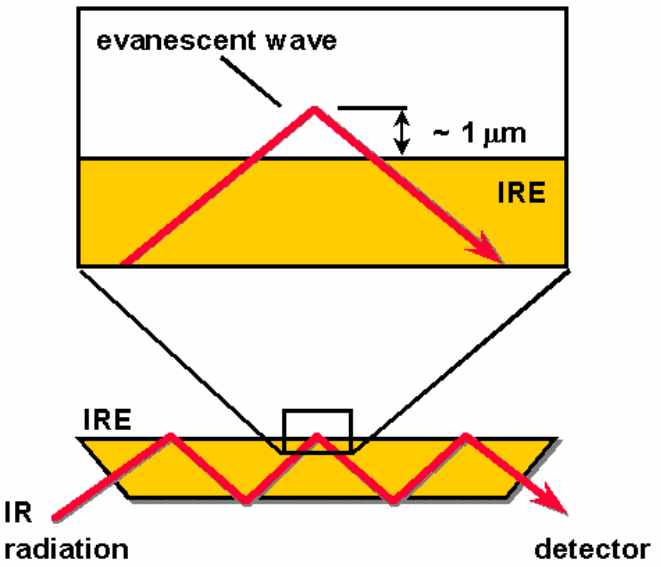


Figure 3 Total internal reflection at the interface of an internal reflection element (IRE, which is the ZnSe crystal in the ATR). Depth of penetration of the evanescent wave is approximately 1 µm. (http://www.micromemanalytical.com/ATR_Ken/ATR.htm)

In this experiment you will be obtaining the IR spectrum of solid benzoic acid via a KBr pellet, of the CCl_4 solution, and of the pure substance and using the ATR micro-sampler. The focus in analyzing the spectra lies in comparing the three sampling techniques. You will also obtain spectra of several unknown plastics and identify them.

Equipment Needed

KBr pellet die kit
Mortar and pestle
Liquid cell
FT-IR spectrometer with ATR accessory and transmission cell holder
Volumetric flask, one 50 mL
Plastic pipets

Reagent Needed

Solid benzoic acid, *pharmaceutical* grade
Solid KBr
CCl_4
0.10 M benzoic acid - dissolve 6.11g benzoic acid in 50.0 mL CCl_4
Unknown plastics

Procedure

Part A Obtain IR spectrum of solid benzoic acid via a KBr pellet

1. Place about 100-200 mg of powdered KBr in the mortar. Add to this about 2 spatula tips (no more than 1-2 mg) of benzoic acid. Mix well and grind together till uniform. Do all this quickly, as the KBr will absorb water from the atmosphere, and this makes it difficult to press a good pellet.
2. Put 1 bolt on the bottom of the pellet holder – note the shiny surface of the bolt, this will form the surface of the pellet so take care and do not scratch. Add about 30-50 mg of the mixture into the cavity. Screw second bolt on top and tighten both with the ratchets.
3. Then loosen and remove the bolts, leaving the pellet in place. Ideally it would be clear, uniform and unblemished; however, if light can shine through the pellet and it doesn't have a hole then it may still be usable. If you want to make another pellet simply rinse the press with water and then follow with acetone and try again.
4. Record a spectrum (after having already collected a background) by placing pellet holder with pellet in place into the transmission cell holder in sample chamber. Your instructor will show you how to navigate the software.

Part B Obtain IR spectrum of benzoic acid via CCl_4 solution

1. Clean the liquid cell with CCl_4.
2. Fill the cell with CCl_4, place it onto the transmission cell holder, and then record the spectrum as background.
3. Empty the liquid cell with a rubber bulb, then fill it with the benzoic acid solution. Record the spectrum.
4. Clean the cell with CCl_4, and dry it under slow flow of N_2. Return the cell to the desiccator after drying.

Part C Using the ATR Micro-Sampler

1. Remove the transmission cell platform and gently insert the ATR accessory. Record a background.
2. Place enough of the benzoic acid on the ZnSe crystal to completely cover the surface of it. You should not see any yellow of the crystal after it is covered. Keep the acid off the surrounding surface. Then gently lower the press onto it. You should feel that the press is tight against the crystal but not so tight as to crack it.
3. Record the spectrum.
4. Lift the pressure application knob; clean the crystal with CCl_4. Let air dry so as not to scratch the crystal.
5. Make 0.10 M benzoic acid solution--- dissolve 6.11 g benzoic acid in 50 mL volumetric flask with CCl_4 and dilute to mark.
6. Place one drop of CCl_4 on the surface of the crystal, record spectrum as background.
7. Place one drop of the benzoic acid solution on the surface of the crystal to completely cover it, record the spectrum.
8. Clean the crystal with acetone.

Part D Acquire spectra of the Unknowns

1. Obtain unknown plastics and a list of possible unknown from your instructor and make a note for the unknown numbers.

2. Record IR spectra of the unknowns using the ATR micro-sampler.

Data Treatment

Part A, B & C

Include all labeled spectra, making sure to indicate sampling technique. Point out differences between the techniques employed and the resulting differences in the spectra.

Part D

For each unknown, tabulate all the wavelengths of significant absorption (select the wavelengths of maximum absorption in the 2- to7 μm region). Deduce the possible chemical structure or functional group for each absorption. Try to match the unknown to the listed polymers given by your instructor, give reasons.

Questions

1. Explain the effect of tightening the pressure against the crystal.
2. Why use KBr to make the pellet? What material is usually used to make the liquid cell window? Why?
3. Discuss the limitations and advantages of each method.

(Note for TA: only performed the Pellet and the ATR method)

Determination of Fe and Zn in Pet Food by Flame Atomic Absorption Spectroscopy

Reference

1. John H. Kennedy, *Analytical Chemistry: Practice*, 1990
2. Sawyer, Heineman, and Beebe, *Chemistry Experiments For Instrumental Methods,* 1984
3. Skoog, Holler & Nieman. Principles of Instrumental Analysis, 5th Ed., 1998
4. Beaty, Richard & Kerber, Jack. Concepts, Instrumentation and Techniques in Atomic Absorption Spectrophotometry, Perkin Elmer Instruments.

Pre-Lab

You are required to read Appendix A before lab.

Introduction

Atomic spectroscopy is used for the qualitative and quantitative determination of trace metals. Sensitivities of atomic methods lie typically in 10^{-6} ppm to 10^{-9} ppm range. In atomic absorption spectroscopy measurement is made of the radiation absorbed by the non-excited atoms in the vapor state. In emission spectroscopy, measurement is made of energy emitted when atoms in the excited state return to the ground state.

Spectroscopic studies of atoms (or of elementary ions) with UV-VIS can be performed only in a gaseous medium in which the individual atoms or ions are well separated from one another. Several techniques are used to atomize the sample. The most widely used is the flame atomization technique. In flame atomic absorption spectroscopy, which is most widely used atomic method, the sample as an aerosol is passed into a 1700 – 3150° C flame, where it is instantaneously converted to an atomic vapor. Light which is characteristic of the element to be determined is shone into the flame; electronic transitions in the atomic state cause some of the incident light to be absorbed. The analyte concentration is determined from the amount of absorption. The most useful radiation source for atomic absorption spectroscopy is the *hollow – cathode lamp* (see Figure 1).

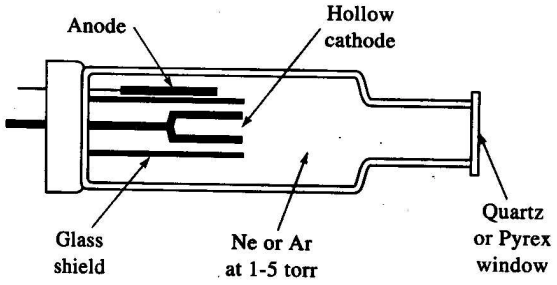


Figure 1 Cross Section of a Hollow Cathode Lamp (Skoog, 1998)

In this experiment, you will use a flame atomic absorption spectrophotometer to analyze the trace amount of Fe in dry cat food. Samples of pet food are "wet ashed" to decompose the organic constituents and solubilize the metal ions. Standard addition method is used instead of a calibration curve to circumvent determination errors caused by matrix effects.

Calibration by Standard Addition Technique

Ideally, the calibration standard is in a similar matrix to that of the sample solution. In the event standards cannot be prepared identical to the unknowns, the technique of standard additions should be used. For example, in an analysis for copper in urine, a known amount of copper is added to the sample so that it contains 10 μg/mL of additional copper. To another portion of sample, copper is added to give an additional 20 μg/mL, etc. After reading the absorbance of these solutions, a plot similar to Figure 2 is made. The line is extrapolated to the value for the copper concentration in the original urine sample.

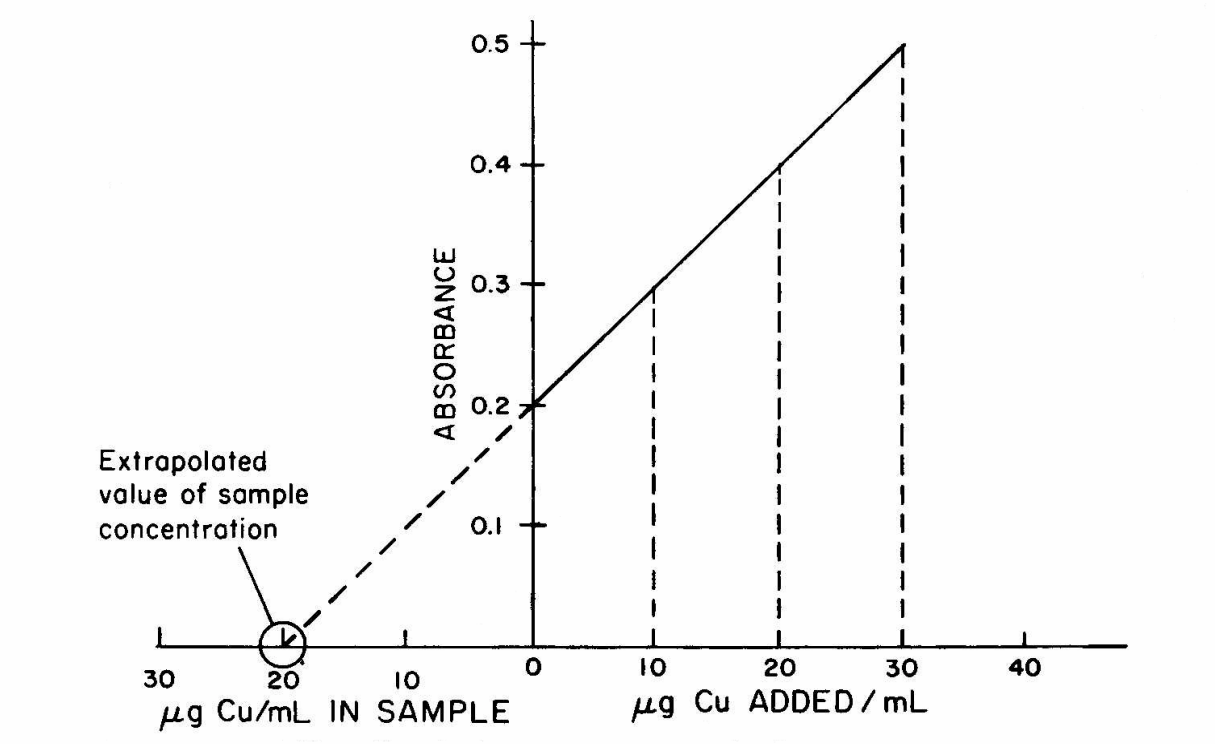


Figure 2 Results of standard addition technique for the determination of Cu in urine (Sawyer, 1984)

Equipment Needed

Atomic absorption spectrometer
Hot plate
Mortar and pestle
Volumetric flasks, 100 mL, 10 mL
Beakers, 20 mL
Graduate cylinder,
Volumetric pipets, 10 mL

Reagent Needed

Concentrated HNO_3
1000 ppm Fe and Zn standard solutions
Dry cat food

Preparation of Solutions

1. Standard solutions (10, 5, 1, 0.1 and 0.01 ppm) of Fe and Zn are prepared by diluting references solutions into 100 mL volumetric flasks and 10 mL of HNO_3 is added before diluting to mark with DI water.
2. Digestion of pet food sample:
   - Thoroughly grind about ~2 g of the dried pet food sample with a mortar and pestle if necessary.
   - To the ground pet food sample, add 10 mL HNO_3, and digest on a hot plate until fully digested or almost dryness. (some binder and fat may not digest completely)
   - Before dryness, add another 10 mL of HNO_3. After digestion, transfer an aliquot of the sample liquid (1 mL) quantitatively to a 100 mL volumetric flask and 10 mL of HNO_3 before diluting to mark with water. (sample diluted 100x)
   - Filter the sample through a polycarbonate filter to remove fat or particulate matter if necessary.
   - Prepare a method blank in the same way (DI water + 10 % of HNO_3).

Procedures

1. Prepare standards for Iron: (please consult with TA) (10, 5, 1, 0.1, 0.01 ppm)
2. Prepare standards for Zinc: (please consult with TA) (10, 5, 1, 0.1, 0.01 ppm)
3. Install the Fe hollow cathode lamp in the spectrometer.
4. Zero the instrument with the blank and aspirate the standard solutions successively, aspirating water between standards. Follow the operation procedures of the spectrometer.
5. Run the sample next, after a 10 fold dilution
6. Install the Zn hollow cathode lamp in the spectrometer
7. Zero the instrument with the blank and aspirate the standard solutions for Zn successively, aspirating water between standards. Follow the operation procedures of the spectrometer
8. Run your sample
9. Save the solutions for use of next laboratory (DCP).

Standard addition method for Fe and Zn:

With a pipet, transfer 5 mL aliquots of the sample solution (100x diluted) to four 10 mL volumetric flaks and add separately 1, 2 and 3 mL of the standard solution containing 1 ppm Fe and Zn and add 1 mL of HNO_3 before diluting to mark with DI water.

Plot the data in a manner similar to Fig 3. Extrapolate the line to value of the sample concentration. Once the concentration is obtained from the graph apply volume correction in order to calculate the corrected concentration of the unknown and report the amount of Na in food in micro gram per gram (μg/g). Compare your results with the amount obtained from the previous method.

Data Treatment

Draw calibration curve for both Fe and Zn respectively. From the calibration curve calculate the concentration of Fe and Zn present in your sample**.** Determine the quantity of Fe and Zn in the pet food in micro gram per gram (μg/g). Compare your results with the labeled value.

Questions

1. What are the other common atomization techniques except flame?
2. Why atomic absorption lines are so sharp compared to the UV absorption spectrum of a molecule dissolved in solution?
3. Both atomic emission and absorption spectra are effected in a complex way by variations in flame temperature. Why is control of flame temperature important in flame spectroscopy?

Operation of the Spectrometer

1. Install the appropriate hollow cathode lamp in the spectrometer.
2. Set **POWER** switch on the spectrometer to ON. Wait until Element Select Mode screen page is presented.
3. If required, key in the date and depress **DATE** soft key.
4. Key in the atomic number of the element to be determined and depress **ELEMENT** soft key. This step can be omitted when a coded source lamp is installed.
5. Select **FLAME MODE**.
6. Depress **Setup** mode key to put the spectrometer into setup mode.
7. Allow the instrument and the radiation source to warm up. Depress **Gain** function key to return the bar graph display to a mid-range value.
8. Depress **Prog** mode key to put the spectrometer into Flame Program Mode. Enter all required operating parameters.
9. Set the air cylinder outlet pressure at 58 psi, nitrous oxide at 58 psi, acetylene at 15psi.
10. Depress **Cont** mode key to put the spectrometer into Continuous Mode.
11. Set up and ignite the flame.
12. Optimize the burner, the nebulizer and the gas flow rates.
13. Depress **Run** mode key to put the spectrometer into Flame Run Mode.
14. Aspirate a blank solution and depress **AUTOZERO** soft key.
15. Aspirate the first sample solution and depress **Read** key.
16. Repeat step 15 for all further sample solutions. Aspirate water between samples.
17. At the end of the laboratory, aspirate water for several minutes. Then shut the flame, turn off all the gas line, and set the **POWER** switch on the spectrometer to OFF.

Note for TA only: Standard Addition method is usually performed in 2nd week if 2 groups are rotated. If coupled with DCP, reference standards should contain all 5 metals and save solutions to be used for both weeks.

Determination of Cu, Fe, Mn, Na and Zn in Pet Food by Direct Current Plasma Atomic Emission Spectroscopy

Reference

1. Skoog, West & Holler, Fundamentals of Analytical Chemistry, 1992.
2. Beaty, Richard & Kerber, Jack. Concepts, Instrumentation and Techniques in Atomic Absorption Spectrophotometry, Perkin Elmer Instruments.

Pre-Lab

You are required to read Appendix B before lab.

Introduction

In last experiment, you determined the quantity of Fe and Zn in pet food by atomic flame absorption spectroscopy; in this experiment direct current plasma atomic emission spectroscopy (DCP) will be used and more elements are analyzed.

In plasma atomic emission spectroscopy, the analyte atoms in solution are aspirated into the excitation region where they are vaporized and atomized by plasma. The plasma (temperature can be as great as 10,000K) provides sufficient energy to promote the atoms into high energy levels. The atoms decay back to lower levels by emitting light. By definition, plasma is a conducting gaseous mixture containing a significant concentration of cations and electrons. In the argon plasma employed for emission analysis, argon ions and electrons are the principle conducting species. Three power sources have been employed in argon plasma spectroscopy: (1) direct current (dc) electrical source, (2) radio-frequency, or inductively coupled plasma source, and (3) microwave-frequency generator. Figure 1 is a diagram of a dc plasma source which will be used in this experiment.

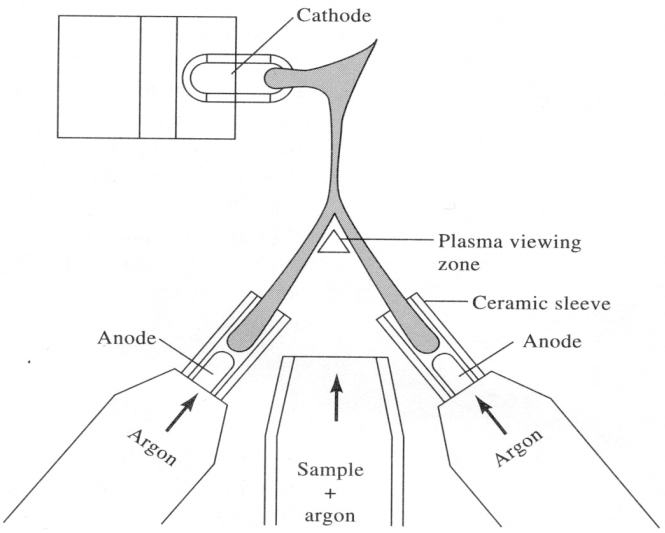


Figure 1 A three electrode dc plasma jet (Skoog, 1992)

The plasma-jet sources consist of three electrodes arranged in an inverted **Y** configuration. A graphite anode is located in each arm of the **Y**, and a tungsten cathode is located at the inverted base. Argon flows from the two anode blocks towards the cathode. The plasma jet is formed when the cathode is momentarily brought into contact with the anodes. Ionization of the argon occurs, and the current that develops (~14A) generates additional ions to sustain the plasma indefinitely. The temperature is around 10,000 K in the arc core and 5000 K in the viewing area. The sample is aspirated into the area between the two arms of the **Y**, where it is atomized, exited, and its spectrum viewed.

Equipment Needed

Volumetric flask, 100 mL
Volumetric pipet

Reagent Needed

Concentrated nitric acid
Pet food sample solution and blank solution,
Cu, Na, Mn, Zn, and Fe reference standards

Procedure

1. Prepare a standard solution containing Cu, Fe, Mn, Na and Zn (please consult TA for concentration) TOGETHER in a single 100 mL volumetric flask. Add 10 mL of concentrated nitric acid before making up to volume with distilled water. Also, please make sure that 4 ppt (part per thousand) lithium is in **ALL** standard + sample solutions.
2. Make dilutions to get at least 2 more points between the blank and your standard solution (Note: need to make up ALL solutions in 10 % nitric acid and 4 ppt lithium salt)
3. Make a blank containing 10 % nitric Acid and 4 ppt lithium salt.
4. Digestion of sample
   - Thoroughly grind if necessary ~2 g of the dried pet food with a mortar and pestle.
   - Add 10 mL HNO_3, and digest on a hot plate until fully digested. (binder and fat may not digest completely) or almost dryness.
   - Before complete dryness, after digestion, add an additional 10 mL of HNO_3 and transfer the 1 mL of liquid sample quantitatively to a 100 mL volumetric flask and add 10 mL of HNO_3 and 4 ppt of lithium salt solution before diluting to mark with DI water.
   - Filter the sample through a polycarbonate filter to remove fat if necessary.
   - Prepare a method blank in the same way (10 % HNO_3 + 4 ppt lithium salt solution)
5. Aspirate the blank and standard solutions with DCP.
6. Measure Cu, Fe, Mn, Na and Zn and concentration in ppm in the sample solution.
7. Please consult your TA for additional standard solutions concentrations if a standard addition method is also performed.

Data Treatment

Draw a calibration curve for each of the analyte under consideration and calculate the concentrations of Cu, Fe, Mn, Na and Zn in the cat food sample.

Questions

1. Describe the basic differences between Atomic Absorption and Atomic Emission Spectroscopy.

Gas Chromatography: Separation and Identification of Organic Unknowns

Reference

Skoog, Holler & Nieman. Principles of Instrumental Analysis, 5th Ed., 1998

Introduction

Chromatography is an important separation technique that permits the scientists to separate, isolate and thus identify components in a complex mixture. Chromatography has been applied to a wide variety of areas. This technique involves the use of a stationary phase and gaseous mobile phase. Component of a mixture is carried through the stationary phase by the flow of the mobile phase; separations are based on the differences in the migration rates among the sample components.

In classical gas chromatography, the components of the sample are transported by the mobile phase through a column packed with solid support particles coated with the stationary phase. High resolution or capillary, gas chromatography employs an open tubular column with a stationary phase film on the inner wall.

The equilibrium involved during separations can be described quantitatively by means of a partition coefficient, K_d. A detector responds to solute concentration and its signal is plotted as a function of time or volume of the added mobile phase, and a series of peaks are obtained. Such a plot known as the chromatogram is useful for both quantitative and qualitative analysis. The position of the peak on the time axis may serve to identify the component in the sample and the area under the peak provides a quantitative measure of the amount of the component. The critical step in developing a method for gas chromatography is determining the resolution required to effect the desired separation. Chromatographic resolution, *R*, is calculated as:

$$R = 2\,(t_{R1} - t_{R2}) / (W_{b1} + W_{b2})$$

where t_{R1} and t_{R2} are the retention time of components 1 and 2, and W_{b1} and W_{b2} are base line width of components 1 and 2. Resolution is a complex interplay of the following chromatographic parameters: efficiency (*N*), selectivity (α) and retention.

$$R = \sqrt{N/4}\,(\alpha - 1/\alpha)(k/k+1)$$

where N = the column efficiency expressed as the theoretical plate number
α = the selectivity factor
k = the solute partition ratio

Separation efficiency is the measure of the broadening of the solute plug as it travels the length of the column. The column efficiency can be expressed in terms of the peak width at half height (W_h) or at the baseline (W_b) as follows:

$N = 5.545\,(t_R / W_h)^2$ $\qquad$ $N = 16\,(t_R / W_b)^2$

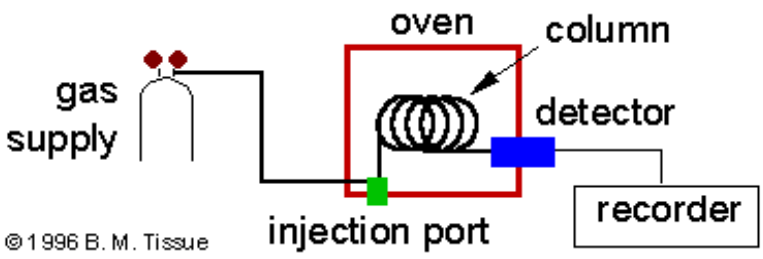
The selectivity factor: $\qquad \alpha = k_2 / k_1$

Capacity Factor: $k = t'_R / t_M$

where t'_R is the adjusted retention time and t_M is the column dead time and can be easily estimated from the retention time of a component un-retained by the column, such as methane.

Instrumentation

The basic components in GC include carrier gas supply, sample injection system, column, oven and detector.



In GC, the sample is vaporized and injected onto the head of a chromatographic column. Elution is brought about by the flow of inert gaseous mobile phase. The sample components are then detected and recorded. The most commonly used detectors in GC are the FID (Flame Ionization Detector) and the TCD (Thermal Conductivity Detector). FID: See figure below. When using an FID the selection of the appropriate make up gas is important to achieve the highest detector sensitivity. Nitrogen is obviously a better choice for an FID than is helium.

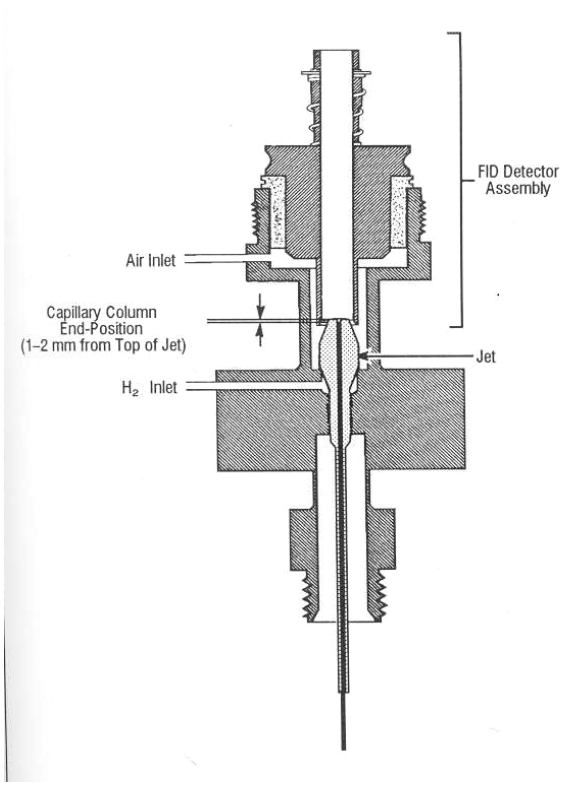


Figure 2 Diagram of a Flame Ionization Detector

TCD: This detector is able to detect any compound (Figure 2). The cell volume and the carrier gas flow rate are critical for the sensitivity of the TCD, because it is a concentration detector. Helium is the recommended carrier and makeup gas for the TCD. Other detectors which are used in GC include Electron Capture Detector, Nitrogen Phosphorus Detector, and Flame Photometric Detector.

Instrumental Information

GC Instrument: HP 5890 series with Flame Ionization Detector (FID)
Capillary column: 30 m of 0.25 mm i.d. column with 0.25 μm film thickness of HP-Innowax (Bonded – phase of polyethyleneglycol)
Carrier gas: Helium

Six vials (1-6) of unknown solutions are given. Choose any two and place 1.0 μL of each solution into 2 mL of CH_2Cl_2 using a micro-syringe. Ensure to record the unknown solution number.

Procedure

1. Determine the average linear velocity of carrier gas, He at 100°C
   Set GC oven at 100°C.
   Inject 0.5 mL of methane using a gas tight syringe.
   Measure tm and calculate the linear velocity (v) in cm/sec:

   $V = L / t_m$
   where L = column length in cm and t_m = retention time of methane in seconds

2. A working standard solution containing ~0.5 μg/mL of each compound in CH_2Cl_2: (Decane, Undecane, Dodecane, Tridecane, 2-octanone, 1-octanol, 2,6-DMP, 2,6-DMA and Naphthalene) has been prepared and will be used to demonstrate basic GC operations.

3. Set the GC condition as follows:
   Injector temperature: 250 °C
   Oven temperature: 100-200 °C @ 5 °C/min
   Detector temperature: 280 °C

4. Inject 0.8 μL of working standard solution using a micro-syringe. Obtain the chromatogram from the integrator.

5. Set another GC condition as follows:
   Injector temperature: 250 °C
   Oven temperature: 100-200 °C @ 10 °C/min
   Detector temperature: 280 °C

6. Inject another 0.8 μL of working standard solution using a micro-syringe. Obtain the chromatogram from the integrator.

7. Inject the prepared unknown solution (~0.8 μL) using a micro-syringe. Obtain the chromatogram from the integrator.

8. For the unknown solution, identify all peaks. Tabulate retention time t_R (min), peak width W (min) and calculate the number of theoretical plate N, capacity factor k, selectivity factor α, for each peak. Calculate resolution R_s, for unknown pairs and discuss.

(Note for TA only: The unknown solutions was a mixture of the above mentioned standards)

Determination of Food additive by High Performance Liquid Chromatography (HPLC): Determination of Caffeine in Beverages

Reference

Sawyer, Heineman, Beebe, *Chemistry Experiments for Instrumental Methods,* 1984

Introduction

HPLC is accomplished by injection of a small amount of liquid sample into a moving stream (termed the *mobile phase*) that passes through a column packed with particles of a *stationary phase*. The separation of the components in the sample occurs as a function of partitioning of each component between the liquid mobile phase and the stationary phases. Each component will partition based on its polarity, as well as that of the stationary and mobile phases. In this experiment, we will be using a non-polar stationary phase and a polar mobile phase. Separation utilizing this arrangement of polar and non-polar phases is termed *reverse-phase chromatography*. The most polar component will thus be retained the shortest time on the column and will be eluted first. We will be using a C_{18} or *n*-octyldecyl column, and 20% methanol**:** 80%water as mobile phase.

In this experiment, reverse phase HPLC is used to determine the concentration of caffeine in coffee, tea, Coca-Cola, and Pepsi-Cola. The traditional method for the determination of caffeine is via extraction with spectrophometric quantitation. Use of the liquid chromatography system permits a fast and easy separation of caffeine from other substances such as tannic acid, caffeine acid and sucrose found in these beverages. Five standards solutions of caffeine are prepared and injected into the HPLC. In addition, the beverages coffee, tea, Coca-Cola, and Pepsi-Cola are prepared and injected into HPLC. From the resulting chromatograms, measurements of retention time t_R and the peak areas are made. If the flow rate and pump pressure are held constant throughout the entire experiment, t_R may be used as a qualitative measure and the peak area as a quantitative measure. A calibration curve for peak area vs. concentration of the caffeine standards can then be employed to determine the concentration of caffeine in the four beverages.

Equipment Needed

High performance liquid chromatograph with UV detector (254 nm)
Reverse phase C_{18} column, 5µ, 4.6x150mm
Syringe, 50µL
Needle port cleaner
Volumetric flasks, five 100mL, three 20mL
Pipets, 2mL, 4mL, 8mL, one of each
Syringe filters, eight
0.45 µ filter paper
Solvent filter degasser assembly
Chromatography sample vials, eight
10-20mL beaker, several, dry and clean

Reagent Needed

HPLC grade methanol and water for mobile phase

Caffeine, regent grade for standards
Coffee, tea, Pepsi and Diet Pepsi

Preparation of solutions

A. Mobile Phase

Prepare 1 L 20 % methanol: 80 % water, and pass through the filter degasser assembly equipped with 0.45 μ filter paper under vacuum.

B. Caffeine Standards

1. Into five clean and dry 100 mL volumetric flasks weigh out accurately the following quantities of caffeine: 2.5 mg, 5.0 mg, 7.5 mg, 10.0 mg and 12.5 mg.
2. Dilute to the mark with previously prepared mobile phase.
3. Shake the five caffeine solutions adequately to insure dissolution and make sure no air bubble in the solutions before injection.

C. Coffee and tea

1. The instructor will supply coffee and tea prepared for this experiment.
2. Into one clean, dry 20 mL volumetric flask pipet 2 mL of coffee, and into another clean, dry 20 mL flask pipet 4 mL of tea.
3. Dilute each volumetric flask to the mark with the mobile phase.

D. Pepsi

1. Pour 10 to 15 mL of Pepsi-Cola into a small clean, dry beaker. Pour this into another clean, dry beaker. Pour the Pepsi back to the original beaker. Continue pouring back and forth until the bubbling ceases. The soda is now adequately decarbonated.
2. Into a clean, dry 20 mL volumetric flask pipet 8 mL of Pepsi (decarbonated), and dilute to the mark with mobile phase.

Procedure

1. Once the sample solutions are prepared, filter an aliquot of each into sample vials using a syringe equipped with a .45 micron syringe tip filter. Dispense the initial 1mL into waste. Dispense the remaining volume into the sample vial.
2. Turn the pump and detector on. Set the detector at 254 nm.
3. Open **Chrom Perfect Spirit 5,** your instructor will show you how to create a method file, format file and how to acquire data with this software.
4. Purge the pump at 5 mL / min for 5 min.
5. Set the pump flow rate at 2.0mL/min. Prior to injection of the standards into the column, allow the mobile phase to pass through the column for 5 to 10 min.
6. Rinse the syringes with two 30 μL portions of the mobile phase. Then prime the syringe with several syringe volumes of the least concentrated standard, dispensing each volume into the overflow waste beaker.
7. Switch the manual injector to the "load" position and push at least three 20 μL volumes of each sample through the sample loop, making certain each volume does not contain air bubbles.

8. With the syringe still in the injector, the injector was turned to the "inject" position, and simultaneously start data acquisition with Chrom Perfect Spirit. Leave the injector in the "inject" position, and remove the syringe.
9. Repeat steps 7 and 8 do a second injection for the least concentrated caffeine standard.
10. Repeat steps 6 to 9 for the rest caffeine standards. Do two injections for every standard.
11. Repeat steps 6 to 8 for coffee, tea and Pepsi-Cola samples.
12. The report of each chromatograph run will be printed with the format specified in the format file you created.
13. At the end of the experiment, flush the needle port with 2mL mobile phase in INJECT position, using the needle port cleaner.
14. Flush the column with 100 % methanol at 1mL / min for 10 min, then with 66% methanol:34 % water (for column storage) for another 10min. Disconnect the column from the system, seal it with the end plugs, and connect the system with a unit.

Data Treatment

1. In tabular form record for the caffeine standards the concentration, retention times, and peak areas. Also, record the average and standard deviation of the times and areas.
2. Plot the concentration in mg/mL versus average peak area for the caffeine standards.
3. From the retention time for the caffeine peak determine which peak on the coffee, tea and Pepsi-Cola chromatograms is due to caffeine.
4. Determine the caffeine concentration in mg/mL in each sample from the calibration curve. Include corrections for dilution in the calculations.
5. In tabular form record the concentration of caffeine present in each beverage.

Questions

1. Consider the use of an internal standard when performing HPLC analysis. Do you think it would improve the results? Why?
2. What could be done to correct for overlapping or poorly resolved peaks?
3. Explain the rationale for using a reverse phase C_{18} column for the determination of caffeine.

Identification of Organics in an Unknown Mixture: an Experiment in FTIR and GC/MS

Reference

Skoog & Leary, Principles of Instrumental Analysis, 1992.

Introduction

Chromatography is an important separation technique that permits the scientists to separate, isolate and thus identify components in a complex mixture. Chromatography has been applied to a wide variety of areas. Gas chromatography involves injection of a small amount of sample into a moving stream of gas, which is termed the *mobile phase* or *carrier gas*. The sample is carried by the gas stream through a column that consists of a tube packed with solid support particles coated with the *stationary phase* (packed column) or a capillary open tubular column with a stationary phase film on the inner wall. Separation of a sample mixture into individual components is achieved if the components are retained in the column to different extents.

GS/MS, as the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. Gas chromatography separates the components of a mixture and mass spectrometry characterizes each of the components individually. GC/MS gives specific information related to the compound. When the MS is used as a GC detector, it responds differentially and more universally than other detectors. The Mass Spectrometer, unlike other GC detectors, responds to mass, a physical property common to all organic compounds. The spectrum consists of a unique bar graph in which the height of the bars represents the relative abundance of the most abundant ions of each individual compound as a function of the mass. These ions give information concerning the molecular weight and the most electronically stable ion fragments from the original molecule. These unique fragments may be matched or interpreted to characterize a molecule based on its atomic structure.

All mass spectrometers have five major components which include an inlet system, ion source, mass analyzer, detector and signal processor. The first four components are typically held at high vacuum (10-5 - 10-8 torr). The inlet system's function is to introduce a small amount of sample (typically 1 micromole or less) into the ionization source with a minimal loss of vacuum. Typical inlets include batch inlets, direct probe inlets and chromatographic inlets. In this experiment, the GC will not only serve as an inlet system but it will also facilitate the separation of our complex mixture before it is introduced to the mass spectrometer.

The ion source is perhaps the most important part of the mass spectrometer. Ion sources are as varied as the types of samples which can be analyzed by mass spectrometry. They can be a simple electron impact (EI) ion source, producing ions from the interaction of analyte molecules with energetic electrons in the gas phase, to the more complicated ionization occurring in a MALDI (Matrix Assisted Laser Desorption Ionization) source useful for nonvolatile samples. You will be using a MS detector with EI source. As the individual compounds elute from the GC column, they enter the electron impact ionization (MS) detector. There, they are bombarded with a stream of electrons causing them to break apart into fragments. These fragments can be large or small pieces of the original molecules. The fragments are actually

charged ions with a certain mass. The mass of the fragment divided by the charge is called the mass to charge ratio (m/z). Since most fragments have a charge of +1, the m/z usually represents the molecular weight of the fragment.

If the ion source is the most important part of the mass spectrometer, the ions produced within it wouldn't provide any useful information without the mass analyzer. The main function of the mass analyzer is to separate ions with different mass to charge ratios (m/z) produced in the ion source. Like ion sources, there are a number of mass analyzers which can be used for this purpose. These are classified as magnetic sector analyzers, double focusing spectrometers, quadrupole mass filters, ion trap analyzers, time of flight analyzers and Fourier Transform instruments. Typically quadrupole mass filters are coupled to chromatographic instruments because they are typically more rugged, lower priced, and more compact than other mass analyzers. Additionally, quadrupole mass filters are capable of scanning a large range of masses in a short time, which is useful for real-time scanning of chromatographic peaks.

A quadrupole consists of a focusing lens stack and four cylindrical metal rods which act as the electrodes of the mass filter (Figure 1). Ions are accelerated and focused into the space between the rods by the lens stack. Opposite rods in a quadrupole are connected electronically, to the positive and negative terminal of a variable dc source. Additionally, there is a variable radio frequency ac potential applied, 180 degrees out of phase, to each pair of rods. The quadrupole acts as a mass filter because only ions with a stable trajectory will remain between the rods and pass to the detector.

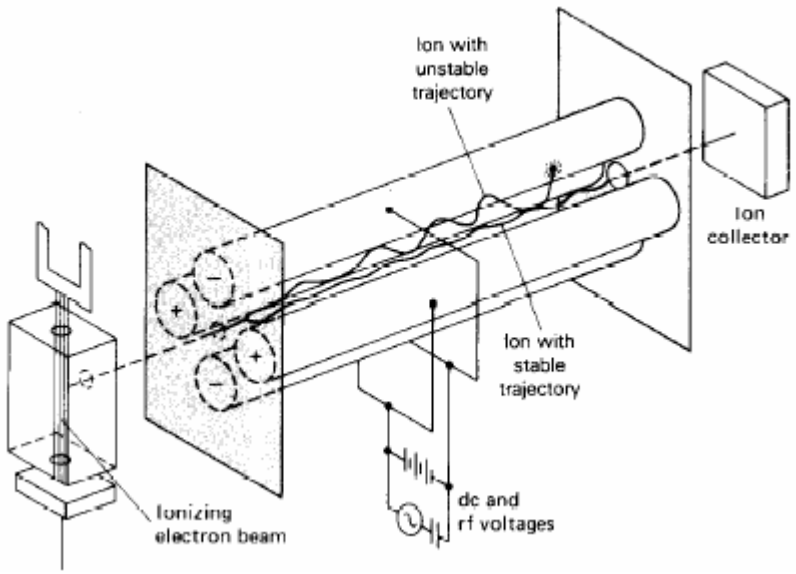


Figure 1 Schematic representation of a quadrupole mass spectrometer
(Skoog & Leary, *Principles of Instrumental Analysis*, 1992)

By simultaneously adjusting the ac and dc potential applied to the rods it is possible to change the m/z of an ion which will pass to the detector. Therefore, by rapidly adjusting the ac and dc potentials it is possible to rapidly scan a range of masses and continually collect a complete mass spectrum of the column eluent.

Once the ions have been separated by the mass analyzer they must be collected and the beam of ions converted into an electrical signal which can be recorded, stored and/or displayed for analysis. Many different types of detectors have been used in mass spectrometers, but the most popular are the discrete dynode electron multiplier and the continuous dynode electron multiplier.

Experiment Needed

- **FTIR**
  Use the ATR micro-sampler, place a drop of the unknown mixture on the surface of the crystal, and record the spectrum of the mixture. By interpreting the IR spectrum, explain what components could be in your unknown mixture.
  .
- **GC-MS Conditions**
  Capillary column: 30 m of 0.25 mm i.d. column with 0.25 μm film thickness of HP-Innowax (Bonded – phase of polyethyleneglycol)
  Carrier gas: Helium
  Detector temperature: 300 °C
  Oven temperature: 100-200 oC @ 5 °C/min
  Column head pressure: 10 psi
  Used deactivated glass liner (gooseneck liner)
  Split injection (split ratio 50:1): already adjusted
  Injection volume: 2μl
  Perform auto tune before sample analysis

- **GC-MS experiment**
  The GC/MS will be operated in the TIC (Total Ion Chromatogram) and the SIM (Selective Ion Monitoring) modes.
  Total Ion Chromatogram (TIC):
  Inject 2μl of the given sample. Identify the components by interpreting the spectrum of each peak, especially the characterizing molecular ions.
  SIM (Selective Ion Monitoring):
  In this mode, only those components which produce the ions you entered will be detected.

Reagent Needed

The unknown mixture contains several components of following compounds in CH_2Cl_2:
Decane, Undecane, Dodecane, Tridecane, 2-octanone, 1-octanol, 2,6-DMP, 2,6-DMA and Naphthalene

Data Treatment

Identify the actual components in your mixtures; list the compound names and structures, and assign them to each peak on the GC chromatogram. For each compound, explain how you identify it.

Your instructor will show you how to operate the GC/MS instrument. (Note for TA; Unable to perform this lab)

Determination of Chloride using Ion-selective Electrode (ISE)

Introduction

In this experiment, you will use a chloride ion-selective electrode to determine the chloride concentrations in given samples of 1) Sea water 2) Tap water 3) Saline solution.

Ion-Selective Electrodes are part of a group of relatively simple and inexpensive analytical tools which are commonly referred to as Sensors. An Ion-Selective Electrode (ISE) produces a potential that is proportional to the concentration of an analyte. Making measurements with an ISE is a form of potentiometry.

Potentiometric methods are based on the measurement of a potential difference between two electrodes immersed in a solution. The electrodes and the solution constitute an *electrochemical cell*. The potential difference between the two electrodes is usually measured with a pH/mV meter. One of the two electrodes is termed an *indicator electrode*, which is chosen to respond to a particular species in solution whose activity is to be measured during the experiment. The other electrode is a *reference electrode* whose half-cell potential is invariant. The most commonly used reference electrodes are *calomel* and *silver-silver chloride electrodes*. See Figure 1.

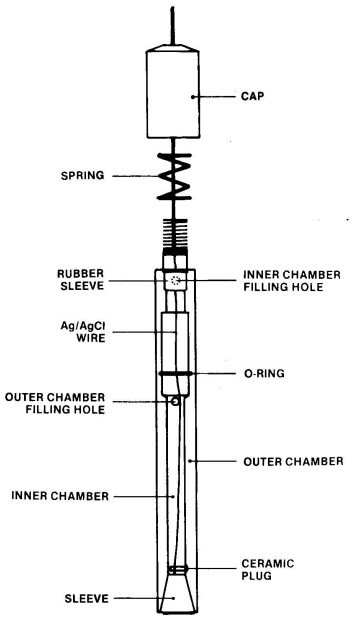


Figure 1 Double Junction Reference Electrode

There are two types of indicator electrodes: *metallic* and *membrane*. It is important that the indicator electrode responds selectively to the species of interest and not to other compounds in the sample that might constitute interference. A large number of electrodes with good selectivity for specific ions are based on the measurement of the potential generated across a membrane (*membrane electrode*). Electrodes of this type are refereed to as *ion-selective electrodes* (ISE), including *glass-membrane electrodes, liquid-membrane electrodes, solid-membrane electrodes,*

gas-sensing electrodes, enzyme electrodes. The membrane is usually attached to the end of a tube that contains an internal reference electrode. The most common ISE is the pH electrode, which contains a thin glass membrane that responds to the H^+ concentration in a solution.

The ISE must be used in conjunction with an external reference electrode to form a complete electrochemical cell. A voltmeter (such as a pH meter) is used to measure the potential difference (ISE vs. reference electrode). Commercial ISE often combine the two electrodes into one unit which can be attached to a pH meter.

The measured potential differences are linearly dependent on the logarithm of the activity of a given ion in solution. For cations,

$$E_{cell} = K + (2.303\ RT / nF)\ log(a)$$

For anions,

$$E_{cell} = K - (2.303\ RT / nF)\ log(a)$$

where K is a constant, R is the gas constant, T is temperature, n is the number of electrons transferred, F is Faraday's constant, and a is the activity of the analyte ion. A plot of measured potential versus log (a) will therefore give a straight line.

Equipment Needed

Chloride ion-selective electrode
Reference electrode
pH meter
Volumetric flask, 250 mL
Plastic beaker, 200 mL
Volumetric pipet, 50 mL, 1 mL
Thermometer
Magnetic stirrer

Reagent Needed

Sodium chloride, reagent grade
Sea water, tap water and unknown saline solution

Preparation of Chloride Standard Solutions

1. Weigh 0.4125 g reagent grade sodium chloride in a beaker, dissolve in about 150 mL distilled water. Quantitatively transfer this solution into a 250 mL volumetric flask, and dilute to mark. This would be the 1000 ppm Cl^- solution.
2. Prepare 250 mL of 100 ppm, and10ppm Cl^- standards from the 1000ppm standard solution by serial dilution.
3. Transfer exactly 50 mL of each solution(10, 100, 1000 ppm) to a beaker and add 1 mL of ionic strength adjuster (ISA) (5M $NaNO_3$) to adjust the ionic strength.

Procedure

A. Connecting electrodes to meter

Fill the reference electrode with inner and outer filling solutions, refer to the instruction sheet in the electrode package. Remove the rubber cap covering the chloride electrode tip. Insert the reference electrode pin-tip connector and the sensing electrode connector into appropriate jacks on the pH meter.

B. Calibration Curve of Cl^-

Allow all standards and samples to come to the same temperature for precise measurement. Measure the potential for each standard solution. Place the electrodes in the solution. Set the function switch on the pH meter to mV. Stir the solution at a rate that will not cause a vortex. When the reading is stable, record the potential in millivolts. Be sure to rinse the electrode with distilled water and blot dry between measurements.

C. Determination of Cl^- in unknowns

Get sea water, tap water, and saline solution. Make dilution if needed, to keep the potential reading within the range of calibration curve. Adjust ionic strength as in procedure B; allow the temperature come to the same of the standards before measurement.

Data Treatment

1. Plot the potential (linear axis, in mV) against the concentrations of the chloride standard solutions (log axis) on standard semi logarithmic paper (or can use excel) to get the calibration curve.
2. From the calibration curve, determine the Chloride concentration in tap water and the original sea water and saline solution.

Questions

1. Why ISA is added to each of the aliquot of samples and please discuss the effects if no ISA was present?
2. What kind of membrane electrode is the chloride ISE you used today? What are the major interferences when measuring chloride ion with an ISE? What interferences could be present in the sea water sample? How do they affect your result?
3. Explain how a glass-membrane electrode (the pH electrode) works.

(Note for TA only: Sea water available in the lab, Saline Solution~ 0.9% NaCl)

Determination of Copper in Various Waters by Graphite Furnace Atomic Spectroscopy

Reference

3. Skoog, West & Holler, Fundamentals of Analytical Chemistry, 1992.
4. Beaty, Richard & Kerber, Jack. Concepts, Instrumentation and Techniques in Atomic Absorption Spectrophotometry, Perkin Elmer Instruments.

Pre-Lab

You are required to read Appendix B before lab.

Introduction

In the previous experiments, we have demonstrated flame atomic absorption and direct current plasma atomic emission are powerful and useful techniques in determining concentration of metals in organic samples. Here, instead of using a flame atomizer, an electrothermal atomizer will be used. The sample is introduced into and evaporated in a graphite furnace (See Figure 1).

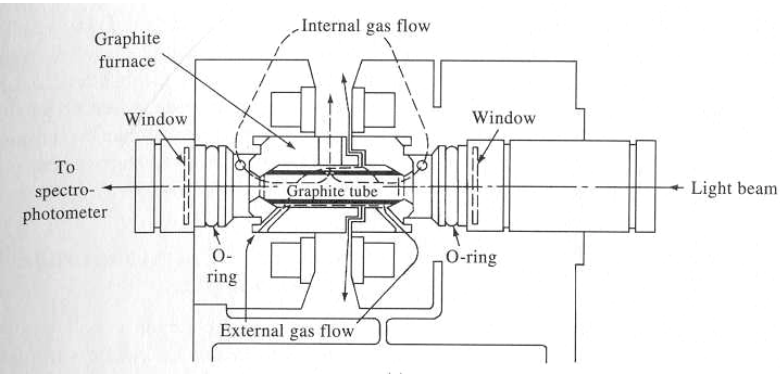


Figure 1 Cross-Section of a Graphite Furnace

An inert carrier gas swept the sample vapor into the detector. Hollow cathode lamps or electrodeless discharge lamps are used for each element of interest. In this experiment, you will be measuring copper in water samples.

Equipment Needed

Volumetric flask
HCLs or EDLs

Reagent Needed

Water samples
Reference standard solutions

Procedure

Your TA will show you how to perform this experiment

Data Treatment

Draw a calibration curve for the analyte under consideration and calculate the concentrations of copper in the water samples.

Questions

Which water sample has the most metals? Please provide some reasons why you think that is the case?

Nuclear Magnetic Resonance Workshop

Introduction

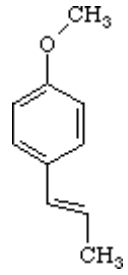
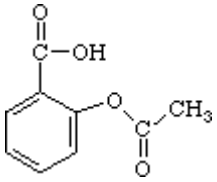
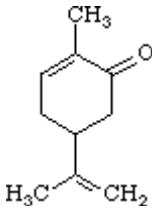
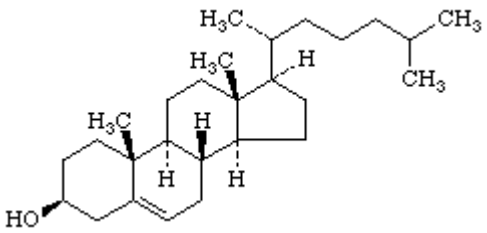
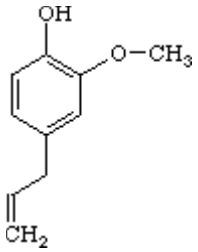
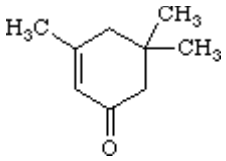
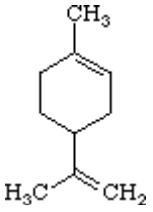
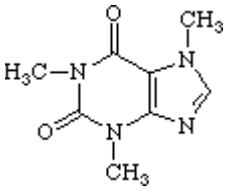
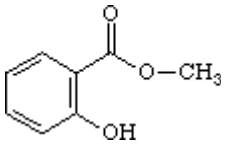
(Your TA will give handouts to you in the lab)

Equipments Needed

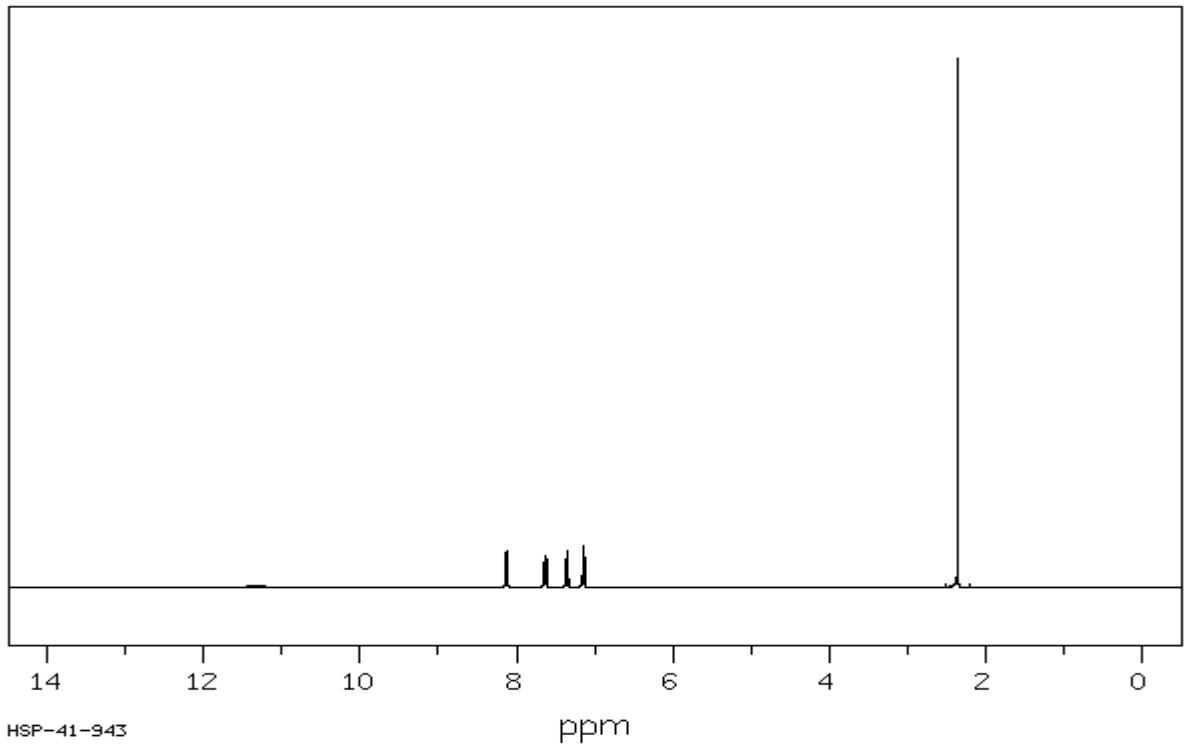
Computer Simulation Software (ChemDraw or ACDLabs)

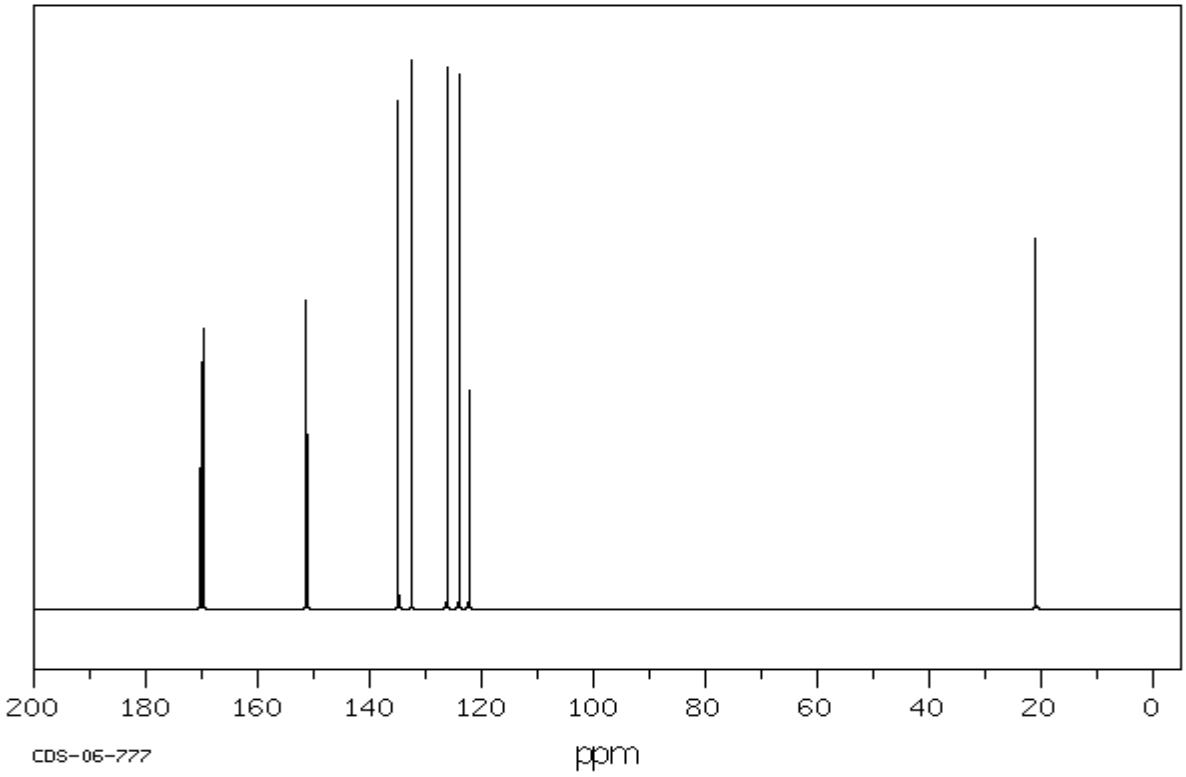
Procedure

Please use the provided computer simulation software to predict the ^{1}H and ^{13}C NMR spectra of the given unknown spectra. The nine possible unknowns are summarized in the table.

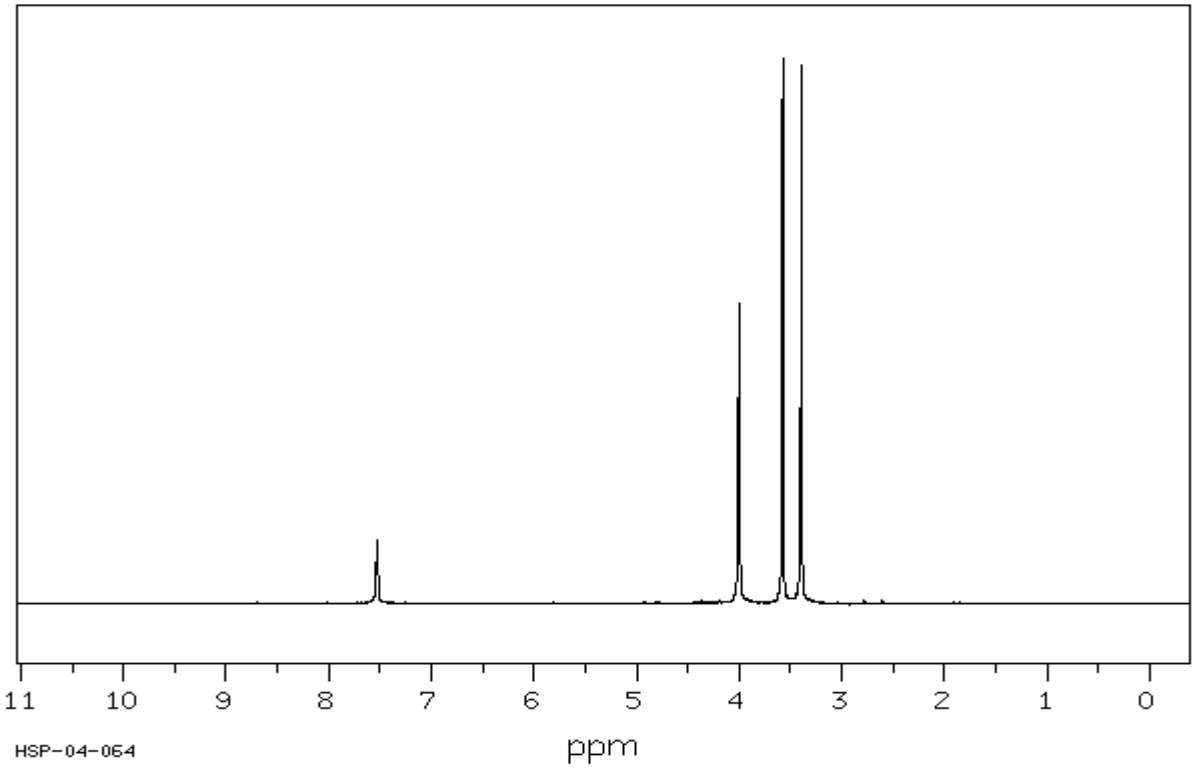
		
Anethole	**Aspirin**	**Carvone**
		
Cholesterol	**Eugenol**	**Isophorone**
		
d-Limonene	**Caffeine**	**Methyl-salicylate**

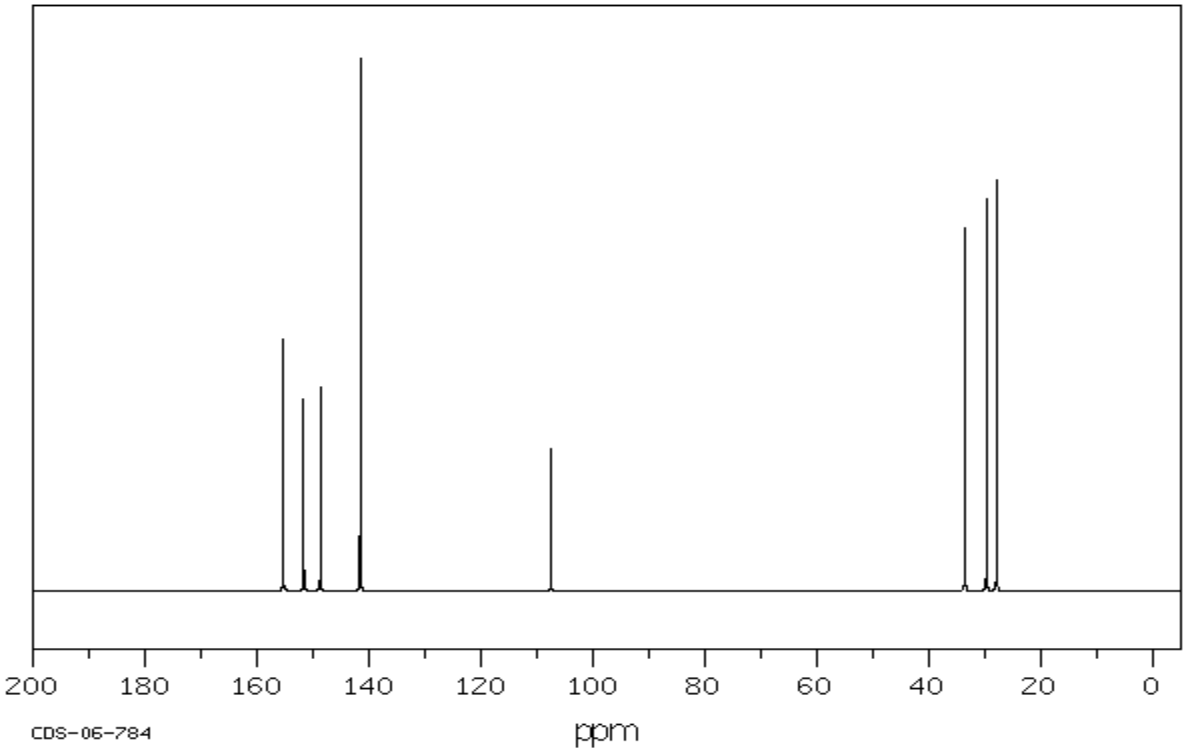
Unknown Spectra 1





Unknown Spectra 2





APPENDIX A

2 ATOMIC ABSORPTION INSTRUMENTATION

THE BASIC COMPONENTS

To understand the workings of the atomic absorption spectrometer, let us build one, piece by piece. Every absorption spectrometer must have components which fulfill the three basic requirements shown in Figure 2-1. There must be: (1) a light source; (2) a sample cell; and (3) a means of specific light measurement.

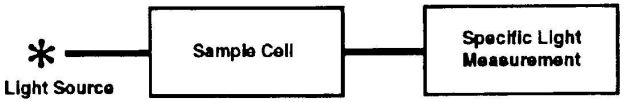


Figure 2-1. Requirements for a spectrometer.

In atomic absorption, these functional areas are implemented by the components illustrated in Figure 2-2. A light source which emits the sharp atomic lines of the element to be determined is required. The most widely used source is the hollow cathode lamp. These lamps are designed to emit the atomic spectrum of a particular element, and specific lamps are selected for use depending on the element to be determined.

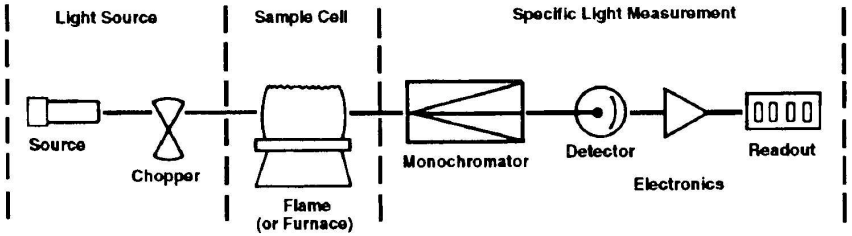


Figure 2-2. Basic AA spectrometer.

It is also required that the source radiation be modulated (switched on and off rapidly) to provide a means of selectively amplifying light emitted from the source lamp and ignoring emission from the sample cell. Source modulation can be accomplished with a rotating chopper located between the source and the sample cell, or by pulsing the power to the source.

Special considerations are also required for a sample cell for atomic absorption. An atomic vapor must be generated in the light beam from the source. This is generally accomplished by introducing the sample into a burner system or electrically heated furnace aligned in the optical path of the spectrophotometer.

Several components are required for specific light measurement. A monochromator is used to disperse the various wavelengths of light which are emitted from the source and to isolate the particular line of interest. The selection of a specific source and a particular wavelength in that source is what allows the determination of a selected element to be made in the presence of others.

The wavelength of light which is isolated by the monochromator is directed onto the detector, which serves as the "eye" of the instrument. This is normally a photomultiplier tube, which produces an electrical current dependent on the light intensity. The electrical current from the photomultiplier is then amplified and processed by the instrument electronics to produce a signal which is a measure of the light attenuation occurring in the sample cell. This signal can be further processed to produce an instrument readout directly in concentration units.

LIGHT SOURCES

An atom absorbs light at discrete wavelengths. In order to measure this narrow light absorption with maximum sensitivity, it is necessary to use a line source, which emits the specific wavelengths which can be absorbed by the atom. Narrow line sources not only provide high sensitivity, but also make atomic absorption a very specific analytical technique with few spectral interferences. The two most common line sources used in atomic absorption are the "hollow cathode lamp" and the "electrodeless discharge lamp."

The Hollow Cathode Lamp

The hollow cathode lamp is an excellent, bright line source for most of the elements determinable by atomic absorption. Figure 2-3 shows how a hollow cathode lamp is constructed. The cathode of the lamp frequently is a hollowed-out cylinder

of the metal whose spectrum is to be produced. The anode and cathode are sealed in a glass cylinder normally filled with either neon or argon at low pressure. At the end of the glass cylinder is a window transparent to the emitted radiation.

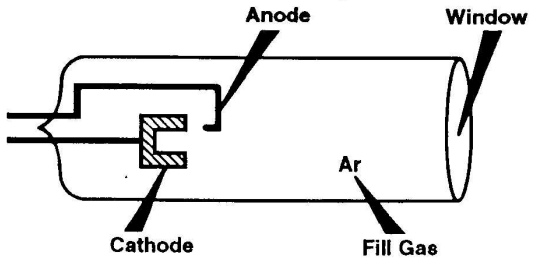


Figure 2-3. Hollow cathode lamp.

The emission process is illustrated in Figure 2-4. When an electrical potential is applied between the anode and cathode, some of the fill gas atoms are ionized. The positively charged fill gas ions accelerate through the electrical field to collide with the negatively charged cathode and dislodge individual metal atoms in a process called "sputtering". Sputtered metal atoms are then excited to an emission state through a kinetic energy transfer by impact with fill gas ions.

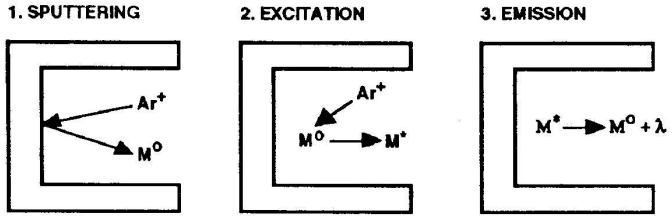


Figure 2-4. Hollow cathode lamp process, where Ar^+ is a positively-charged argon ion, M^0 is a sputtered, ground-state metal atom, M^* is an excited-state metal atom, and λ is emitted radiation at a wavelength characteristic for the sputtered metal.

Hollow cathode lamps have a finite lifetime. Adsorption of fill gas atoms onto the inner surfaces of the lamp is the primary cause for lamp failure. As fill gas pressure decreases, the efficiency of sputtering and the excitation of sputtered metal atoms also decreases, reducing the intensity of the lamp emission. To prolong hollow cathode lamp life, some manufacturers produce lamps with larger internal volumes so that a greater supply of fill gas at optimum pressure is available.

The sputtering process may remove some of the metal atoms from the vicinity of the cathode to be deposited elsewhere. Lamps for volatile metals such as arsenic, selenium, and cadmium are more prone to rapid vaporization of the cathode during use. While the loss of metal from the cathode at normal operating currents (typically 5-25 milliamperes) usually does not affect lamp performance, fill gas atoms can be entrapped during the metal deposition process which does affect lamp life. Lamps which are operated at highly elevated currents may suffer reduced lamp life due to depletion of the analyte element from the cathode.

Some cathode materials can slowly evolve hydrogen when heated. As the concentration of hydrogen in the fill gas increases, a background continuum emission contaminates the purity of the line spectrum of the element, resulting in a reduction of atomic absorption sensitivity and poor calibration linearity. To eliminate such problems, most modern hollow cathode lamps have a tantalum "getter" on the anode which irreversibly adsorbs evolved hydrogen as the lamp is operated.

The cathode of the hollow cathode lamp is usually constructed from a highly pure metal resulting in a very pure emission spectrum of the cathode material. It is sometimes possible, however, to construct a cathode or cathode insert from several metals. The resulting "multi-element" lamp may provide superior performance for a single element or, with some combinations, may be used as a source for all of the elements contained in the cathode alloy. However, not all metals may be used in combination with others because of metallurgical or spectral limitations.

Special consideration should be given before using a multi-element lamp as analytical complications may result. Often the intensity of emission for an element in a multi-element lamp is not as great as that which is observed for the element in a single-element lamp. This loss of intensity could be a disadvantage in applications where high precision or low detection limits are required. The increased spectral complexity of multi-element lamps may require that alternate wavelengths or narrower slits be used, which may also adversely affect sensitivity or baseline noise.

Each hollow cathode lamp will have a particular current for optimum performance. In general, higher currents will produce brighter emission and less baseline noise. As the current continues to increase, however, lamp life may shorten and spectral line broadening may occur, resulting in a reduction in sensitivity and linear working range. The recommended current specified for each lamp will usually provide the best combination of lamp life and performance. For demanding analy-

ses requiring the best possible signal-to-noise characteristics, higher currents can be used for the lamp, up to the maximum rated value. Lower lamp currents can be used with less demanding analyses to prolong lamp life.

Confusion over exactly what current is being used for a hollow cathode lamp may occur due to the method used for lamp modulation. As explained earlier, the source for atomic absorption must be modulated in order to accomplish selective amplification of the lamp emission signal. This can be accomplished mechanically by using a rotating chopper or electronically by pulsing the current supplied to the lamp, as illustrated in Figure 2-5. Both methods produce similar results; however, in some instruments the use of electronic modulation may create the impression that a lower lamp current is being applied than is actually the case.

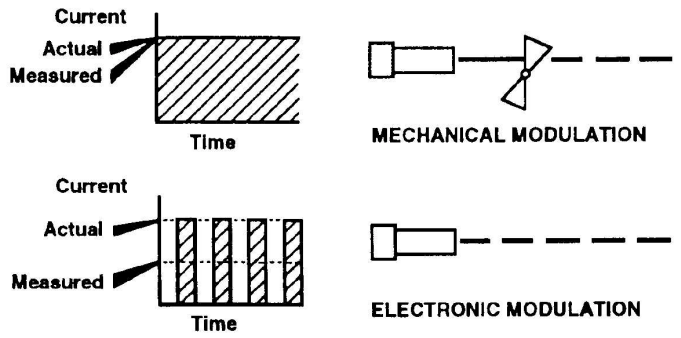


Figure 2-5. Mechanical vs. electrical modulation.

The cause for the apparent difference in measured currents with mechanically and electronically modulated systems is also shown in Figure 2-5. For mechanical modulation, the lamp is run at a constant current. Under these conditions, an ammeter reading of lamp current will indicate the actual current flow. For electronic modulation, the current is switched on and off at a rapid rate. An ammeter normally will indicate the time-averaged current rather than the actual peak current which is being applied.

While some instruments are designed to apply a correction factor automatically to electronically modulated lamp current readings to provide true peak current values, many do not. For electronically modulated systems without such correction, the actual peak current can be approximated from the measured current by dividing it by the "duty cycle", the fraction of time that current is applied to the lamp. For example, for a duty cycle of 40% and a measured lamp current of 10 milliamperes, the actual peak operating current for an electronically modulated system is:

10 milliamperes/0.4 = 25 milliamperes

Specified lamp current settings may appear to be lower for atomic absorption instruments which modulate the source electronically and do not apply correction. The only valid basis of comparison between the current settings used by two different systems is one which includes compensation for the duty cycle, as shown above.

The Electrodeless Discharge Lamp

For most elements, the hollow cathode lamp is a completely satisfactory source for atomic absorption. In a few cases, however, the quality of the analysis is impaired by limitations of the hollow cathode lamp. The primary cases involve the more volatile elements where low intensity and short lamp life are a problem. The atomic absorption determination of these elements can often be dramatically improved with the use of brighter, more stable sources such as the "electrodeless discharge lamp".

Figure 2-6 shows the design of the Perkin-Elmer System 2 electrodeless discharge lamp (EDL). A small amount of the metal or salt of the element for which the source is to be used is sealed inside a quartz bulb. This bulb is placed inside a small, self-contained RF generator or "driver". When power is applied to the driver, an RF field is created. The coupled energy will vaporize and excite the atoms inside the bulb, causing them to emit their characteristic spectrum. An accessory power supply is required to operate an EDL.

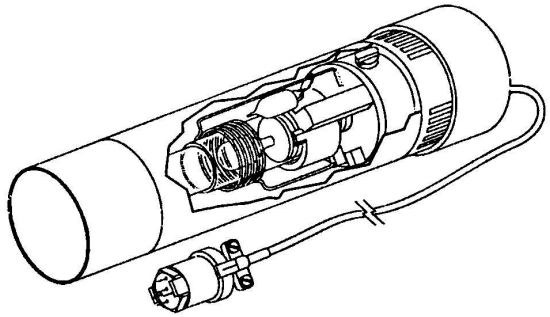


Figure 2-6. Electrodeless discharge lamp.

Electrodeless discharge lamps are typically much more intense and, in some cases, more sensitive than comparable hollow cathode lamps. They therefore offer the analytical advantages of better precision and lower detection limits where an analysis is intensity limited. In addition to providing superior performance, the useful lifetime of an EDL is typically much greater than that of a hollow cathode lamp for the same element. It should be noted, however, that the optical image for the EDL is considerably larger than that in a hollow cathode lamp. As a result, the performance benefits of the EDL can only be observed in instruments with optical systems designed to be compatible with the larger image.

Electrodeless discharge lamps are available for a wide variety of elements, including antimony, arsenic, bismuth, cadmium, cesium, germanium, lead, mercury, phosphorus, potassium, rubidium, selenium, tellurium, thallium, tin and zinc.

OPTICAL CONSIDERATIONS

Photometers

The portion of an atomic absorption spectrometer's optical system which conveys the light from the source to the monochromator is referred to as the *photometer*. Three types of photometers are typically used in atomic absorption instruments: single-beam, double-beam and what might be called compensated single-beam or pseudo double-beam.

Single-Beam Photometers

The instrument diagrammed in Figure 2-7 represents a fully functional "single-beam" atomic absorption spectrometer. It is called "single-beam" because all measurements are based on the varying intensity of a single beam of light in a single optical path.

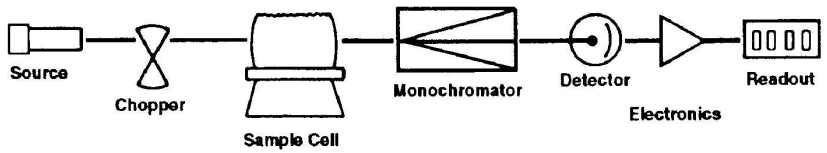


Figure 2-7. A single-beam AA spectrometer.

The primary advantage of a single-beam configuration is that it has fewer components and is less complicated than alternative designs. It is therefore easier to construct and less expensive than other types of photometers. With a single light path and a minimum number of optical components, single-beam systems typically provide very high light throughput. The primary limitation of the single-beam photometer is that it provides no means to compensate for instrumental variations during an analysis, such as changes in source intensity. The resulting signal variability can limit the performance capabilities of a single-beam system.

Double-Beam Photometers

An alternate photometer configuration, known as "double-beam" (Figure 2-8) uses additional optics to divide the light from the lamp into a "sample beam" (directed through the sample cell) and a "reference beam" (directed around the sample cell). In the double-beam system, the reference beam serves as a monitor of lamp intensity and the response characteristics of common electronic circuitry. Therefore, the observed absorbance, determined from a ratio of sample beam and reference beam readings, is more free of effects due to drifting lamp intensities and other electronic anomalies which similarly affect both sample and reference beams.

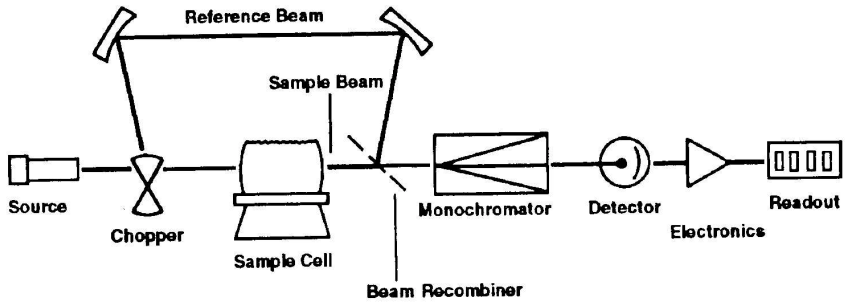


Figure 2-8. A double-beam AA spectrometer.

Modern atomic absorption spectrometers are frequently highly automated. They can automatically change lamps, reset instrument parameters, and introduce samples for high throughput multielement analysis. Double-beam technology, which automatically compensates for source and common electronics drift, allows these instruments to change lamps and begin an analysis immediately with little or no

lamp warm-up for most elements. This not only reduces analysis time but also prolongs lamp life, since lamp warm-up time is eliminated. Even with manual analyses, the ability to install a lamp or turn on the instrument and start an analysis almost immediately is a decided advantage for double-beam systems.

Double-beam photometers do divert some source energy from the sample beam to create the reference beam. Since it is the signal:noise ratio of the sample beam which determines analytical performance, modern double-beam instruments typically devote a much higher percentage of the source emission to the sample beam than to the reference beam. For example, a modern double-beam system which uses a beam splitter to generate sample and reference beams may use 75% of the source emission for the sample measurement and only 25% for the reference measurement. Using such techniques, modern double-beam instruments offer virtually the same signal-to-noise ratio as single-beam systems while enjoying the high-speed automation benefits and operational simplicity of double-beam operation.

Alternative Photometer Designs

There are several alternative system designs which provide advantages similar to those of double-beam optical systems and the light throughput characteristic of single-beam systems. Such systems can be described as compensated single-beam or pseudo double-beam systems. One such design uses two mechanically-adjusted mirrors to alternately direct the entire output of the source through either the sample path (during sample measurements) or through a reference path (Figures 2-9 and 2-10).

These alternative photometer designs provide light throughput comparable to that provided by single-beam photometer systems. They also compensate for system variations in a manner similar to that of double-beam photometers—similar, but not the same. This type of photometer performs compensation for drift much less frequently than do double-beam systems, typically only once per analytical reading. Double-beam systems typically provide drift compensation at rates in excess of 50 times per second. The lower compensation frequency limits the ability of alternative photometer systems to compensate for large, quickly changing variations in source intensity such as those that frequently occur when a source is first lighted.

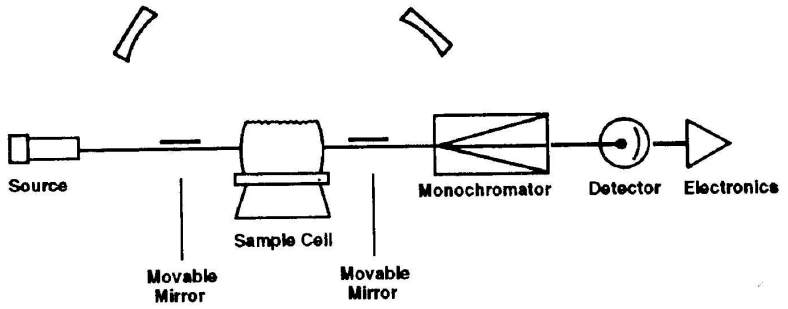


Figure 2-9. A compensated single-beam system with source light directed through the sample path.

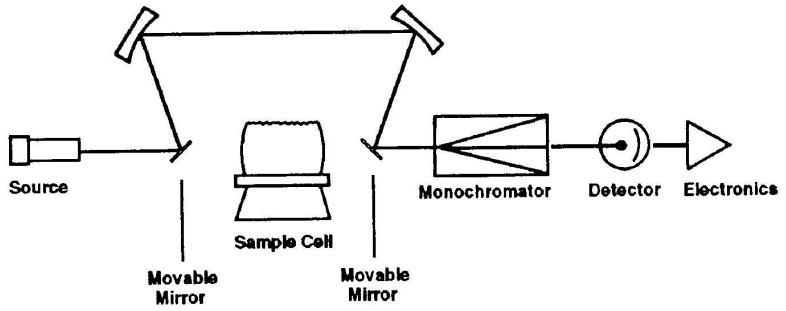


Figure 2-10. A compensated single-beam system with source light directed through the reference path.

Optics and the Monochromator System

As previously discussed, an important factor in determining the baseline noise in an atomic absorption instrument is the amount of light energy reaching the photomultiplier (PMT). Lamp intensity is optimized to be as bright as possible while avoiding line broadening problems. The impact of single-beam and double-beam photometer systems has been discussed above. But the impact of other components must also be considered to determine the capabilities of the complete optical system.

Light from the source must be focused on the sample cell and directed to the monochromator, where the wavelengths of light are dispersed and the analytical line of interest is focused onto the detector. Some energy is lost at each optical surface along the way. Front-surfaced, highly reflective, mirrors can be used to control the focus of the source lamp and the field of view of the light detector precisely and with minimal light loss. Alternately, focusing can be accomplished by refraction instead of reflection by using a lens system. Since the focal length of a lens varies with wavelength, additional optics (which may further reduce energy throughput) or complex optical adjustments must be used to obtain proper focus over the full spectral range for atomic absorption.

Particular care must be taken in the monochromator to avoid excessive light loss. A typical monochromator is diagrammed in Figure 2-11. Wavelength dispersion is accomplished with a grating, a reflective surface ruled with many fine parallel lines very close together. Reflection from this ruled surface generates an interference phenomenon known as diffraction, in which different wavelengths of light diverge from the grating at different angles. Light from the source enters the monochromator at the entrance slit and is directed to the grating where dispersion takes place. The diverging wavelengths of light are directed toward the exit slit. By adjusting the angle of the grating, a selected emission line from the source can be allowed to pass through the exit slit and fall onto the detector. All other lines are blocked from exiting.

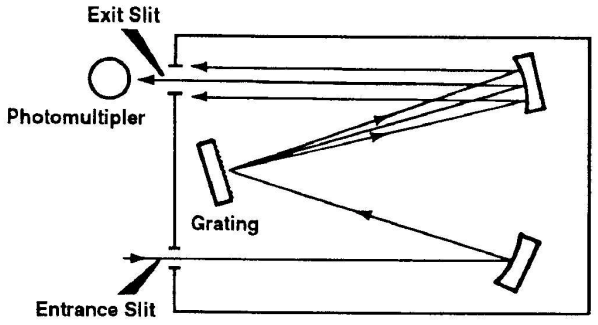


Figure 2-11. A monochromator.

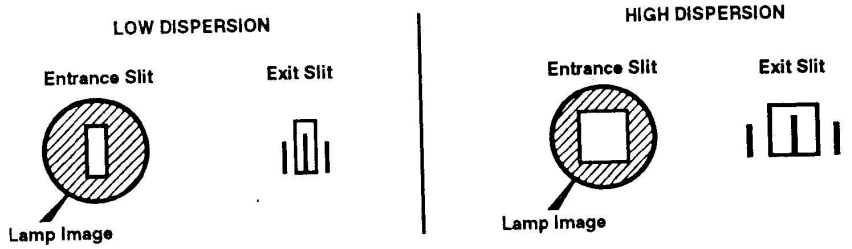


Figure 2-12. Advantages of high dispersion.

The angle of dispersion at the grating can be controlled by the density of lines on the grating. Higher dispersion will result from greater line density, i.e., more lines/mm. High dispersion is important to good energy efficiency of the monochromator, as illustrated in Figure 2-12.

The image of the source focused on the entrance slit and dispersed emission lines at the exit slit are shown for both a low-dispersion and a high-dispersion grating. In order to isolate a desired line from nearby lines, it is necessary to use a narrower exit slit in the low-dispersion example than is required in the high-dispersion case. Good optical design practices dictate that the entrance and exit slits be similarly sized. The use of a larger entrance slit will overfill the grating with the source image, while the use of a smaller entrance slit restricts the amount of light entering the monochromator. Both reduce the amount of energy available at the exit slit. For a low dispersion grating, this means that the size of the monochromator entrance slit is limited to the narrow size demanded of the exit slit to exclude nearby lines. Thus, much of the available light energy is prevented from ever entering the monochromator. In contrast, the greater wavelength separation provided by a high-dispersion grating allows the use of wider slits, which make use of more of the available light without any sacrifice in resolution.

To a first approximation, the energy throughput of a monochromator is proportional to the illuminated ruled grating area and inversely proportional to the *reciprocal linear dispersion*. To obtain the full energy benefit of high dispersion, it is necessary to use a grating with a ruled surface area large enough to capture all of the light from the magnified slit image. Large, quality gratings of high dispersion are difficult and expensive to make. Therefore, the incentive is great to accept smaller gratings with lesser line densities and poorer dispersion for atomic absorption instrumentation. However, better instruments take advantage of the superior energy throughput afforded by larger gratings.

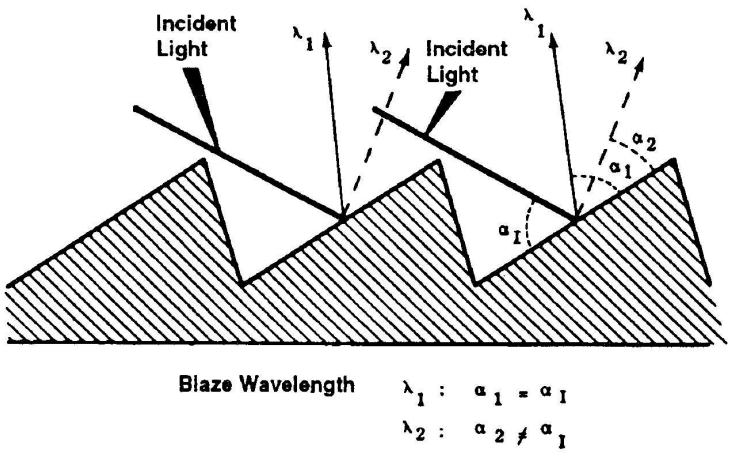


Figure 2-13. Grating blaze angle.

Another factor affecting the optical efficiency of the monochromator is the blaze angle of the grating, whether it is mechanically ruled or holographically generated. An illustration of a mechanically-ruled blaze angle appears in Figure 2-13.

Mechanical grating rulings are in the form of V-shaped grooves carved into the surface of the grating. As discussed earlier, an interference phenomenon causes light of different wavelengths to diverge from the grating at different angles. The particular wavelength which diverges from the blazed surface at an angle corresponding to specular reflectance (i.e., angle of reflection equals angle of incidence) will suffer the least loss in intensity as a result of the diffraction process. A grating can be constructed for a blaze at any desired wavelength by controlling the angle of cut during ruling. The farther removed a given wavelength of light is from the wavelength for which a grating is blazed, the greater will be the extent of monochromator light loss at that wavelength.

The useful atomic absorption wavelength range runs from 189 to 851 nanometers. With one grating blazed somewhere in the middle of this range, significant energy fall-off occurs at the wavelength extremities due to energy inefficiencies in the diffraction process. One technique used to overcome this problem and to provide enhanced energy throughput at the wavelength extremities is to equip the instrument with two gratings, one blazed in the ultraviolet and the other blazed in the visible region of the spectrum. Then by choosing the grating blazed nearest the working

wavelength, the optimum energy throughput can be achieved. Alternately, a single "dual-blazed" grating can be used, with two regions blazed for the two spectral regions. As the dual blazed grating rotates from one wavelength extreme to another, the region blazed for the current working wavelength is brought into alignment with the optical beam, thereby offering improved efficiency compared with a single grating blazed at one wavelength.

THE ATOMIC ABSORPTION ATOMIZER

Pre-Mix Burner System

The sample cell, or atomizer, of the spectrometer must produce the ground state atoms necessary for atomic absorption to occur. This involves the application of thermal energy to break the bonds that hold atoms together as molecules. While there are several alternatives, the most routine and widely applied sample atomizer is the flame.

Figure 2-14 shows an exploded view of an atomic absorption burner system. In this "premix" design, sample solution is aspirated through a nebulizer and sprayed as a fine aerosol into the mixing chamber. Here the sample aerosol is mixed with fuel and oxidant gases and carried to the burner head, where combustion and sample atomization occur.

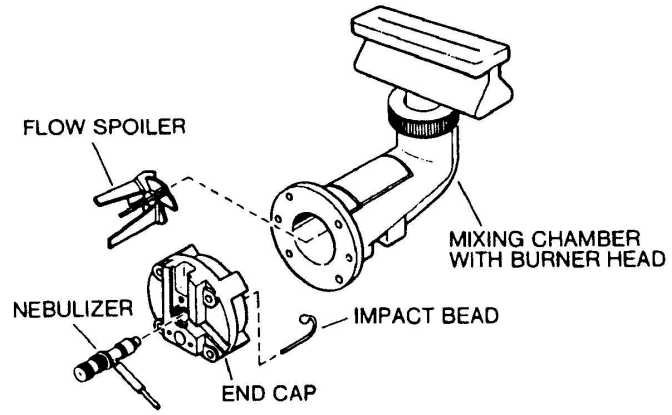


Figure 2-14. Premix burner system.

Fuel gas is introduced into the mixing chamber through the fuel inlet, and oxidant enters through the nebulizer sidearm. Mixing of the fuel and oxidant in the burner chamber eliminates the need to have combustible fuel/oxidant in the gas lines, a potential safety hazard. In addition to the separate fuel and oxidant lines, it is advantageous to have an auxiliary oxidant inlet directly into the mixing chamber. This allows the oxidant flow adjustments to be made through the auxiliary line while the flow through the nebulizer remains constant. Thus, for a burner system with an auxiliary oxidant line, the sample uptake rate is independent of flame condition, and the need to readjust the nebulizer after every oxidant flow adjustment is eliminated.

Only a portion of the sample solution introduced into the burner chamber by the nebulizer is used for analysis. The finest droplets of sample mist, or aerosol, are carried with the combustion gases to the burner head, where atomization takes place. The excess sample is removed from the premix chamber through a drain. The drain uses a liquid trap to prevent combustion gases from escaping through the drain line. The inside of the burner chamber is coated with a wettable inert plastic material to provide free drainage of excess sample and prevent burner chamber "memory." A free draining burner chamber rapidly reaches equilibrium, usually requiring less than two seconds for the absorbance to respond fully to sample changes.

Impact Devices

The sample aerosol is composed of variously sized droplets as it is sprayed into the mixing chamber. Upon entering the flame, the water in these droplets is vaporized. The remaining solid material must likewise be vaporized, and chemical bonds must be broken to create free ground state atoms. Where the initial droplet size is large, the sample vaporization and atomization process is more difficult to complete in the short time in which the sample is exposed to the flame. Incomplete sample vaporization and atomization will lead to increased susceptibility to analytical interferences.

Impact devices are used to reduce droplet size further and to cause remaining larger droplets to be deflected from the gas stream and removed from the burner through the drain. Two types of impact device are used typically, impact beads and flow spoilers.

Impact bead systems are normally used to improve nebulization efficiency, the percentage of sample solution converted to smaller droplets. The impact bead is normally a spherical bead made of glass, silica or ceramic. Glass or quartz impact beads may be less corrosion resistant and may cause more contamination problems than more chemically inert ceramic beads.

The impact bead is positioned directly in the nebulizer spray as it exits the nebulizer. The sample spray contacts the impact bead at high speed, causing some of the larger droplets to be broken up into smaller droplets. The design and positioning of the impact bead are critical in determining how well it will work. Properly designed impact bead systems will improve nebulization efficiency and remove many of the remaining large droplets from the spray. However, poorly designed or positioned impact beads may have little or no effect on nebulization efficiency and may be very inefficient at removing larger droplets from the spray. The increased population of large droplets in the aerosol may create undesirable effects, including poorer precision and increased interferences. Additionally, burner systems using an impact bead may exhibit memory problems with high concentration solutions or solutions with high dissolved solids content.

The quality of an impact bead system can frequently be determined by the increase in sensitivity it provides for selected elements. A poorly designed system will provide improved sensitivity for easily atomized elements simply because more sample is transported to the flame and less to the drain. However, there normally will be little or no improvement in sensitivity for the less easily atomized elements. A properly designed impact bead system will provide improved nebulization efficiency and improved sensitivity for all elements.

Flow spoilers normally do not improve nebulization efficiency. The primary use of a flow spoiler is to remove the remaining large droplets from the sample aerosol. The flow spoilers used in atomic absorption burner systems normally are placed between the nebulizer and the burner head. They typically have three or more large vanes constructed from or coated with a corrosion resistant material. Smaller droplets are transported through the open areas between the vanes while larger droplets contact the vanes and are removed from the aerosol.

For routine atomic absorption analyses where maximum sensitivity is not required, use of an efficient flow spoiler alone will provide the required analytical stability and freedom from interference. A burner system optimized for maximum sensitivity and performance should include both a high nebulization efficiency ceramic impact bead and an efficient flow spoiler.

Nebulizers, Burner Heads and Mounting Systems

Several important factors enter into the nebulizer portion of the burner system. In order to provide efficient nebulization for all types of sample solution, the nebulizer should be adjustable. Stainless steel has been the most common material used for construction of the nebulizer. Stainless steel has the advantage of durability and low cost but has the disadvantage of being susceptible to corrosion from samples with a high content of acid or other corrosive reagents. For such cases, nebulizers constructed of a corrosion resistant material, such as an inert plastic, platinum alloys or tantalum should be used.

Burner heads typically are constructed of stainless steel or titanium. All-titanium heads are preferred as they provide extreme resistance to heat and corrosion.

Different burner head geometries are required for various flame or sample conditions. A ten-centimeter single-slot burner head is recommended for air-acetylene flames. A special five-centimeter burner head with a narrower slot is required when a nitrous oxide-acetylene flame is to be used. Burner heads also are available for special purposes, such as use with solutions that have exceptionally high dissolved solids contents.

In addition to the flame, there are several options for atomic absorption atomizers. These options are discussed in detail in Chapter 4. Most of these options require removal of the premix burner system and replacement by an alternate atomizer in the spectrometer sample compartment. Since these alternate atomizers offer complementary and extended analytical capabilities, it is likely that the analyst will want to alternate between the use of flame AA and one or more of the other systems. A "quick change" atomizer mount is an important item to facilitate convenient changeover from one device to another without the use of tools. In addition to convenience, a "quick change" mount may reduce or eliminate entirely the need for realignment of the atomizer when it is replaced in the sample compartment.

ELECTRONICS

Precision in Atomic Absorption Measurements

We have already discussed the effects of light energy on the precision of an atomic absorption measurement. The analyst will have little control over these optical factors, as they are an inherent part of the instrument design. However, the analyst can exercise some degree of control over precision by proper selection of integration time with flame atomic absorption.

Observed precision will improve with the period of time over which each sample is read. Where analyte concentrations are not approaching detection limits, integration times of one to three seconds will usually provide acceptable precision. When approaching instrument detection limits where repeatability is poor, precision can be improved by using even longer integration times, up to 10 seconds. In most instances; however, there is little advantage to using integration times longer than 10 seconds. (To a first approximation, improvement in signal:noise ratio is proportional to the square root of the ratio of integration times.)

Since the detection limit is defined based on the observed precision, the detection limit also can be improved by increasing the integration time. The analyst, therefore, has control over the priorities for a particular analysis, maximum speed or optimum precision and detection limits.

Current instruments offer statistical functions for averaging and calculating standard deviation and relative standard deviation or coefficient of variation of replicate measurements. These functions can be used to determine the precision under various experimental conditions, allowing the analyst to optimize method parameters for each individual requirement.

Calibration of the Spectrometer

Most modern atomic absorption instruments include microcomputer-based electronics. The microcomputer provides atomic absorption instruments with advanced calculation capabilities, including the ability to calibrate and compute concentrations from absorbance data conveniently and accurately, even for nonlinear calibration curves. In the linear region, data on as little as one standard and a blank may be sufficient for defining the relationship between concentration and absorbance. However, additional standards are usually used to verify calibration accuracy. Where the relationship becomes nonlinear, however, more standards are required. The accuracy of a calibration computed for a nonlinear relationship depends on the number of standards and the equations used for calibration.

For the equation format which optimally fits atomic absorption data, it has been experimentally shown that accurate calibration can be achieved with a minimum of three standards plus a blank, even in cases of severe curvature. Figure 2-15 illustrates the accuracy of microcomputer-calculated results for cobalt with single standard "linear" and three-standard "nonlinear" calibrations. After the instrument was calibrated using the specified procedure, a series of standards were analyzed. Figure 2-15 shows plots of the actual concentrations for those standards versus the measured values for both calibration procedures. The results obtained

with "linear" calibration are accurate only where the absorbance:concentration relationship is linear, up to about 5 µg/mL. The results obtained with three-standard "nonlinear" calibration are still accurate at 30 µg/mL, significantly extending the useful working range. For versatility, current instruments allow fitting of more than three standards to these same basic equations.

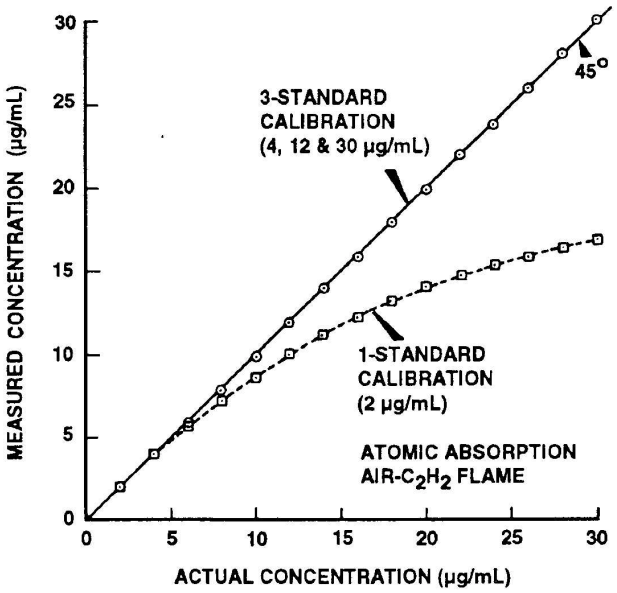


Figure 2-15. Cobalt Calibration Accuracy

AUTOMATION OF ATOMIC ABSORPTION

Automated Instruments and Sample Changers

One of the greatest contributions to the efficiency of the analytical laboratory is the automated atomic absorption spectrometer. Automatic samplers were the first step in freeing the analyst from the monotonous task of manually introducing each and every sample.

However, the real advancement in analysis automation came in the late 1970's, when automated multielement atomic absorption was introduced. In addition to automatic sample introduction, these instruments offer automatic setup of instrument parameters to preprogrammed values. These instrument "programs" can be accessed sequentially, making it possible to analyze a tray full of samples for multiple elements, without any operator intervention.

Automated Sample Preparation

While automated instrumentation has meant considerable time savings to the analyst, analytical throughput (i.e., the number of samples which can be analyzed in a given time) frequently is limited by the time required to prepare the sample. Even when the sample is available in a suitable solution form, there often are pretreatment steps which must be performed prior to analysis. The introduction of commercial systems based on techniques such as flow injection have directly addressed the need for automated sample preparation capabilities. Flow injection techniques can be used to automate relatively simple procedures such as dilution or reagent addition. They can also be used to automate complex chemical pretreatments, including analyte preconcentration and cold vapor mercury and hydride generation procedures.

The Stand-alone Computer and Atomic Absorption

Stand-alone and personal computers have extended the automation and data handling capabilities of atomic absorption even further. These computers can interface directly to instrument output ports to receive, manipulate, and store data and print reports in user selectable formats. Also, data files stored in personal computers can be read into supplemental software supplied with the system or third party software such as word processor, spreadsheet and database programs for open-ended customization of data treatment and reporting.

APPENDIX B

5 INTRODUCTION TO GRAPHITE FURNACE ATOMIC ABSORPTION

CONSIDERATIONS IN ULTRA TRACE ANALYSIS

Performance Criteria

Differences in graphite furnace performance characteristics require redefinition of some basic AA performance criteria. Since the magnitude of the graphite furnace signal observed depends on analyte mass rather than concentration, the term "characteristic mass" is used as a measure of the sensitivity of the furnace. Characteristic mass is analogous to characteristic concentration for flame AA except that mass, rather than concentration, is related to absorbance. The characteristic mass of an analyte is defined as the mass of analyte in picograms required to produce a peak height signal of 0.0044 absorbance or an integrated peak area signal of 0.0044 absorbance-seconds (A·s).

Similar to characteristic concentration for flame AA, characteristic mass may be used as an indicator of instrument optimization. Typical characteristic mass values for a properly adjusted instrument are usually given in the instrument documentation. Experimental values for characteristic mass (m_o) can be determined for comparison by measuring the peak area absorbance of a known mass of analyte and calculating m_o according to the following equation.

$$m_o\ (pg) = \frac{\text{Sample vol. } (\mu L) \times \text{Analyte Conc. } (\mu g/L) \times 0.0044\ A{\cdot}s}{\text{Observed Peak Area } (A{\cdot}s)}$$

Note that for this equation to be valid, the analyte mass used to measure m_o must produce a signal in the linear range of the calibration curve. By comparing the experimental value obtained from this equation to the reference value given in the instrument documentation, the state of instrument optimization can be evaluated. If the calculated m_o is significantly greater than the reference value, adjustments should be made to improve the sensitivity of the measurement. Calculated values for m_o which are significantly less than the reference value, suggesting better than specified sensitivity, may in fact be a warning sign of analyte contamination in the standard.

Another term characterizing graphite furnace instrument performance is "detection limit". Similar to flame AA, the detection limit is an indicator of the lower limits of analyte detectability and is limited by the instrument signal-to-noise ratio. Graphite furnace detection limits are usually stated in mass units (pg) rather than concentration units, again reflecting the furnace signal's dependency on analyte mass, rather than concentration. However, in real analytical situations the detection limit will depend both on the system sensitivity (characteristic mass) and the maximum sample size which can be accommodated.

Graphite Furnace Applications

The sensitivity of graphite furnace atomic absorption makes it the obvious choice for trace metal analysis applications. Routine determinations at the µg/L level for most elements make it ideal for environmental applications. Advances in instrumentation and techniques have made it possible to analyze very complex sample matrices, such as those frequently found in biological and geological samples. The microliter sample sizes used offer additional benefits where the amount of sample available for analysis is limited, as in many clinical analyses.

COMPONENTS OF THE GRAPHITE FURNACE SYSTEM

The graphite furnace is made up of three major components, the atomizer, the power supply, and the programmer. The atomizer is located in the sampling compartment of the atomic absorption spectrometer, where sample atomization and light absorption occur. The power supply controls power and gas flows to the atomizer under the direction of the programmer, which is usually built into the power supply or spectrometer. A description of each of these major components follows.

The Graphite Furnace Atomizer

A basic graphite furnace atomizer is comprised of the following components:

- graphite tube
- electrical contacts
- enclosed water cooled housing
- inert purge gas controls

A graphite tube is normally the heating element of the graphite furnace. The cylindrical tube is aligned horizontally in the optical path of the spectrometer and serves as the spectrometer sampling cell. A few microliters (usually 5-50) of sample are measured and dispensed through a hole in the center of the tube wall onto the inner tube wall or a graphite platform. The tube is held in place between two graphite contact cylinders, which provide electrical connection. An electrical potential applied to the contacts causes current to flow through the tube, the effect of which is heating of the tube and the sample.

The entire assembly is mounted within an enclosed, water-cooled housing. Quartz windows at each end of the housing allow light to pass through the tube. The heated graphite is protected from air oxidation by the end windows and two streams of argon. An external gas flow surrounds the outside of the tube, and a separately controllable internal gas flow purges the inside of the tube. The system should regulate the internal gas flow so that the internal flow is reduced or, preferably, completely interrupted during atomization. This helps to maximize sample residence time in the tube and increase the measurement signal. Figure 5-1 illustrates one type of atomizer assembly, a longitudinally-heated furnace.

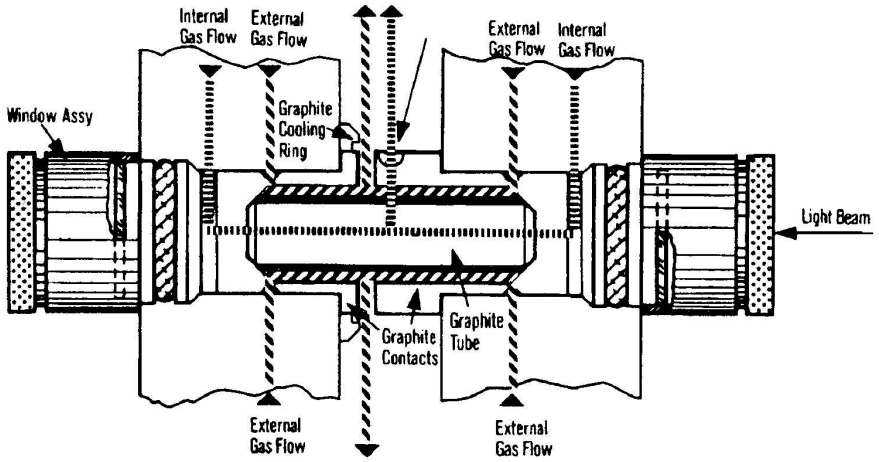


Figure 5-1. Longitudinally-heated graphite furnace atomizer.

The tube in Figure 5-1 is heated by passing electrical current from the graphite contacts at the ends of the tube through the length of the tube. This type of furnace is similar to the original design of Massmann, which is the basis for most currently available commercial graphite furnace systems.

The longitudinally-heated furnace has a major liability. The electrical contacts at each end of the tube must be cooled. As a result, there must always be a temperature gradient along the length of the tube, the tube ends adjacent to the electrical contacts being cooler than the central portion. This temperature gradient can cause vaporized atoms and molecules to condense as they diffuse to the cooler tube ends. This may produce interferences, the most common type being the incomplete removal of analyte or matrix from the tube. Incomplete removal of matrix during pyrolysis can increase the magnitude of background absorption during atomization. Incomplete removal of analyte during atomization is more serious. It creates "carryover" or "memory", wherein a portion of the analyte in the current sample remains in the tube and contributes to the analytical signal for the following sample. This produces erroneously high analytical results and poor precision.

To minimize carryover, most longitudinally-heated furnace heating programs use one or more cleanout steps after the atomization step. A cleanout step involves the application for several seconds of full internal gas flow and a temperature equal to or greater than that used for atomization to remove residual sample components. While this technique works well for the more easily atomized analytes, it is not always successful with those analytes that require higher atomization temperatures. The use of a high temperature cleanout step may also reduce tube lifetime.

The transversely-heated graphite furnace eliminates many of the problems associated with the longitudinally-heated furnace. The graphite tube of the transversely-heated furnace, shown in Figure 5-2, includes integral tabs which protrude from each side. These tabs are inserted into the electrical contacts. When power is applied, the tube is heated across its circumference (transversely). By applying power in this manner, the tube is heated evenly over its entire length, eliminating or significantly reducing the sample condensation problems seen with longitudinally-heated furnace systems.

An additional advantage of the transversely-heated furnace is that it allows the use of longitudinal Zeeman-effect background correction. As described in Chapter 3, longitudinal Zeeman offers all of the advantages of transverse Zeeman correction without the need to include a polarizer in the optical system. This provides a significant improvement in light throughput.

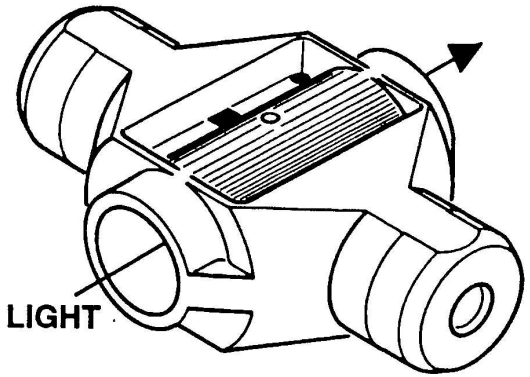


Figure 5-2. A graphite tube for a transversely-heated furnace.

The Graphite Furnace Power Supply and Programmer

The power supply and programmer perform the following functions:

- electrical power control
- temperature program control
- gas flow control
- spectrometer function control

The power supply controls the electrical current supplied to the graphite tube, which causes heating. The temperature of the tube is controlled by a user-specified temperature program. Through the programmer the operator will enter a sequence of selected temperatures vs. time to carefully dry, pyrolyze, and finally atomize the sample. The program may also include settings for the internal inert gas flow rate and, in some cases, the selection of an alternate gas. Certain spectrometer functions, such as triggering of the spectrometer read function, also may be programmed and synchronized with the atomization of the sample in the furnace.

SUMMARY OF A GRAPHITE FURNACE ANALYSIS

A graphite furnace analysis consists of measuring and dispensing a known volume of sample into the furnace. The sample is then subjected to a multi-step temperature program. When the temperature is increased to the point where sample atomi-

zation occurs, the atomic absorption measurement is made. Variables under operator control include the volume of sample placed into the furnace and heating parameters for each step. These parameters include:

1) temperature	final temperature during step
2) ramp time	time for temperature increase
3) hold time	time for maintaining final temperature
4) internal gas	gas type and flow rate

In addition to the above, spectrometer control functions can be programmed to occur at specified times within the graphite furnace program. While the number of steps within each program is variable, 6 steps make up the typical graphite furnace program. These steps include:

1) Drying
2) Pyrolysis
3) Cool down (optional)
4) Atomization
5) Clean out
6) Cool down

Figure 5-3 illustrates a typical graphite furnace program. The following paragraphs will discuss each operator controlled variable, and how they may affect the analysis.

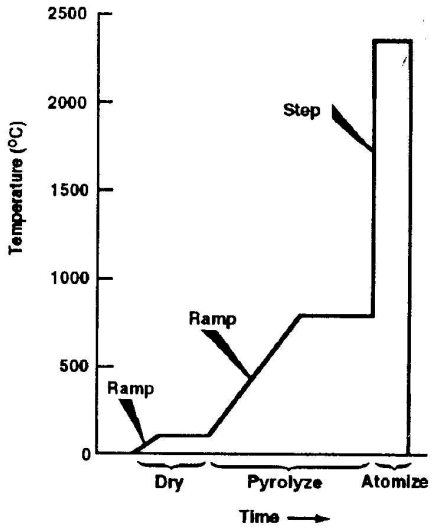


Figure 5-3. A graphite furnace temperature program.

Sample Size

Since the graphite furnace signal depends on analyte mass, the operator has an effective degree of control on measured absorbance by controlling the sample volume. Larger volumes of sample solution contain more analyte and result in greater signals. The analytical range of furnace analysis can therefore be controlled, to some extent, by varying sample volume. For very low concentrations, the maxi-

mum volume of analyte can be used, while for higher concentrations, the sample volume can be reduced. Smaller sample volumes can also be used where sample availability is limited or where background absorption is excessively large.

The maximum volume of sample usable will depend on the tube configuration. Where the graphite platform is not used, sample volumes up to 100 µL can be used, depending on the type of tube and sample. With the platform in place, a sample volume of less than 50 µL is recommended. A convenient sample volume for most analyses is 20 µL. Where larger volumes are required, i.e., for improved detection limits, multiple injections can be used with appropriate drying and pyrolysis steps between each injection to increase the effective sample size.

The use of an autosampler is strongly recommended for dispensing samples into a graphite furnace. While skilled operators may obtain reasonable reproducibility by manual injection on a short term basis, autosamplers have been proven to provide superior results. With many graphite furnace systems, autosamplers can also generate working standards from stock standard solutions; add appropriate reagents; and provide method of additions analyses or recovery measurements, all automatically.

The Drying Step

After the sample is placed in the furnace, it must be dried at a sufficiently low temperature to avoid sample spattering, which would result in poor analytical precision. Temperatures around 100-120 °C are common for aqueous solutions.

Use of a temperature "ramp" provides a variable time over which the temperature is increased. A longer ramp time provides a slower, more "gentle" increase in heating. When a platform is used, the temperature lag of the platform versus the tube walls provides a natural "ramping" effect. Therefore shorter ramp times are usually used with the platform. Longer ramp times are used when the sample is to be atomized from the tube wall.

After the temperature ramp, the furnace is held at the selected drying temperature until drying is complete. Since only a few microliters of sample are used, the drying "hold" time is usually less than a minute.

During the drying process, the internal gas flow normally is left at its default maximum value (250-300 mL per minute) to purge the vaporized solvent from the tube.

The Pyrolysis Step

The purpose of the pyrolysis step (sometimes referred to as the ashing, char or pretreatment step) is to volatilize inorganic and organic matrix components selectively from the sample, leaving the analyte element in a less complex matrix for analysis. During this step, the temperature is increased as high as possible to volatilize matrix components but below the temperature at which analyte loss would occur.

The temperature selected for the pyrolysis step will depend on the analyte and the matrix. Suggested temperatures normally are provided in the documentation supplied with the graphite furnace. The internal gas flow is again left at 250-300 mL per minute in the pyrolysis step, to drive off volatilized matrix materials. For some sample types, it may be advantageous to change the internal gas, e.g., to air or oxygen, during the pyrolysis step to aid in the sample decomposition.

The Pre-atomization Cool Down Step

With longitudinally-heated furnaces, it is frequently advantageous to cool the furnace prior to atomization since the heating rate is a function of the temperature range to be covered. As the temperature range is increased, the rate of heating also increases. The use of a cool down step prior to atomization maximizes the heating rate and extends the isothermal zone within the tube immediately after heating. The extended isothermal zone has been shown to improve sensitivity and reduce peak tailing for a number of elements, including those which characteristically are difficult to atomize in the graphite furnace.

A pre-atomization cool down step normally is not required for transversely-heated furnaces as the isothermal zone extends the length of the tube with that type of system.

The Atomization Step

The purpose of the atomization step is to produce an atomic vapor of the analyte elements, thereby allowing atomic absorption to be measured. The temperature in this step is increased to the point where dissociation of volatilized molecular species occurs.

The atomization temperature is a property of the analyte element. By following recommended procedures of analysis, it is usually possible to use the temperatures provided in the graphite furnace documentation without further optimization. Care should be taken to avoid the use of an excessively high atomization tempera-

ture, as the analyte residence time in the tube will be decreased and a loss of sensitivity will occur. Also, the use of excessively high atomization temperatures can shorten the useful lifetime of the graphite tube.

For atomization, it is desirable to increase the temperature as quickly as possible. Therefore, ramp times normally will be set to minimum values to provide the highest heating rate. It also is desirable to reduce or, preferably, to totally interrupt the internal gas flow during atomization. This increases the residence time of the atomic vapor in the furnace, maximizing sensitivity and reducing some interference effects. At the beginning of this step, the spectrometer "read" function is triggered to begin the measurement of light absorption.

The Clean Out and Cool Down Steps

After atomization, the graphite furnace may be heated to still higher temperatures to burn off any sample residue which may remain in the furnace. An optional cool down step then allows the furnace to return to near ambient temperature prior to the introduction of the next sample. With some systems, a preset cool down step is automatically included in each furnace cycle, and need not be programmed separately.

FAST FURNACE ANALYSIS

The most time consuming portions of a graphite furnace AA analysis are the drying and pyrolysis steps. Analysts have long sought some means to reduce or eliminate the time required for these pretreatment stages. For years, an inability to compensate for the high background signals generated with incomplete pretreatment and matrix-related interferences precluded reducing the time required for these steps.

The situation changed dramatically with the introduction of two developments: Zeeman effect background correction and Stabilized Platform Graphite Furnace (STPF) technology, which is described in detail in Chapter 6. Zeeman effect background correction is far superior to continuum source background correction in its ability to correct accurately for high levels of background absorption. The use of STPF technology provides almost constant characteristic mass values independent of sample matrix. The combination of these two techniques was the key to providing faster graphite furnace analyses.

In fast furnace analysis, the pyrolysis step and matrix modification are usually eliminated. The drying step is minimized by injection of the sample onto a preheated platform. Drying time is also minimized by using as small a sample size as possible consistent with the required analytical precision and detection limits.

To further reduce the furnace program time, the cool down step at the end of the program is frequently reduced or eliminated. This is possible since most furnace systems typically require 20 seconds or more between the end of a temperature program and the point at which the next sample is ready to be introduced into the furnace. That 20 second period is usually sufficient to allow the furnace to cool reproducibly to the preheated drying temperature required for the next sample.

Using these procedures, typical furnace program times frequently can be reduced from 2-4 minutes to 30 seconds or less per determination, a considerable time savings, without sacrificing analytical precision or accuracy.

Fast furnace analysis techniques are not compatible with all AA instrumentation. To fully realize the benefits of fast furnace analysis, the instrumentation used must provide Zeeman background correction, be capable of handling very high absorbance measurements and be compatible with the requirements of STPF technology. Also, the fast furnace technique may not be compatible with all sample types. Very complex matrices may still require at least a short pyrolysis step and use of a matrix modifier for optimum results.

MEASURING THE GRAPHITE FURNACE AA SIGNAL

Nature of the Graphite Furnace Signal

In flame atomic absorption, the absorption signal is steady state. That is, as long as solution is aspirated into the flame, a constant absorbance is observed. For graphite furnace analyses, however, the signal is transient. As atomization begins, analyte atoms are formed and the signal increases, reflecting the increasing atom population in the furnace. The signal will continue to increase until the rate of atom generation becomes less than the rate of atom diffusion out of the furnace. At that point, the falling atom population results in a signal which decreases until all atoms are lost and the signal has fallen to zero. To determine the analyte content of the sample, the resulting peak-shaped signal must be quantitated.

Peak Height Measurement

For many years, measuring the height of the transient signal was the only practical means for quantitating furnace results. The constantly changing signal was monitored on a strip chart recorder, and peak height was measured manually in chart divisions. Later instrumentation allowed peak height to be measured directly by electronic means.

While peak height does depend on the analyte concentration in the sample, it is also affected by other factors. Peak height is only a measure of the maximum atom population which occurred in the furnace during atomization. If matrix components in the sample affect the rate of atom formation, the maximum atom population and the peak height are also affected, as shown in Figure 5-4 for the determination of lead in blood. While the two solutions contain identical amounts of lead (0.2 ng), the peak shapes and appearance times are dramatically different.

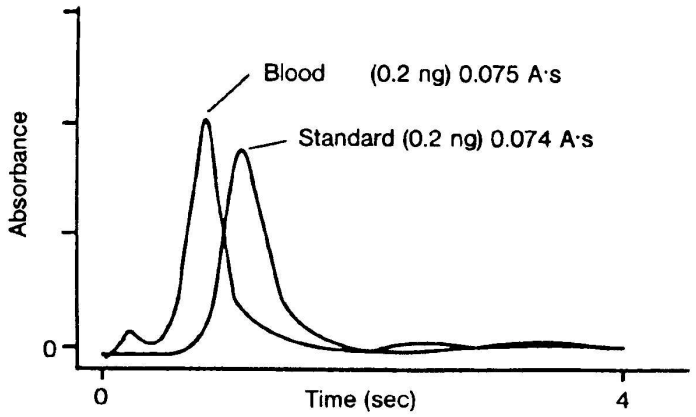


Figure 5-4. Effect of matrix on peak height and area.

This susceptibility to matrix effects makes graphite furnace AA vulnerable to interferences when peak height measurement is used for quantitation. Therefore, peak height measurements are seldom used with modern graphite furnace AA systems.

Peak Area Measurement

Modern instrumentation provides the capability to integrate absorbance during the entire atomization period, yielding a signal equal to the integrated peak area, that is, the area under the peak signal. If the temperature in the furnace is constant during the measurement process, the peak area will represent a count of all atoms present in the sample aliquot, regardless of whether the atoms were generated early or late in the atomization process. Integrated peak area measurements (A·s) are independent of the atomization rate, and are therefore much less subject to matrix effects as shown in Figure 5-4. As a result, peak area is preferred for graphite furnace analysis.

SOLID SAMPLING WITH THE GRAPHITE FURNACE

The ability to analyze solid samples directly with little or no sample preparation offers a number of potential benefits. It reduces total analysis time, minimizes the potential for contamination or analyte loss, and reduces the cost of sample pretreatment reagents and their disposal.

The first published information on the use of graphite furnace AA for the direct analysis of solid samples appeared in 1971. However, the technique was fraught with problems. The small sample sizes required (1-2 mg) frequently were not representative of the original sample due to inhomogeneous distribution of the analyte. Samples were difficult to handle as insertion of the sample into the furnace was a cumbersome, manual procedure. Analytical results frequently were not reproducible as each replicate had to be sampled, weighed and transferred separately. Finding a suitable means of calibrating the determination varied from difficult to impossible. Aqueous standards or the method of additions frequently could not be used. Matrix-matched standards (if available) were required for many determinations.

Today, many of the problems associated with solid sampling with the graphite furnace have been overcome. The key to removing the previous limitations was the development of slurry sampling. With slurry sampling, the sample is reduced to a fine powder, usually by freeze-drying or grinding depending on the sample type. The powder is weighed directly into an autosampler vial, and a measured amount of liquid is added. The vial is then placed into the tray of the furnace autosampler. Just prior to the analysis, the mixture is agitated to form a slurry. Most commonly, the agitation is performed using an ultrasonic probe. The slurry is then sampled by the autosampler capillary.

Slurries can be analyzed exactly as though they were true solutions. Replicates can be measured from the same sample vial. Matrix modifiers and spikes can be added. Sample size can be varied conveniently. True Stabilized Temperature Platform Furnace (STPF) conditions are maintained, allowing calibration against simple aqueous standards.

Even with slurry sampling, however, some limitations remain. Although a larger portion of the original sample is weighed relative to direct solid sampling, sample homogeneity problems can still occur. Particle size may affect reproducibility and accuracy if it is inconsistent or too large. The degree to which the solvent used may extract the analyte can also affect analytical results. However, the major hurdles to solid sampling with the graphite furnace have been overcome.

7 ALTERNATE ANALYTICAL TECHNIQUES

Atomic absorption is a mature analytical technique. Its interferences are well documented and, for the most part, easy to control. The various atomizer alternatives make atomic absorption one of the most versatile analytical techniques, capable of determining a great number of elements over wide concentration ranges. Even so, there are competing techniques which should be considered as alternatives or complements to AA for certain applications. In this chapter, we will examine the advantages and disadvantages of these techniques compared to atomic absorption. A summary comparison is given in Table 7-1.

DIRECT CURRENT PLASMA (DCP) EMISSION

DCP is an atomic emission technique. Sample is aspirated into a premix spray chamber through a nebulizer using a system very similar to that for atomic absorption. However, instead of combustible gases, argon is used as a transport gas for the sample. The sample aerosol in a stream of argon is directed at a set of electrodes, across which a high voltage electrical potential is applied. The resulting electrical discharge between the electrodes supplies enough energy to ionize the argon into a "plasma" of positively charged argon ions and free electrons. The thermal energy of the plasma, in turn, atomizes sample constituents and creates excited state atoms, which emit their characteristic atomic emission spectra.

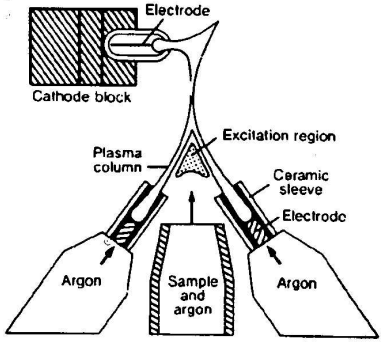


Figure 7-1. The DCP Torch

DCP was the first plasma technique applied to routine atomic emission analyses. In its early days, it was an especially valuable complement to atomic absorption in that DCP provided good detection limits

for the refractory elements, for which atomic absorption was not particularly sensitive. DCP is also capable of simultaneous multielement analysis and qualitative, as well as quantitative, analysis.

DCP carries with it some significant disadvantages, however. The electrodes which form the DC arc are continually eroded and burned away during operation. This imposes a maintenance problem of continual adjustment and replacement of the electrodes. In addition, the very high temperatures for which plasmas are known are not fully realized in the DCP design. Due to a highly resistant "skin effect", the sample does not penetrate into the hottest part of the plasma, but is instead deflected around it. The analytical measurement normally is made just underneath the hottest part of the plasma, where temperatures are hot enough to provide good sensitivity for refractory elements but not hot enough to eliminate chemical and ionization effects. Procedures for reagent addition usually are prescribed to deal with these interferences. Because these limitations are not normally encountered with inductively coupled plasma (ICP) systems, ICP emission is normally a preferred emission technique.

INDUCTIVELY COUPLED PLASMA (ICP) EMISSION

Similar to DCP, ICP is an atomic emission technique using an argon plasma as an excitation source. However, the design of the source is completely different. Sample is again introduced into a premix spray chamber, where it is directed up the central tube of the ICP "torch". The torch consists of concentric tubes with independent argon streams flowing through each. The top of the torch is centered within a radio frequency (RF) induction coil, which is the source of energy for the system.

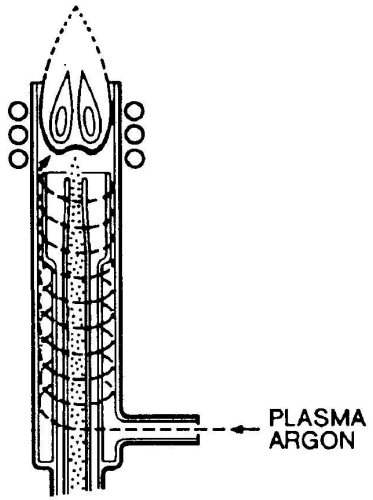


Figure 7-2. The ICP torch.

After ignition, the plasma is propagated through inductive coupling with the RF field generated from the coil. Unlike DCP, there are no electrodes to maintain and replace. Further the ICP torch is designed specifically to promote penetration of the plasma skin by the sample, allowing sample atoms to experience the full energy of the plasma source.

The high temperatures provided by the ICP provide excellent sensitivities for refractory elements and also essentially eliminate chemical interferences. Like all emission techniques, there are no source lamps. By monitoring several wavelengths, either all at once or in a programmed sequence, many elements can be determined in one automated analysis. ICP emission, therefore, offers significant speed advantages over atomic absorption for multielement analyses. Except for the refractory elements, which may be substantially better than even graphite furnace AA, ICP detection limits are comparable to flame atomic absorption.

The high temperatures of the ICP carry one disadvantage. The plasma is so effective in generating excited state species that the rich emission spectra produced increase the probability of spectral interferences. High resolution monochromators and sophisticated software for background and interelement correction are used to deal with this potential problem. Another limitation of ICP emission is the initial cost of the instrumentation. The price for basic ICP systems starts at about the same level as the prices for top-of-the-line automated AA systems. More sophisticated instrumentation can cost two to four times the price of basic systems.

INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY (ICP-MS)

ICP-MS is one of a growing number of "hyphenated techniques", where the output of one technique becomes the input of another. For ICP-MS, the ICP is used as the ion source for a mass spectrometer. The ions are then spatially separated according to their mass and charge, and measured individually.

The major attractiveness of ICP-MS is its exceptional sensitivity combined with high analysis speed. For most elements, ICP-MS offers detection limits which are comparable to or better than those of graphite furnace AA. But ICP-MS can determine many elements in the time required for the determination of one element by graphite furnace AA. ICP-MS also offers the ability for isotopic analysis.

As with the other techniques, ICP-MS also has its limitations. The relative newness of ICP-MS means, while the required instrumentation is well developed, many developments in analytical methodology are yet to be made. This translates into additional effort for the analyst in adapting the technique to his or her particular analytical needs. Since ICP-MS is not a spectroscopic technique, spectral interferences do not occur. Interferences from mass overlaps due to other isotopes and polyatomic species do occur, however, and may provide erroneous results un-

less properly corrected. The major limitation of ICP-MS at this time, however, may be its cost. ICP-MS systems typically are two to four times as expensive as basic ICP emission systems. However, the unique abilities of ICP-MS to provide graphite furnace detection limits with the analytical speed of ICP emission and to perform isotopic analysis capabilities frequently provide the justification needed to overcome cost limitations.

SUMMARY

While other analytical techniques may offer specific advantages over atomic absorption, it can be seen from the above comparison that no single technique offers all of the advantages. The versatility, moderate cost, and established methodology of atomic absorption will continue to make it a valuable tool for the laboratory.

Table 7-1
Comparison of Analytical Techniques

Maturity:	
Flame AA	well established
Furnace AA	well established
DCP	superseded by ICP
ICP	established and growing
ICP-MS	new and growing
Speed:	
Flame AA	fast (single-element)
Furnace AA	slow (single-element)
DCP	fast (multi-element)
ICP	fast (multi-element)
ICP-MS	fast (multi-element)
Sensitivity:	
Flame AA	moderate; poor for refractories
Furnace AA	excellent; limited for refractories
DCP	moderate; very good for refractories
ICP	moderate; excellent for refractories
ICP-MS	excellent
Interferences:	
Flame AA	few; well understood
Furnace AA	many; controlled with STPF
DCP	many
ICP	spectral
ICP-MS	moderate, mass overlap
Relative Cost:	
Flame AA	low to moderate
Furnace AA	moderate to high
DCP	moderate to high
ICP	moderate to very high
ICP-MS	very high