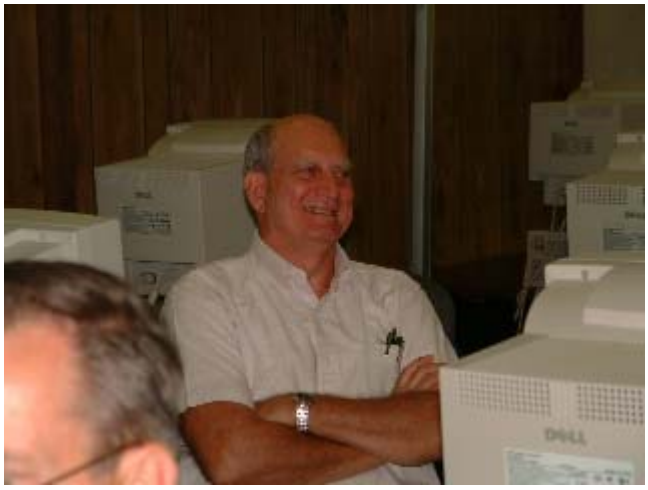
An Introduction to Instrumental Analysis:
A laboratory manual for CHM 205 and FOS 205



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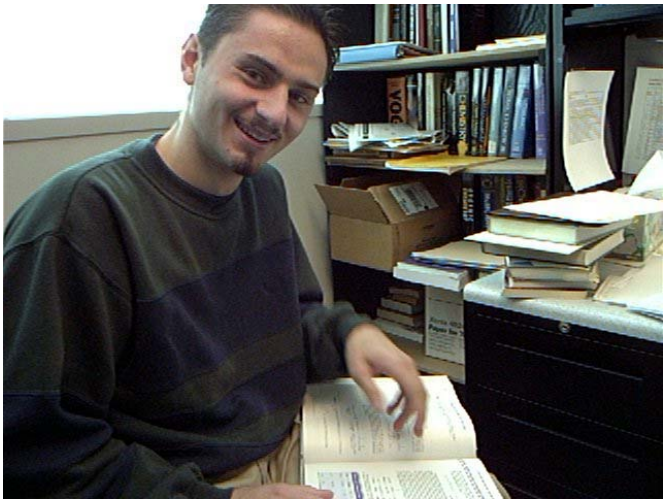

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Spring 2004

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This manual is for sophomore-level students who are studying forensic sciences or chemistry. The manual was prepared with support from the National Science Foundation under Grant DUE-0202431.

Introduction to Forensic Chemistry: Instrumental Analysis
Chemistry 205 and Forensic Sciences 205

Preface Chemistry is not one of the formal disciplines known as forensic sciences. However, many forensics scientists use chemistry as part of their daily routine. For example, a combination of gas chromatography and mass spectrometry (GC-MS), two instrumental techniques that will be introduced in this course, was used recently by toxicologists[1,2] to conduct forensic analyses. Our intent is to introduce you to five instruments that have wide applicability in the forensics field, as well as in other disciplines. The focus of this course will be on fundamental or basic laboratory analyses that make use of our available instruments. You will be introduced to rudimentary forensics analyses that make use of these instruments with the objective of preparing you for more advanced training or to be an end point if your interests lie elsewhere.

Chime: The Chime plug-in allows you to view molecules in the Chime format. You must first download the free Chime plug-in. Open your Internet browser, minimize it, and come back to this page. You are going to download Chime 2.6(SP3). Depress the Ctrl key on your keyboard and click on the following link. It will take you to the Chime site, where you should register and follow the directions for downloading the plug-in.
Chime Download (http://www.mdlchime.com/downloads/downloadable/index.jsp)
Note: If you cannot go directly to the download site, then copy the above URL and paste it into your browser to go to the download site.
Test your plug-in by clicking twice on the following icon. After the animation opens, use the left mouse key and shift key simultaneously to enlarge the molecule. Use the left mouse key to manually rotate the molecule up and down and in circular motions. Right click on the structure and explore all of the options available to manipulate the molecule, a model of bromocyclohexane.


Spartan.pdb

Chemistry Information from the Net:

The following link goes to a web page in the Google search engine. That page provides additional links to interesting sites that offer vast amounts of information and data. Click on the link to see what's available.

http://directory.google.com/Top/Science/Chemistry/Software/Web_Publishing/

[1,2] **JOURNAL OF FORENSIC SCIENCES,** Volume 47, No. 5, 2002, pp. 1133-1134; JFS**, 47** (6), 2002, pp. 1380-1387.

Reference Spectra: The following site allows you to obtain spectral data on a compound by typing in its chemical name or formula. The spectra include: infrared (IR) spectra, proton magnetic resonance (^{1}H NMR) spectra, carbon-13 magnetic resonance (^{13}C NMR) spectra, and mass spectra (MS). This site should prove very useful during your study of spectroscopy.

http://www.aist.go.jp/RIODB/SDBS/menu-e.html

Blackboard: Blackboard is the name of a company that provides PGCC with computer services. Among other things, you can find your point scores on all exams and labs, the course syllabus, and answers to exams. Your instructor will go over how to use Blackboard at the class's first meeting. The initial login instructions are given below.

Login Instructions:

http://pgcconline.blackboard.com

Blackboard is a secure site. You must be enrolled in the course and have a user id and password to login. The initial user id is made from your name and birthday. For example, if your name is Jay Gross and your birthday is April 22. Then your userid becomes the alphanumeric: rgro0421 and is determined by the first letter of the first name, followed by the first three letters of last name and the MMDD of birthday.

Userid: jgro0422 **R**ay **Gro**ss Birthday April **04** Twenty-first **22**

The initial password is your 9 digit social security number: Password: SSN xxxxxxxxx

Course Syllabus: The course syllabus is always available on Blackboard. Consult the syllabus for details about grading labs, etc.

Dr. Gross's Web Site:

http://academic.pg.cc.md.us/~rgross/

Laboratory Schedule

Lab Number	Week #	Activity
1	1	Introduction to Gas Chromatography
2	2	Explorations in Gas Chromatography
3	3	Forensic Analysis by Gas Chromatography
4	4	Introduction to Infrared Spectroscopy

5	5	Explorations in Infrared Spectroscopy
6	6	Forensic Analysis by Infrared Spectroscopy
7	7	Introduction to Ultraviolet-Visible Spectroscopy
8	8	Explorations in Ultraviolet-Visible Spectroscopy
9	9	Forensic Analysis by Ultraviolet-Visible Spectroscopy
10	10	Introduction to High-Pressure-Liquid Chromatography
11	11	Explorations in High-Pressure-Liquid Chromatograph
12	12	Forensic Analysis by High-Pressure-Liquid Chromatograph
13	13	Introduction to Gas Chromatography-Mass Spectroscopy
14	14	Explorations in Gas Chromatography-Mass Spectroscopy
15	15	Forensic Analysis by Gas Chromatography-Mass Spectroscopy
16	16	Final Exam Week

Weekly Lab Requirements: Each experiment is followed by a problem set that contains questions about the experiment, related lecture topics, review, or preview questions. You must work these problem sets and turn them in at the beginning of the next lab period. If a lab report is due, you will staple the problem set to the report. This manual contains all of the information you will need for a given lab period after the first lab. This includes the introductory materials and lab procedures. Thus, as a minimum, you must print the lab procedure for each week's lab and bring it with you to the lab. Then you can follow the written instructions. Likewise, you must print each Lab Report and each Problem Set in order to turn in your work. You may type your answers to the extent possible before printing.

Summary of Weekly Requirements:

1. Read the references and discussions that go with that week's lab.
2. Complete any pre-lab assignments.
3. Print the procedure and bring it with you to lab.
4. Conduct the experiment, collecting any data in your lab notebook.
5. Write up your lab report.
6. Work the problem set, print it, and attach it to your report.
7. Turn in the completed report at the beginning of the next lab period. If no report is due, turn in the completed problem set alone.

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1 Introduction to Gas Chromatography

(Qualitative and Quantitative Analysis of an Unknown Alcohol[1])

A Summary of this Week's Lab During this lab period, you will be introduced to gas chromatography and its application to forensic analysis. You will learn how a gas chromatograph operates, what data you can get from it, and how to analyze the data to get qualitative and quantitative results. As your introduction to the instrument, you will be allowed to prepare and inject three samples. The first sample is a mixture that contains four known alcohols. You will be given the names of the four alcohols and the order in which they appear on a chromatogram (the retention times or relative order of elution). The second sample is an unknown alcohol, which is one of the four possible known alcohols. You will compare the retention time of your unknown to the retention times of the four known alcohols and make a preliminary determination about the identity of your unknown from the comparison. You will complete the identification of your unknown by preparing and injecting a third sample. You will prepare the third sample by adding a small amount (~ 1μL) of your unknown to the known mixture of alcohols. You will inject the third sample and obtain a chromatogram. You will identify the unknown on the basis of the peak-enhancement you observe. You will be able to conclusively identify the unknown, because you know it to be one of only four possible alcohols. If you did not know that the unknown is one of four alcohols, then you could state only that your unknown and one of the known alcohols have identical retention times. An identification of this type, in which you identify an unknown, is called ***qualitative analysis***. For home work, you will be given a set of gas-chromatographic data about an alcohol from which you will prepare a calibration curve, using a TI-83 graphing calculator. You will be given additional data pertaining to a GC analysis of a sample that contains an unknown amount of the alcohol. You will find the exact amount of the alcohol in the unknown sample from the calibration curve, using the TI-83. An analysis of this type, in which you find an exact amount (within the limits of analysis) of a known chemical, is called ***quantitative analysis***. At the beginning of the next lab period, you will turn in a report of your laboratory findings (See the Introduction to this Manual). You will attach your homework to the report.

The Major Uses of Gas Chromatography

There are three major applications of gas chromatography. One use is to determine the number of component compounds in a mixture; another use is to determine whether or not a specific compound is present in a sample; and the third use is to determine the specific amount of a given compound in a sample. In short, these three uses are the separation of a mixture, a qualitative analysis, and a quantitative analysis.

General Considerations of Chromatographic Analysis

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1. [1] This three-lab block of instruction was modified from: Meloan, C.E, James, R.E, and Saferstein, R., Lab Manual for *Criminalistics: An Introduction to Forensic Science*, 7th ed., p. 225-238.

Chromatography literally means to separate by colors, because early separations were detectable by the colors of the various compounds that were separated. The word chromatography as it is used today still implies a separation technique. The words that lead in to chromatography define how the separation is accomplished. For example, ***thin layer chromatography*** (TLC) is a separation technique in which a mixture of compounds is separated on a thin plate that is coated with silica or a similar solid. ***Column chromatography*** is a lab technique in which larger mixtures can be separated by gravity elution of a liquid solution of a mixture through a column packed with a material such as silica gel. Gas chromatography, also called vapor-phase chromatography, is the technique we will study in this block of instruction.

Chromatographic separations involve two phases (recall that phases are solids, liquids, or gases). In chromatography, one of the two phases is a ***stationary phase*** (i.e., it does not move during the analysis), and the other phase is a ***mobile phase***, which moves through the stationary phase. The mixture of compounds to be separated, let's say Compound A, Compound B, and Compound C, is placed on the stationary phase and is separated into pure A, pure B, and pure C as the mobile phase carries them from the starting point to the ending point. The chemicals to be analyzed are called ***analytes***. The chemical principle that is involved in all separations of this type is that the properties of compounds are a function of their structure. Hence, compounds that differ in structure will differ in the rates at which they travel during the chromatographic process and will be separated over time. If compounds A, B, and C differ sufficiently in structure for the chromatographic technique employed, they can be separated. However, you should be aware that a mixture of compounds, very similar in structure, might be inseparable by standard chromatographic techniques. For example, a mixture of sterols might appear to be a pure compound by TLC. Thus, finding the right column to separate a novel mixture might require some experimentation to find the parameters that work. For the alcohol analyses, we will not be changing columns; we will use the one installed in our instrument.

Gas Chromatography (GC)

Gas chromatography is accomplished with an instrument known as a ***gas chromatograph***. Today, many options are available to an investigator. For example, one vendor, SRI, (http://www.srigc.com/catalog/products.htm) offers 15 GC models pre-configured for common applications. SRI's literature includes the following statements, "Up to 4 detectors, from a choice of 14, can be mounted simultaneously. Up to 5 injector types, from a choice of 15, can be installed at the same time." As you continue your studies in gas chromatographic analysis, you will learn more and more about the myriad options that are available. We will focus our attention on the basics or fundamentals of GC and on the equipment we have on hand at PGCC (i.e., the equipment that you will use in the laboratory). The following discussion is an introduction to your laboratory work and is not intended to be a comprehensive treatment of gas chromatographic analysis.

As the name implies, a gas is the mobile phase in GC. This gas is commonly called the ***carrier gas***, because it carries the mixture through a stationary phase. The carrier gas must be inert (i.e., non reactive) to our mixture of compounds. The stationary phase is prepared by packing a long (~10 m), small diameter (6 mm) coiled metal tube, called a column, with a high-boiling compound that will be in the liquid phase when the separation is carried out. Fortunately, you will not have to prepare a column, because a variety of them are commercially available, depending upon the separation to be accomplished. Thus, the chromatographic system is a ***gas-liquid system***. The mobile phase is the

gas, and the stationary phase is the liquid. Helium is a commonly used carrier gas, because it is readily available and is an inert chemical. You select a column from those available that will best separate your mixture. A typical column contains ground up fire brick, which serves as a support for a high-boiling liquid phase, which is a material such as Carbowax. Carbowax is essentially non-volatile, meaning that it will not boil away during its use and will remain stationary (i.e., the stationary phase); however, it will be a liquid at the temperature of the analysis. The separation begins by the injection of the mixture from a syringe through a silicon septum onto the column, which is heated by an oven. The oven can be programmed to rise in temperature during the analysis but is often set at a constant temperature when the sample is a simple mixture of known structures. The sample is vaporized by a heating element as it is injected. That is, the temperature of the heating element immediately causes all components of the liquid sample to boil, thus making certain that they are in the vapor or gaseous state (i.e., the gas phase). The carrier gas enters the column near the injection port and sweeps the sample through the column. The various compounds in the mixture vary in their solubility in the stationary liquid phase. Thus, the compounds in the mixture will travel through the column according to their solubility in the liquid phase. The compounds being separated first dissolve in the liquid and then vaporize from it and continue this process of dissolving and vaporizing until they emerge from the column. In the vapor form, the helium moves the compound along. Thus, the more soluble a compound is in the stationary liquid, the longer it stays dissolved in the liquid during each dissolution-vaporization cycle, and the longer it takes for it to pass through the column. The time it takes for a given compound to pass through the column from the time it is injected to the time it is detected is called the ***retention time***. That is, the retention time is how long the compound is retained on the column. The greater the compound's affinity for the column, the longer is its retention time. As a compound reaches the end of the column, it is sensed by a detector, which sends an electronic signal either to a strip recorder where the signal is recorded as a peak on the strip chart or to a computer data station where the signal is captured electronically. This kind of analysis in which the signal is proportional to the concentration of the analyte is called a ***concentration technique***. The strip chart of the entire sample, called a ***chromatogram*** provides documentation of the analysis. Likewise, an electronic chromatogram, captured by a computer data station, allows an investigator to save the data for follow-on interpretation and analysis. When the mixture is totally separated into its components, the chromatogram shows each compound as a separate peak. The area under a given peak is proportional to the amount of the compound that causes the signal. Hence, this technique can be used quantitatively to determine amounts or concentrations as well as to separate the components of a mixture. Modern instruments perform the integration function for the analyst.

Schematic of Gas Chromatograph Gas chromatography includes three basic operational steps. They are injection, separation, and detection. Please click on the link below to view several schematics[2] of the basic operations involved in gas chromatography. Look at the schematics, labeled as follows in blue on the web.

GC
Inject: FVI (Flash Vaporization Injection Port)
Separate: (Wall Coated Open Tubular with animation of separation)
Detect:
TCD (Thermal Conductivity Detector, interactive)
FID (Flame Ionization Detector, interactive)

[2] The schematics are copyrighted products of the University of Richmond.

Web site:
http://oncampus.richmond.edu/academics/as/chemistry/CMoR/info/newmod/GC/

Chromatogram A chromatogram can be very simple or very complex, depending on the nature of the sample. An example of a simple chromatogram[3] is shown at the following web site. The researchers have isolated a fraction from an aquatic plant that is active in killing fish. The chromatogram shows peaks for the fraction, including 1,4-hydroquinone, which they believe, is the bioactive component.

http://www.agnet.org/library/image/bc45016f5.html

The chromatogram[4] shown on the following web site is from a sample of opium collected in Iran. The investigators were surprised to find the synthetic, non-narcotic, drug antipyrine in the Iranian opium. Their studies involve the search for a non-narcotic marker in opium that might help law enforcement identify the source of illegal opium. Antipyrine might be such a marker.

http://www.undcp.org/odccp/bulletin/bulletin_1976-01-01_1_page007.html

The Hewlett Packard (HP) 6890 Gas Chromatograph (GC)
The visible physical components of our GC are shown in the following pictures. They include the data station, the gas chromatograph itself, and the gas cylinders. The gas chromatograph is a metal case that contains the components shown in the schematic (i.e., the injection ports, the column, the oven, and the detector).

[3] The chromatogram is copyrighted by the Food & Fertilizer Technology Center, Taipei, Taiwan.

[4] The chromatogram is copyrighted by the United Nations Office for Drug Control and Crime Prevention.

The Data Station for a HP 6890 Gas Chromatograph

The HP 6890 Gas Chromatograph


The Gases: Hydrogen, Helium, and Argon/Methane

Procedure:
Start-up of GC [Adapted for HP 6890 series GC with flame ionization detector. The three main components are the gas cylinders, the instrument and the data station. All must be adjusted before starting a run.]:

1. Open the gas cylinders containing the gases hydrogen, helium, argon/methane, and compressed air. The tank pressure should be about 2000 psi for each cylinder. The step-down pressures should be around 20-40 psi.

2. Turn on the GC by pressing the white on/off button that protrudes from the lower-left corner of the instrument. When the button is "on," the instrument starts a self-check. The self-check is visible on the small LCD screen located in the upper right corner of the instrument. When the instrument is ready, the display reads, "Power on successful."

3. To start the data station, depress the on/off button located at the lower-left corner of the data station (computer terminal). Icons will appear on the screen. Select "Instrument I online."

4. Prior to injecting a sample into the GC, the method to be used must be determined. It can be a new method or a pre-existing method. Our method is already loaded into the system. It is good practice to check the parameters to make sure they are correct. Click on the icon for each component in turn. Check the parameters for the inlets, oven and detectors and also make sure the front column and front inlet are selected.

5. As you click on the various icons, the previous one will display a blue check mark, indicating that parameter is ready. If a red X is displayed and does not disappear within five minutes, the instrument is not ready and you should check with the instructor.

6. When all of the systems are ready, the red field in the upper-left corner of the window will change to green and read "ready." Click on start and another small window will come up. Click on "Yes" and the field in the upper left corner will turn pink and display "Waiting for injection."

The operating conditions have been preset, so that you can inject a sample of a mixture of low molecular-weight alcohols and get a chromatogram in a reasonably short period of time.

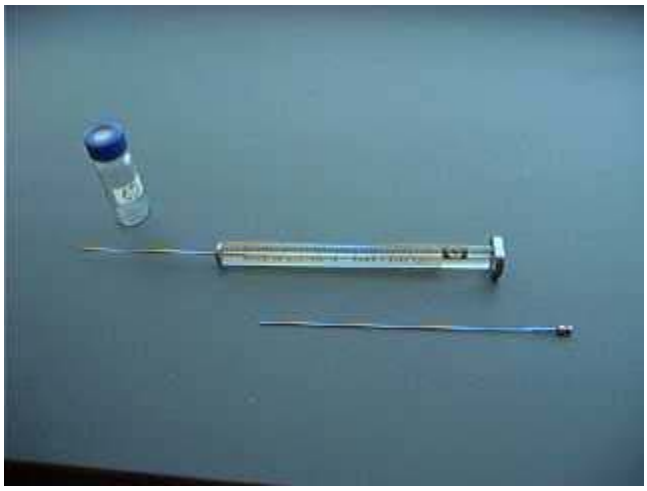
Preparation of the Mixture of Alcohols:

1. Thoroughly clean four 25-mL Erlenmeyer flasks and rinse them with acetone. Wipe the outsides of the flasks with a paper towel and allow them to dry as much as possible.
2. Rinse each Erlenmeyer flask several times with HPLC water, which will serve as the solvent for your four alcohols.
3. Into one of the flasks, weigh exactly 0.060 g (60.0 mg) of methanol.
4. Add approximately 10 mL of HPLC water to the flask and mix the contents thoroughly.
5. Clean and prepare a 100-mL volumetric flask by rinsing it with acetone and then HPLC water. It's okay to leave a small amount of HPLC water in the flask, because you are going to fill the volumetric flask with HPLC water momentarily.
6. Pour the contents of the Erlenmeyer flask into the clean 100-mL volumetric flask with the aid of a short stem or burette funnel and rinse the Erlenmeyer with two or three 2-mL portions of HPLC water. Pour each rinse solution, in turn, into the volumetric flask. This is a ***quantitative transfer***. We want every molecule of the alcohol to be transferred.
7. You will now add the other three alcohols to the volumetric flask in the same way you added methanol.
8. Starting with one of the remaining clean Erlenmeyer flasks, repeat steps 3-5 with ethanol.
9. Starting with one of the two remaining clean Erlenmeyer flasks, repeat steps 3-5 for 1-propanol (also known as propanol or *n*-propanol or propyl alcohol).
10. Repeat steps 3-5 for 2-propanol (isopropyl alcohol), using the last remaining clean Erlenmeyer flask.
11. The 100-mL volumetric flask is now more than half full.
12. Swirl the volumetric flask to ensure the contents of the flask are thoroughly mixed.
13. Add HPLC water to the flask until the mixture reaches the neck of the flask, and then remove the funnel and add HPLC water with a medicine dropper or controllable pipette until the concave meniscus in the neck of the flask just touches the calibration mark on the 100-mL volumetric flask.
14. Place the lid into the volumetric flask and shake the flask vigorously to ensure that the contents of the flask are thoroughly mixed.
15. You now have a standard solution. A ***standard solution*** is one for which the concentration is known exactly. The concentration of your sample is 60.0 mg/100 mL HPLC water for each alcohol. Keep your standard solution in the volumetric flask until you are ready to inject it onto the gas chromatograph.

The Syringe

The syringe is similar to but more delicate than those used by medical technicians to remove blood from a patient. Syringes contain two very delicate components. One component of a syringe is basically a slender metal tube or needle that is attached to calibrated glass housing. The glass part is

about 3 inches long and the diameter of a pencil. The other component is a plunger. The plunger is a very slender and flexible wire that slides back and forth lengthwise through the glass part of the syringe. The plunger has a small cap affixed to one end, so that an analyst can depress the cap to eject a sample from the syringe.



The syringe. A 10-μL syringe with plunger adjacent to a sample vial.

The syringe is ready for use when both components are clean and the plunger is inserted into the glass portal. The analyst uses the syringe to transfer exact amounts of liquid samples. The needle is immersed into a liquid sample with the plunger fully inside the glass housing. The sample is drawn into the syringe when the analyst slowly pulls the plunger outward. The exact amount of sample is determined by how far the plunger is pulled out. The amount is read from the calibrated syringe by the analyst to the correct number of significant figures. The amount is usually pre-determined by the analyst, so that the sample is simply drawn up into the syringe to the pre-determined point. For example, if the syringe holds a maximum of 10 μL (microliters), the analyst may withdraw any pre-determined amount between 0 and 10 μL. The diameter of the hole inside the syringe just accommodates the plunger, so that a vacuum is created when the plunger is withdrawn, sucking the sample into the syringe. The diameter of the hole is constant throughout the length of the hole; therefore, the volume drawn into the syringe is proportional to the linear calibration on the side of the glass. Thus, we read a volume (e.g., 1.0 microliter) when we draw a sample into a syringe. As an analyst, you must take care of your equipment. Do not force a plunger; it should glide back and forth with ease. If it doesn't glide smoothly, it is probably not clean. The syringe should be cleaned before every use for two reasons. The first is so that it will operate properly, and the second is to ensure that our sample is not contaminated by chemicals left on the syringe.

Cleaning the Syringe

1. Examine the syringe. If the plunger cannot be removed with a gentle tug, check with your instructor on what to do next.
2. You will clean the syringe with alcohol, because you are analyzing for alcohol today. Fill the syringe to its maximum capacity with ethanol. Expel the ethanol onto a paper towel. Wipe the needle with a lab tissue such as a Kimwipe.
3. Repeat *Step 2* several times and then repeat *Step 2* with HPLC water.
4. Expel all liquid from the syringe and wipe the needle clean.

5. The syringe is now ready for transferring your standard solution of four alcohols to the gas chromatograph.

Preparing the Sample for Injection:
The sample is delivered to the GC column by injecting it onto the column with the syringe. You will draw your sample into a calibrated syringe and inject the sample directly onto the column through a tiny plug in the end of the column called a ***septum***.

1. Transfer a small amount of your standard solution from the 100-mL volumetric flask to a clean and dry 25-mL Erlenmeyer flask that you have fitted with a stopper covered with aluminum foil. Rinse the Erlenmeyer flask with two small portions of your standard solution. Dispose of the rinse by pouring it into the non-halogen waste jar.
2. Transfer a small amount of your standard solution to the 25-mL Erlenmeyer and stopper the flask. (It may be necessary to wait for another student to clear the instrument.)

Pre-operational Check of the Gas Chromatograph:

This method is adapted for a HP 6890 series gas chromatograph equipped with a flame ionization detector. The column is a proprietary HP (now Agilent) Blood Alcohol Column (7.5 m x 0.320 mm ID—part number 19091 S-510). Before injecting your sample, call up the method called "Blood-Alcohol Analysis" by following the steps in the section above called "General Operating Procedures." Then, verify that the following parameters have been entered for the method.

Inlet System:

Split Inlet:	50:1 split ratio
Volume to inject:	1μL aqueous Standard of Alcohols
Sample:	0.10 % (w/v) of Methyl, Ethyl, Isopropyl, *n*-Propyl (internal standard-ISTD) and *n*-Butyl Alcohols in Water
Inlet Temperature:	250^{o}C
Inlet Pressure:	(Constant Pressure Mode Helium) Column Pressure 6 psi @ 120^{o}C 2.00 mL/min He (55 cm/sec)
Detector Temperature:	FID temperature, 300^{o}C
Column Oven Temperature Program:	120^{o}C (1 min) 25oC/min to 165^{o}C (1 min)
Time between injections:	A minimum of 5 min

Injecting the Samples and Obtaining Chromatograms:

1. Rinse the syringe with the sample solution by filling the syringe twice with the sample solution and expelling it onto a paper towel.
2. When you are ready to inject your sample onto the gas chromatograph, remove the stopper and draw 2 μL of sample into the 10-μL syringe. Draw the sample by dipping the tip of the needle into the standard solution. Slowly pull on the plunger until you have withdrawn 2.0 μL
3. With the ECP on "Ready," carefully inject the needle through the septum. With a continuous but smooth motion, depress the plunger to expel all of the contents onto the column.
4. Cleanly withdraw the syringe from the septum.
5. The chromatogram of the sample should clearly show peaks attributable to each of the four alcohols.
6. Carefully transfer exactly 10.00 mL of your standard stock solution to a clean dry 25-mL Erlenmeyer flask.
7. Tare the Erlenmeyer flask (be sure to close the sliding glass window on the balance before you tare it) on a balance that weighs to 0.0000 g.
8. Clean and rinse a 10-μL syringe with acetone. Wipe it clean and withdraw 8.0-μL of your unknown into the syringe. If you have an ample amount of unknown sample, expel the unknown from the syringe onto a paper towel. If not, continue.
9. Add exactly 8.0 μL of your unknown to the Erlenmeyer flask while the flask remains on the balance. Close the glass door and record the mass of the added unknown.
10. Carefully swirl the contents of the flask to ensure even mixture and a homogeneous concentration.
11. Inject a 1-μL sample that is enhanced with your unknown onto the GC column.
12. Compare the chromatogram of the enhanced sample with that of the four known alcohols and determine which your unknown is.

Shut-down Procedure:

1. Close all windows on the computer data station for "Instrument One" application. Depress the on/off switch to off.

2. Fully close all four gas cylinders

3. Allow the GC to cool for about 30 min, and then depress the on/off button on the GC to the off position.

Lab Report #1
Unknown Alcohol

Student Name_____

Unknown Sample Number_____

Identity of the Unknown Alcohol_____

Data: Complete the following sections by filling in the indicated information. For Table 1 insert the experimentally observed retention or migratory times for the four alcohols.

1. **Table 1 Retention Time/Migration Time (RT/MT) and Peak Heights for Alcohols**

Compound	**RT/MT**	**Peak Height**
Methanol		
Ethanol		
2-Propanol		
1-Propanol		
Remarks: Column: Oven Temp: Flow Rate: Other:		

2. The experimentally determined RT/MT for the unknown alcohol is _____.

3. For Table 2, insert the data you obtained from the sample you enriched with the unknown. The RT/MT for the four alcohols in Table 1 should closely match the data you enter into Table 2. Three of the alcohols should give peak heights close to their values in Table 1; the unknown's peak height should be significantly enhanced.

Table 2 Peak Heights for the Sample Enriched with the Unknown Alcohol

Alcohol	**MT/RT**	**Peak Height**	**Enhanced**
Methanol			Yes No
Ethanol			Yes No
2-Propanol			Yes No
1-Propanol			Yes No
Remarks:			

1 Homework

1. The GC data in Table 1 is for the purpose of preparing a calibration curve for blood-alcohol determinations. Standard solutions of alcohol in blood were injected into a GC and the peak height of each standard solution was recorded. The concentration of ethanol in blood is expressed as the number of milligrams (mg) of alcohol in 100 milliliters (mL) of blood. Each peak height is expressed in millimeters (mm).

Table 1 Alcohol Standards

Peak Height (mm)	Concentration (mg EtOH/100 mL Blood)
17.4	30.0
23.6	40.0
29.6	50.0
35.6	60.0
41.6	70.0
47.8	80.0
53.8	90.0
60.0	100.0
71.8	120.0
84.0	140.0
95.5	160.0

Enter the data from Table 1 into a TI-83 calculator. A manual titled "Data Handling and Analysis on the TI-82 and TI-83/83 Plus Graphing Calculators: A Resource for Science and Mathematics Students" is available in room 100, Chesapeake Hall. Enter the peaks heights in L_2 and the concentrations in L_1 of the calculator. Observe your data in the trace format on the calculator's screen. Then, conduct a linear regression analysis with diagnostic on and find the value of a, b, r^2 and r. Record the values of a, b, r^2 and r in the spaces indicated below.

a = _____ and b = _____

r^2 = _____ and r = _____

2. Using the values you obtained for a and b, convert the equation $y = ax + b$ from your calculator into an equation in which h (peak height) replaces x, and C (concentration) replaces y. Round off the values of a and b to the corresponding number of significant figures in your data. The new equation is:

_____

3. Use your equation that shows concentration C as a linear function of peak height h to calculate values for the concentration of ethanol corresponding to each peak height recorded in Table 2. The GC operator must be approved/certified by the state, the machine must be calibrated on a frequent basis and a record of the calibration maintained for review by inspectors. The instrument must be

maintained and a maintenance log kept. The analyst should participate in frequent blind tests to demonstrate and maintain proficiency.

Table 2 Date: 10/26/02; Operator: RAG; GC Number: 02; Other:

Run	Time	Lab Number	Forensic Id. Number	Experiment Peak Height (mm)	Calculated Concentration (mg/100 mL)	Remarks
1	0815	BA-2144	CID-5R-02544	43.0		Standard injected and method validated
2	0830	BA-2145	FBI-DC-13	26.0		
3	0900	BA-2146	ATF-ATL-1125	21.2		
4	1000	BA-2147	PGCL-2222	65.6		
5	1030	BA-2148	MCCL-322	52.6		
6	1045	BA-2149	Alex-03333	53.8		

4. When a person in the state of Alaska is suspected by law enforcement officials of being under the influence of intoxicating liquor, a sample of blood is drawn by a person qualified to do so. The sample is analyzed and data like that in Table 2 is obtained. The state of Alaska draws three possible conclusions about the person, depending upon the results of the blood alcohol analysis. The following quotations are from the state of Alaska's official web site and are state laws.

"(1) If there was ….40 milligrams or less of alcohol per 100 milliliter's of the person's blood,…it shall be presumed that the person was not under the influence of intoxicating liquor."

"(2) If there was ….in excess of 40 but less than 80 milligrams or less of alcohol per 100 milliliter's of the person's blood,…that fact does not give rise to any presumption that the person was or was not under the influence of intoxicating liquor..."

"(3) If there was ….80 milligrams or more of alcohol per 100 milliliter's of the person's blood,…it shall be presumed that the person was under the influence of intoxicating liquor."

Using the state of Alaska's criteria for the presumption of intoxication and your data in Table 2, render a conclusion (in the space below) about the persons whose blood samples were analyzed in runs 1, 3, and 6.

Run 1:

Run 2:

Run 3:

5. According to the Alaska's criteria, a person with a blood-alcohol concentration of exactly 80 mg/100 mL would be presumed to be_____
_____ .
(Complete this statement.)

6. Given samples of ethanol and HPLC water and all of the lab equipment you need, explain how to make a standard solution that contains exactly 80.0 mg of ethanol in 100 mL of solution of ethanol in HPLC water.

7. Sketch a chromatogram of a hypothetical solution that contains equal amounts of pentane, hexane, heptane and octane dissolved in a suitable solvent. Show peaks for each compound and the point at which the injection was made.

8. Label the peaks on your sketch with the names of the compounds in the order that you expect they would emerge from the GC on a structure—property basis.

9. What physical property of the hydrocarbons in problem 7 can we correlate with the order of their elution on GC?_____

10. Once you have a calibration curve and corresponding equation that relates the concentration C to the peak height h, what readily available software can you use to automate the calculation (i.e., you enter the data, and the program calculates C for you)?

_____

2 Explorations in Gas Chromatography

(Varying GC Parameters)

A Summary of this Week's Lab You learned last week how to do qualitative and quantitative analyses by gas chromatography, but your interaction with the gas chromatograph was limited. The instrument was pre-programmed by the instructor. In today's lab, you will have the opportunity to vary the controls on the instrument and learn for yourself how changes in the control settings affect the output of the instrument. As an operator of a gas chromatograph, you can control several variables that together dictate what kind of peaks you obtain from a given column and carrier gas. You will be allowed to change the flow rate of the carrier gas and the temperature of the column. You will also inject three different volumes of sample to see how the output changes with sample size. The objective is to optimize the control settings and amount injected to get the best peak for an alcohol sample. Our considerations of GC analysis will be expanded to include various column and detector options for specific analyses. In the classroom, you will learn the kinds of evidence that are suitable for gas-chromatographic analysis from research reports of analyses in which forensic evidence was successfully analyzed by GC. For homework, you will be given a set of hypothetical GC analyses. You will determine what kind of column and which carrier gas is suitable for these analyses. In many cases, more than one answer is acceptable because many columns can be used for a diversity of compounds. Likewise, helium, hydrogen, or nitrogen is suitable as a carrier gas for many applications. Your search for existing information in books, periodicals, the Internet, etc., to find specific information about an analysis is called a ***literature search.*** The combination of a literature search to determine the appropriate column and carrier gas together with the hands-on expertise to determine the appropriate instrument settings completes your introduction to gas-chromatographic analysis. You will prepare a report of your experimental findings and attach your solutions to the homework. Next week, you will conduct a blood-alcohol (simulated) analysis.

Forensics Applications of Gas Chromatography

All three major uses of a gas chromatograph, separation, qualitative analysis, and quantitative analysis might be used in forensic chemistry. In last week's lab, we saw how gas chromatographic analysis can be used to determine the concentration of alcohol (ethanol) in blood. As we shall learn more about later, a major use of a gas chromatograph in forensics is the separation of a mixture into its components for their immediate follow-on analysis by mass spectroscopy. The combination of a gas chromatograph and a mass spectrometer is shortened to GC-MS (i.e., a hyphenated technique). What are some applications of gas chromatography?

The following examples of the use of GC in both forensics and non-forensics applications were taken at random from the Internet to give you an idea of the diversity of applications that are available.

1. "*Test by Taste and Smell.* Large volume static headspace (LVSH), in conjunction with gas chromatography, has managed to duplicate olfactory detection limits in food and flavor analysis. (Now, it's not only the nose that "knows".) Based on gas chromatography (GC) and GC coupled with mass spectroscopy (MS) technology, LVSH allows the visualization of contaminants, impurities, and other compounds. LVSH requires a 3-stage pre-concentrator: automating GC analysis of gas-phase samples, cold trapping of the analyte, and complete heating of the tube. Lower

temperatures and milder adsorbents reduce analyte loss and chemical rearrangements versus the classical adsorbent trapping, which requires higher temperatures and causes material loss.
This technology proved useful for the analysis of coffee and yogurt, and the comparison of garlic versus garlic powder and chocolate versus carob. A 3-step process involves analysis of 100cc of the "good" food product(s); analysis of 100cc of the bad or spoiled food product(s); and picking the markers or those compounds that differ or change between the good and bad food materials. When good grapes were compared with spoiled grapes, there were two obvious compound changes—ethyl acetate increased by 120% in the spoiled product and the production of limonene dropped off. Overall, LVSH maintains the sample in a more natural state and allows the measurement and detection of those compounds responsible for odor and taste[5]"

2. "Portable Gas Chromatograph-Quadrupole Mass Spectrometer (GC-QMS) System
The Technical Support Working Group (TSWG), via Lawrence Livermore National Laboratory (LLNL) has developed a lightweight portable Gas Chromatograph-Quadrupole Mass Spectrometer (GC-QMS) for use by organizations to detect and identify hazardous substances related to terrorist attacks, demilitarization, and law enforcement. Current devices available on the market are either too cumbersome or do not detect and identify all the necessary substances.

GC-QMS analysis is routinely utilized in most analytical labs worldwide for the quantitative determination and the characterization of both known and total unknown chemicals. Most commercial GC-QMS systems are non-portable laboratory-based systems, or are only transportable when the necessary support equipment (gas supply, vacuum system, computer, etc.) The GC-QMS system is a portable 65 lb., robust, and field reliable/serviceable GC-QMS system for on-site analysis.[6]"

Four Common Detectors

Flame Photometric Detector (FPD)
As the name implies, a FPD measures photons or light (i.e., photometric = the measurement of light). Where does the light come from? The light (photons) is produced as the sample burns in the hydrogen flame. Some of the byproducts of the combustion are excited by the flame. Excited means some of the electrons in a sample move from one orbital location to another. The final orbital is at higher energy level than is the initial orbital. The heat of the flame provides the energy to elevate the electrons to the higher energy level. The electrons are normally at the lower level or ground state. The electrons have the energy of their orbital. Thus, "excited" electrons have more energy than do ground-state electrons. The energy difference between two orbital locations is a discrete or fixed amount, just at the number of steps between two floors of a building is a constant number. As excited electrons "fall" back to their more stable ground state, they transition from a higher to lower energy orbital. The excess energy (i.e., the energy difference between the two orbitals) is liberated or emitted, not as heat, but as electromagnetic radiation. Some of the electron transitions result in the emission of visible light. That is, the energy emitted is visible to the human eye. This only happens when the energy difference between the two orbitals involved in the transition produces electromagnetic radiation that falls within the visible range. We will consider electromagnetic radiation in more detail in the blocks on infrared and ultraviolet radiation.

[5] http://pubs.acs.org/hotartcl/tcaw/00/apr/miller.html
[6] http://www.tswg.gov/tswg/cbrnc/gcqms.htm

A sample is separated into its components as it travels through a GC column. The first compound to emerge from the column is burned in a hydrogen-rich flame. It is important to note that the FPD destroys the sample by combustion. Hence, this detector is not suitable if you wish to conduct a follow-on analysis such as mass spectroscopy, unless you split the sample and burn only a portion of it while the remainder is directed to the mass spectrometer.
As noted above, some of the combustion products are excited by the flame and emit visible light as they are swept from the hot flame to a relatively cold zone. The emitted visible light is filtered (i.e., only certain specific wavelengths of light pass through the filter). The light that passes through the filter impinges on a photomultiplier tube (PMT). The photomultiplier tube does two things. It converts the tiny amount of light energy from the sample into electrical energy, and then it multiplies the electrical signal many-fold so that it can be recorded digitally. Just as your music amplifier multiplies the sound that you hear, the PMT amplifies the signal it receives from the burned sample. The amplified signal is sent to the data station as a digital signal that is recorded and stored in a computer file. Each component of the mixture is detected in this manner.

Flame Ionization Detector (FID)
This is the kind of detector you will use in lab. The FID is similar to the FPD. Again, the sample is burned in a hydrogen-rich flame. The combustion of organic compounds produces ions in a large excess of the number of ions produced by the carrier gas. A voltage is applied across the gas stream such that the ions are attracted to a collector near the flame. The ions produce an electrical current (i.e., ions are charged particles). The current produced is proportional to the number of ions, and the number of ions is proportional to the sample size. The current is sensed by an electrometer, converted into a digital form, and sent to the data station for storage in a computer file.

Thermal Conductivity Detector (TCD)
As the name implies this detection system involves the conduction of heat (thermal = heat). The TCD compares the thermal conductivity of the carrier gas to that of the carrier gas enriched in a sample. The sample is also called the column effluent. The TCD is manufactured so that a switch automatically flips back and forth allowing the pure carrier gas and the effluent to alternately pass over an electrically heated filament. The temperature of the filament is hotter than the rest of the surrounding detector. As a pure gas passes over the filament, the filament is cooled and a certain amount of electrical energy must be supplied to the filament in order for it to maintain a constant temperature. The amount of cooling by the gas is a function of how efficiently the gas conducts heat. Helium and hydrogen are good thermal conductors. When they are used as the carrier gas, the effluent will cause the thermal conductivity to decrease. This decrease in thermal conductivity results in a change in the amount of electrical energy necessary to keep the filament at a constant temperature. The electrical change is proportional to the sample size and is measured digitally and sent to the data station. Unlike helium and hydrogen, nitrogen is a poor thermal conductor relative to an effluent. Therefore, an effluent increases the thermal conductivity when nitrogen is the carrier gas. Our GC uses a tungsten-rhenium filament when it is configured with a TCD. A TCD is non-destructive, which means it does not consume the effluent. The effluent simply passes over a filament. Consequently, the effluent can be directed to another detector. The idea is to separate the components of a mixture by gas chromatography and then send a pure compound to another detector that is best used with pure compounds instead of mixtures. It is generally true that it is easier to

interpret data obtained from a pure compound than it is to interpret similar data from a mixture. Hence, at the fundamental level gas chromatography's most essential role in analysis is separation.

Electron Capture Detector (ECD)

As the name implies, electron capture involves the "capture" of electrons. Where do the electrons come from, how are these electrons captured, and what captures them? The electrons come from a radioactive source that produced a predictable and reproducible stream of β particles (electrons). The source is a β emitter. Early scientists used the Greek alphabet to describe radiation in which particles were emitted by a source. Later, it was learned that an alpha (α) particle is a helium nucleus (He^{2+}) and that a beta (β) particle is an electron. The alpha and beta terms are still in use. A typical beta emitter for an ECD is ^{63}Ni, a radioactive isotope of nickel. The electrons from the radioactive isotope in the detector collide with molecules from the carrier gas. The result is somewhat like a billiard or pool ball striking a set of racked balls. The initial collision produces a series of follow-on collisions. One high-energy β particle produces approximately 100 low-energy electrons from the carrier gas. The electrons produced in this manner create small electrical current. This is the reference or standing current. It will remain constant until an effluent is detected. When the effluent passes the detector, some of the available electrons may be captured by the sample, creating negative ions. When the number of measurable electrons decreases, the current also decreases. This change in current is measured and sent to the data station as a digital signal.

Summary of Detectors

Table 1compares the detectors described above. The information was extracted from a Hewlett Packard manual.[7]

Columns

A variety of columns are available to the analyst. Like the detector, the column selection will depend upon the application. One column might be suitable for hydrocarbon analysis in the absence of water, whereas another column might be suitable for alcohol analysis in the presence of water. To pick a column, first determine what you want to analyze. Then try to marry the requirement with an available column. You should start by checking with the vendor whose instrument you are using. You may find that other vendor's columns do not fit your machine. The data in Table 1 are extracted from a textbook[8] to give you an idea of the types of columns that are available.

Table 1. Some GC Stationary Phases

Stationary Phase	Use	Solvent	Max. Temp.
Apiezon L.	Multi-purpose Orgo	Benzene/Toluene	300
DEGA	Polar H-bonders	Acetone	200
Carbowax 20M	Multi-purpose Orgo	Chloroform	250
DC 710 Silicone Oil	Inorg and Orgo	Acetone/Chloroform	300

[7] HP Operating Manual, Volume 3, Detectors, page 2.
[8] Shriner, R. L., Fuson, R. C., Curtain, D. Y., and Morrill, T. C., *The Systematic Identification of Organic Compounds*, 6th Ed., (1979).

SE 30	Inorg and Orgo	Chloroform/Toluene	350

For our simulated blood-alcohol analyses, we are using a proprietary column made by HP (now Agilent Technologies) especially for alcohol/water solutions. You will not be changing columns or detectors during your analyses today. These parameters, perhaps the most important ones, will remain constant throughout your GC studies. What parameters will we vary? We will vary the parameters that are easily changed in the method by computer. The components of our system are laid out in Table 2. The parameters that we will vary are highlighted in blue.

Table 2. HP 6890 series GC/FID Parameters for Alcohol in Water Analysis (A Simulated Blood Alcohol Analysis)

Parameter	**Initial Setting (Used in Last Week's Expt.)**
Inlet System **Split Inlet** **Volume Injected** **Sample**	 50:1 Split Ratio 1 µL Aqueous Alcohol 0.02-0.18 % (w/v) Alcohols in Water
Inlet Temperature	250°C
Inlet Pressure	Constant Pressure Mode Helium or Hydrogen He: 6 psi @ 120°C 2.00 mL/min He (55 cm/sec) H: 3 psi @ 120°C 50 mL/min H (63 cm/sec)
Detector Temperature	FID temperature, 300°C
Column Oven Temperature	Program: 120°C (1 min) 25°C/min to 165°C (1 min)

Figures 1-3 show photos of different kinds of columns as they appear when mounted inside a GC's oven. Figure 1 shows our model 6890 HP GC outfitted with two capillary columns. They are visible as copper-colored coils. Figure 2 shows a GOW-MAC GC. It is fitted with a TCD. Figure 3 shows two columns mounted inside the GOW-MAC's oven. They are standard size columns. The distinction between capillary and standard columns is easily discernable in the photos.

Figure 1. Capillary Columns in HP 6890 GC

Figure 2. A GOW-MAC GC

Figure 3. Macro-Columns in a GOW-MAC GC

Experimental:
Open the method called "Blood-Alcohol Analysis" by following the steps in the GC Lab #1 called "General Operating Procedures" and verify that the initial parameters shown in Table 2 have been entered for the method.
Because the cycle time for an analysis is approximately 7 min, the number of injections a given student gets to make will be dependent upon the number of students in the class. The instructor may have students share data (i.e., allow one student to collect data from three injections, another student to collect different data from three injections, and so on, until all of the requisite data is collected.

Variations in Sample Size

1. With the instrument parameters set as indicated in Table 2 above, inject a 1-µL sample containing 60 mg ethanol/100 mL water and print the output.

2. Repeat step 1 with a 2-µL sample.

3. Repeat step 1 with a 4-µL sample.

Variations in Flow Rate

1. In the parameters box for the method, change the Inlet Pressure condition from He: 6 psi @ 120°C 2.00 mL/min He to He: 5 psi @ 120°C 2.00 mL/min He.

2. Inject a 1-µL sample containing 60 mg ethanol/100 mL water and print the output.

3. Change the Inlet Pressure condition to He: 7 psi @ 120°C 2.00 mL/min He.

4. Inject a 1-µL sample containing 60 mg ethanol/100 mL water and print the output.

Variations in Initial Temperature

1. In the parameters box, change the initial temperature of the column from 120°C to 95°C. First change the initial 120°C settings in under Inlet Pressure in Table 2 to read He: 6 psi @ 95°C 2.00 mL/min He (55 cm/sec). Then, change the temperature in the Column Oven Temperature to read Program: 95°C (1 min).

2. Inject a 1-µL sample containing 60 mg ethanol/100 mL water and print the output.

3. In the parameters box, change the initial temperature of the column from 120°C to 155°C. First change the initial 120°C settings in under Inlet Pressure in Table 2 to read He: 6 psi @ 155°C 2.00 mL/min He (55 cm/sec). Then, change the temperature in the Column Oven Temperature to read Program: 155°C (1 min).

4. Inject a 1-µL sample containing 60 mg ethanol/100 mL water and print the output.

Lab Report #2 and Homework
Variations in GC Operating Parameters

1. Briefly describe how the change in sample size affected your results.

2. Briefly describe how the change in flow rate affected your results.

3. Briefly describe how the change in initial column temperature affected your results.

4. Make a general statement about the effect of varying the three parameters on your GC output. Indicate which of the three variables appeared to be most important for getting optimal results.

5. Suppose you are a forensics analyst and you are asked by your boss to develop a new method for separating a mixture of C_6-C_{10} hydrocarbons by gas chromatography. What factors should you consider as you set about accomplishing the task?

For each pair of compounds listed in the following table, list a specific gas chromatographic column that an analyst can use to separate the compounds. Provide sufficient detail to your answers so that a reader can order the column (i.e., include the vendor of the column and the corresponding part number or other identifying characteristic). Indicate the reference (e.g., URL or web site, supply catalog, etc.) where you found the information.

Table 1. GC Columns for Various Separations

Compounds to be Separated	GC Column and Description	Reference
5. Steric Acid and Oleic Acid		
7. Methyl Butyrate and Methyl Pentanoate		
8. Ethyl Alcohol and Isopropyl Alcohol		
9. Isooctane and Nonane		
10. *p*-Dinitrobenzene and *m*-Dinitrobenzene		

3 Forensic Analysis by Gas Chromatography

(Analysis of Blood Alcohol)

A Summary of this Week's Lab You have completed your training in gas-chromatographic analysis. This week you will apply your training. You will determine the alcohol content in a simulated blood sample. The alcohol is ethyl alcohol (ethanol), the component of alcoholic beverages that makes them intoxicating. The simulated blood is water (i.e., HPLC water). The blood sample contains an unknown amount of ethanol dissolved in water. The sample was obtained, hypothetically, by a law-enforcement officer and is considered to be forensic or criminal evidence. Accordingly, you must maintain absolute control of the evidence at all times. All analysts and other persons who handle criminal evidence are responsible for maintaining the integrity of that evidence. When all who handle the evidence maintain its integrity, we say they have maintained a ***chain-of-custody***. Accordingly, a written record is maintained by a forensic chemist to document the chain-of-custody. Thus, prosecutors can assure themselves and juries that only responsible people have had access to the evidence. As a person in the chain, you have a professional responsibility to keep an accurate written record of your handling of the evidence. You will record both your own analysis number and the evidence number that comes with the sample so that you can cross-reference your work to the appropriate criminal case. You will prepare several samples of ethanol in propanol in which the concentration of ethanol varies from zero to just above the maximum concentration encountered in blood. You will inject these samples and prepare a calibration curve of peak height vs. concentration or of peak area vs. concentration, whichever you decide. You will then inject the forensic sample and determine the concentration of ethanol in the sample from your calibration curve. You will use the TI-83 Graphing Calculator to construct your calibration curve and to determine the concentration of ethanol in the sample. This activity is very much like the homework in "Introduction to Gas Chromatography." You will prepare a report of your findings and turn it in at the beginning of your next lab period.

A Cautionary Note to Would-be Forensic Analysts

As you continue in your studies, please be aware that you are entering an adversarial profession. That is, you will likely find yourself on one side or the other of a criminal case.

Many people make good money as expert witnesses paid to refute your work. For example, consider the following quotation from one such expert. The yellow highlights were not part of the original quote but were added in this manual for emphasis.

"Forensic Accuracy of GC/MS:

Gas chromatography/mass spectrometry is extremely and highly accurate if done correctly. A laboratory, which performs the test, must be NIDA certified or CAP (College of American Pathologists) certified. All of the labs that perform the gas chromatography-mass spectrometry on site can be NIDA certified. Labs that send samples to another laboratory for gas chromatography-mass spectrometry confirmation are ineligible, I repeat, ineligible, for NIDA certification. Therefore one must be very careful when looking at the test results to see whether the laboratory is NIDA/CAP certified. Furthermore, some labs do not properly and thoroughly clean the GC-MS equipment. Some labs don't even do GC-MS confirmation. Some labs use cheap alternative methods to increase

profits and reduce expenses. Therefore you (the paid expert) must be in a position to aggressively cross examine the laboratory director and technician.[9]"

In a similar vein, but from a different viewpoint, the editor of the *Journal of Forensic Sciences* has recently commented on the growing need for the certification of individuals and the need for accreditation of crime labs. "These professional credentials will become more and more necessary as a defense against lawyers' attacks on one's individual skills and on one's lab's trustworthiness. I believe your best defense is to be the best analyst possible, to include participating in blind tests, so that your proficiency can be documented and substantiated."

It is highly recommended that you seek personal certification for any forensic analytical skill in which you become proficient. Likewise, you should seek to have your lab accredited by a bona-fide accrediting agency such as ASCLD-LAB. ASCLD is the American Society of Crime Lab Directors.

Standards

One of the most important aspects of quantitative and qualitative analysis is to know the source and purity of the standards you are using to compare with an unknown sample. Standards can come in variety of ways. One way is for the authenticity and purity of the sample to be attested to by a competent authority. For example, you obtain a set of weights that are certified by the manufacturer. The weight marked 1 gram weighs exactly 1.000 g.

You might also obtain a chemical that is certified to be 99.99 % pure by the manufacturer.

In the case of lengths, a platinum bar is maintained in Paris, France, which is inscribed with two marks that are exactly one meter apart. This platinum bar is the primary standard for a meter. All other standards are secondary in that they are derivatives of the primary standard. Thus, a ***primary standard*** is the one from which all other standards are derived.

In the case of chemicals, you might use one such as KHP, potassium acid phthalate, to prepare a standard solution of NaOH(*aq*). Then, you might use the NaOH(*aq*) to prepare a standard solution of HCl(*aq*). The KHP would be the primary standard and the two solutions would be secondary standards. Even though they are called secondary standards, their purity and concentration is known within the limits of the burets, pipets, etc., that were used to prepare these secondary standards. When the exact concentration of a solution is known, the solution is called a ***standard solution.***

Sometimes, reference standards have a slightly different connotation. If an investigator collects a piece of carpet from a crime scene, that piece of carpet then becomes the referece standard to which suspect carpet fibers would be compared. In this case, the reference standard might not be pure at all. In fact, grease stains on the carpet might be the link between the reference and the suspect carpet. Thus, it is important to keep the words reference and standard in proper context when using them in reports of scientific findings. Thus, it is important to define the terms as you are using them in your results to help avoid misinterpretation of what you actually mean.

Pre-Experimental Preparation:

[9] http://www.environmentaldiseases.com/article_intoxication_drugs_of_abuse_testing_forensic_application.html

A. Solutions needed for the Analysis of Alcohol in Blood (Water as Blood Simulant) to be Analyzed on a "Blood Alcohol" GC Column

Standard stock solution

1. Weigh exactly 640 mg (0.640 g) of absolute ethanol in a tarred 100.00-mL volumetric flask.

2. Add about 25 mL of spectroscopic grade (HPLC) water and gently swirl the flask to ensure the contents are well mixed.

3. Fill the volumetric flask to the mark with spectroscopic grade water.

4. Insert the glass stopper and thoroughly mix the contents of the flask by shaking it vigorously.

You have a stock solution that has a concentration of 640. mg of ethanol in 100.00 mL of pure water (simulated blood) or 6.40 mg/mL.

Preparation of 11 Standard Solutions from the stock solution

The eleven standard solutions that are needed for alcohol in blood (simulated) are prepared by diluting exactly 1.00 mL of the stock solution with pure water. The amounts of pure water to add to 1.00 mL of stock solution of concentration 6.4 mg/100 mL is given in the following table.

The concentration of the final solution is obtained from the following equation.

(Volume of Stock)(Conc. of Stock) = (Conc. of Standard)(Final Volume of Standard)

or

Final Volume of Standard = (Volume of Stock)(Conc. of Stock)/ (Desired Conc. of Standard)

For a desired concentration of 0.30 mg/100 mL and 1.00 mL of a stock of 6.4 mg/100 mL, the final volume is as shown below:

$$\text{Final Vol. Standard} = \frac{(1.00\ \text{mL stock})(6.4\ \text{mg}/100\ \text{mL stock})}{(0.30\ \text{mg}/\ 100\ \text{mL})} = 21.33\ \text{mL}$$

The final Volume of the Standard = mL stock + mL pure water added

mL pure water to add = final volume – mL stock = 21.33 mL – 1.00 mL = 20.33 mL

Table 1. Volume of Pure Water to Add to 1.00 mL of Stock to Make the Standard Solution Indicated

Concentration of Standard	Volume Pure Water to Add to 1.00 mL of Stock	Final Volume of Standard Solution

	Solution	
#1. 20.0 mg EtOH/100 mL B	**31.00 mL**	**32.00 mL**
#2. 30.0 mg EtOH/100 mL B	**20.33 mL**	**21.33 mL**
#3. 40.0 mg EtOH/100 mL B	**15.00 mL**	**16.00 mL**
#4. 50.0 mg EtOH/100 mL B	**11.80 mL**	**12.80 mL**
#5. 60.0 mg EtOH/100 mL B	**9.67 mL**	**10.67 mL**
#6. 70.0 mg EtOH/100 mL B	**8.14 mL**	**9.14 mL**
#7. 80.0 mg EtOH/100 mL B	**7.00 mL**	**8.00 mL**
#8. 90.0 mg EtOH/100 mL B	**6.11 mL**	**7.11 mL**
#9. 100 mg EtOH/100 mL B	**5.40 mL**	**6.40 mL**
#10. 120 mg EtOH/100 mL B	**4.33 mL**	**5.33 mL**
#11. 160 mg EtOH/100 mL B	**3.00 mL**	**4.00 mL**

Overall: The minimum amount of ethanol and pure water needed to make one set of standards is as follows. The mass of ethanol is 640 mg or 0.640 g. The volume of water = the sum of the amounts in blue plus 100 mL for the stock solution = 226 mL.

B. Obtain a Forensic Sample from the instructor and record its voucher number in your notebook. Also assign the sample your own reference number. Pick a number so that you can easily find your data when given the number later on.

Experimental:

Open the method called "Blood-Alcohol Analysis" by following the steps in the GC Lab #1 called "General Operating Procedures." Then, verify that the initial parameters shown in the following table have been entered for the method. If any parameter is different from that shown in the table, change it to the tabular value with the instructor's permission.

Table 2. HP 6890 series GC/FID Parameters for Alcohol in Water Analysis (A Simulated Blood Alcohol Analysis)

Parameter	Initial Setting (Used in Last Week's Expt.)
Inlet System **Split Inlet** **Volume Injected** **Sample**	 50:1 Split Ratio 1 µL Aqueous Alcohol 0.02-0.18 % (w/v) Alcohols in Water
Inlet Temperature	250°C
Inlet Pressure	Constant Pressure Mode Helium or Hydrogen He: 6 psi @ 120°C 2.00 mL/min He (55 cm/sec) H: 3 psi @ 120°C 50 mL/min H (63 cm/sec)
Detector Temperature	FID temperature, 300°C
Column Oven Temperature	Program: 120°C (1 min) 25°C/min to 165°C (1 min)

Calibration Curve Data

Because of the limitations imposed by the turn-around time for a blood alcohol analysis by this method, each student will participate in the preparation of the calibration curve by injecting the number of samples specified by the instructor. For example, it might be determined for a three-student class that Student 1 injects samples # 1, #4, and #8 from Table 2. Student 2 would inject samples #2, #5, and #9, and Student 3 would inject #3, #6, and #10. Each student's samples would bracket the range of alcohol concentrations, and the 9 samples would give a satisfactory total number of data points.

1. Following the techniques you learned for using a syringe and injecting a sample in GC Lab #1, inject the specified standard solution and print out the computer output.

2. Repeat step 1 for each calibration standard and for your unknown.

3. Clean your lab bench and common work areas.

4. Complete the lab report and turn it in at the beginning of the next lab period.

Lab Report #3 and Homework

Concentration of Alcohol in a Suspect's Blood

Name_____

Voucher Number_____

Your Sample Number_____

The concentration of the sample in mg alcohol/100 mL blood _____

Data:

For each sample that you actually ran to determine the calibration curve, enter the corresponding peak area from your computer printout into the appropriate column in the following Table 1.

Concentration of Standard	**Peak Area from GC Printout**
#1. 20.0 mg EtOH/100 mL B	
#2. 30.0 mg EtOH/100 mL B	
#3. 40.0 mg EtOH/100 mL B	
#4. 50.0 mg EtOH/100 mL B	
#5. 60.0 mg EtOH/100 mL B	
#6. 70.0 mg EtOH/100 mL B	
#7. 80.0 mg EtOH/100 mL B	
#8. 90.0 mg EtOH/100 mL B	
#9. 100 mg EtOH/100 mL B	
#10. 120 mg EtOH/100 mL B	
#11. 160 mg EtOH/100 mL B	

Data Reduction: Enter the data into L_1 and L_2 in your TI-83 calculator, as you did in the homework of GC Lab #1. Observe your data in the trace format on the calculator's screen. Then, conduct a linear regression analysis with diagnostic on and find the value of a, b, r^2 and r. Record the values of a, b, r^2 and r in the spaces indicated below.

a = _____ and b = _____

r^2 = _____ and r = _____

2. Convert the equation $y = ax + b$ into an equation in which A (peak area) replaces x, and C (concentration) replaces y. Round off the values of a and b. The number of significant figures in a and b should correspond to the number of significant figures in your data. The new equation becomes _____ .

3. Use your equation that shows concentration C as a linear function of peak area A to calculate the concentration of ethanol corresponding to the peak area you obtained for your forensic blood sample. Your calculated C =_____mg/100 mL. Record this value in the space above for your sample's blood alcohol concentration.

4. Use the data in Table 3 to prepare a calibration curve, using Excel instead of the TI-83 calculator.

Table 2 Alcohol Standards

Peak Height (mm)	Concentration (mg EtOH/100 mL Blood)
17.4	30.0
23.6	40.0
29.6	50.0
35.6	60.0
41.6	70.0
47.8	80.0
53.8	90.0
60.0	100.0
71.8	120.0
84.0	140.0
95.5	160.0

5. Use your calibration curve from problem 4 to determine the concentration of an unknown that gives a peak height of 4.97 cm. Concentration = _____ .

6. Explain how a flame ionization detector (FID) works.

7. What advantages does a FID have over a thermal conductivity detector?

8. List the three principal uses of gas chromatography and give a forensics application of each one.

A.

B.

C.

9. What is the main consideration when choosing a column for a GC application?

10. What is the difference between a primary standard and a secondary standard?

4 Introduction to Infrared (IR) Spectrophotometry

(Identification of Organic Families)

A Summary of this Week's Lab During this lab period, you will be introduced to infrared spectrophotometry and its application to forensic analysis. You will learn how an infrared spectrophotometer operates, what kind of data it produces, and how to analyze the data to characterize organic families of compounds. You will learn what kinds of samples are appropriate for infrared analysis. You will prepare four liquid samples for analysis and take their infrared spectra. These compounds will be pre-dissolved in a solvent suitable for IR analysis or be neat liquids or solids. The instrument will be adjusted by the instructor, and you will take the spectra with the instrument settings fixed. The four samples will be comprised of organic compounds from four different organic families. The families are acids, alkanes, alcohols, and ketones. You will be given the family name of each of your four compounds before you analyze them. Because alkanes are saturated hydrocarbons, we expect them to have absorptions in the IR that are common to all carbon and hydrogen containing compounds. Each family contains unique structural features that produce IR bands, which are characteristic of that family. You will compare each of your four spectra, in turn, to two spectra of compounds from the same family. The two spectra that you are given will be labeled with the name of the compound. Your spectrum for a given compound will match one of the two you are given. You will then identify which of the two known compounds you analyzed for each family. The instructor will verify that you have correctly identified each of your four samples by name. For your lab report, you will analyze each of your four spectra in the region between 1650 cm^{-1} and 4000 cm^{-1}. For the alkane, you will associate absorptions with the presence of C and H. The absorptions (wavenumbers) you associate with alkanes will not be used in the identification of other families. You will identify the major IR bands in this region, known as the functional-group region (functional group equates to family). You will find the point of maximum IR absorption for each major band and record its wave number in reciprocal centimeters (cm^{-1}). You will describe each major band as sharp or broad, depending on whether it forms a spike or is very rounded. You will describe the intensity (length) of each major band, depending on how far the band extends from the baseline. You will then prepare a table of this data. The completed table will be comprised of the IR spectral characteristics of the acid, alkane, alcohol, and ketone families. When you finish, you will have prepared an IR correlation chart, which shows the wavenumbers you have associated with specific structural features. Next week, you will use an IR correlation chart from a textbook.

Theory:

Infrared spectrometry is one of the most useful tools available for the analysis of organic compounds. An infrared spectrum helps the analyst determine the functional group(s) present in the compound as well as something about the structural environment of the group. When an infrared analysis is conducted on a sample dissolved in a solvent, the technique is non-destructive, which means the sample is not destroyed by the analysis. Thus, once an IR spectrum has been taken on the unknown compound, the entire sample may be recovered from the solvent for further analysis.

What is Infrared Radiation? Like visible light, infrared radiation is electromagnetic radiation. The difference among the various kinds of electromagnetic radiation is how energetic the radiation is. Very high-energy radiation such as that found in cosmic rays is at one end of the electromagnetic spectrum and very low-energy radiation such as that found in radio waves is at the other end.

Infrared radiation is less energetic than visible light. As you know, visible light is absorbed by certain materials. This absorption produces the complementary color in the human eye. We cannot see infrared radiation; therefore, it is not detectable by the human eye. We need an instrument called an infrared spectrometer to measure the amount of infrared radiation that is absorbed by a given compound.

What is the energy of infrared radiation? Because the energy (E), frequency (ν), and wavelength (λ) of electromagnetic radiation are related as shown by equation 1, any of these variables may be used by an analyst to represent energy.

$$E = h\nu = hc/\lambda \quad (1)$$

E is the energy of any electromagnetic radiation in joules (J). Planck's constant, h, is 6.63×10^{-34} J s, ν (nu) is the frequency of the radiation or wave in reciprocal seconds s^{-1}, c is the speed of light 3×10^{10} cm s^{-1}, and λ (lambda) is the wavelength of the radiation in cm. As shown in equation 1, E is inversely proportional to λ. Therefore, the term wavenumber was created. Wavenumber $\bar{\nu}$ (nu bar) is the reciprocal of wavelength. Thus, nu bar is directly proportional to energy, and the greater nu bar the greater the energy of the IR radiation.

$$\bar{\nu} = 1/\lambda \quad (2)$$

The units for nu bar are reciprocal centimeters or cm^{-1}.

How are IR peaks reported? For the past few years, chemists have attempted to use a common set of units. For IR radiation, the standard unit for reporting the position of absorption of a given IR band is nu bar, which is reported in reciprocal centimeters. Thus, an absorption that corresponds to the presence of a carbonyl group in a ketone might be expressed as 1715 cm^{-1}. This is an energy-equivalent unit. The reciprocal of 1715 cm^{-1} is 1/1715 = 0.000583 cm. Clearly, it is more user friendly to describe an absorption as 1715 as compared to 0.000583. Therefore, nu bar or reciprocal centimeters is the preferred unit. An older unit called a micrometer or micron might be encountered. A micron (μm) is one-millionth of a meter or 1×10^{-6} m, which equals 1×10^{-4} cm. Thus, 0.000583 cm = 5.83
μm. A 1715 cm^{-1} wave is a 5.83 μm wave. In this course, we shall use wavenumbers in reciprocal centimeters as the standard for reporting IR spectral bands.

How does IR radiation interact with molecules? Two atoms joined by a covalent bond can be considered as two masses joined by a spring. The vibration of two such masses joined by a spring can be described by Hooke's law. According to Hooke's law, the stretching frequency of two masses joined by a spring is directly proportional to the strength of the spring and inversely proportional to masses joined by the spring. For two atoms, the bond strength is analogous to the strength of a spring. Thus, bonds involving hydrogen such as C—H and O—H have higher stretching frequencies than do C—O and C—C bonds. Double and triple bonds are stronger than the corresponding sigma bonds holding two atoms together. Therefore, the stretching frequencies for C=C and C=O are higher than those for C-C and C-O, respectively. Likewise, triple bonds, C≡C or C≡N are at even higher frequency than double bonds, but lower than C—H or O—H stretching frequencies. Thus, IR radiation causes vibrations in molecules, particularly at the natural stretching frequencies of the bonds. Stretching is one kind of vibration known as a mode of vibration. A mode of vibration is how

the vibration occurs. Stretching is one mode, but bending and scissoring are also modes of vibration. The most intense stretching vibrations occur when the stretch involves a change in the dipole moment of a bond. A carbonyl group, C=O, undergoes a large change in its dipole moment when the double bond in the group is stretched, leading to one of the most intense IR absorptions for a carbonyl group as compared to other IR absorptions. On the other hand, a symmetrically substituted triple bond such a the one in 2-butyne, $CH_3C{\equiv}CCH_3$, produces a very weak IR signal, if any, because there is no change in the dipole moment when this kind of a symmetrical molecule is stretched at the triple bond.

How does an instrument record the data? The infrared region of the electromagnetic spectrum is scanned from 4000 cm^{-1} to about 800 cm^{-1}. Infrared radiation interacts strongest with a molecule when the IR radiation corresponds to a mode of vibration in the molecule (i.e., stretching, bending, or scissoring, etc.) The sample is placed in the IR beam, and the IR region scanned by the instrument. As the region from high to low energy is swept, both the location and intensity of the absorption is registered. Think about walking down a street with numbers on the houses. You might find Smith at 3500 and Jones at 1700. That is the number may be correlated with a family. Exactly the same thing is true of IR spectra. As the spectrum is obtained, significant absorption occurs at numbers that can be correlated with organic families. For example, alcohols are found at 3300-3500 cm^{-1} and ketones are found at 1680-1800 cm^{-1}. IR is most useful to us in the correlation of peaks we find in a spectrum with the families or structural features that we know absorb at these wavenumbers.

Experimental:
Follow the local directions for the start up of the Optical Bench.

1. Obtain four unknown samples from the instructor and record the numbers of the unknowns in your notebook. The family names of the four compounds will be known.

2. Fill a sample cell with one of the unknowns, using the instructor-demonstrated technique for filling the cell.

3. Obtain the IR spectrum of the unknown and print it out.

4. Clean the cell with chloroform and obtain IR spectra for the other three unknowns.

5. Clean up all of the equipment and sample cells are return them to their storage locations.

5. Compare the spectra of you unknowns with the spectra provided by the instructor.

6. Identify your four unknowns by comparing your spectra band for band with those provided.

7. Verify the identity of the four unknowns with your instructor.

8. Complete the lab report and turn it in at the beginning of the next lab period.

Lab Report # 4 and Homework
Unknown Compounds by IR

Student Name_____

1. **Table 1 The Identification of Unknown Compounds by their IR Spectra**

Unknown Number	Family Name	Result of Comparative Analysis (Name of Unknown)
	Acid	
	Alkane	
	Alcohol	
	Ketone	

2. In Table 2, write in the wavenumbers of the significant IR bands you find in the functional-group region of each spectrum. Use the following methodology. Find the wavenumber of maximum absorption for a given peak. In the example below, 2200 cm^{-1} is the maximum. Then find the limits of the "band" by tracing up both sides of the peak until you reach the baseline or the beginning of another absorption band. Place tick marks to identify these limits and record the wavenumber as a range. The tick marks define the range.

The range is 2200-2300 cm^{-1} for the example.

Likewise, indicate the shape of the band (i.e., sharp, intense, sharp and intense or broad).

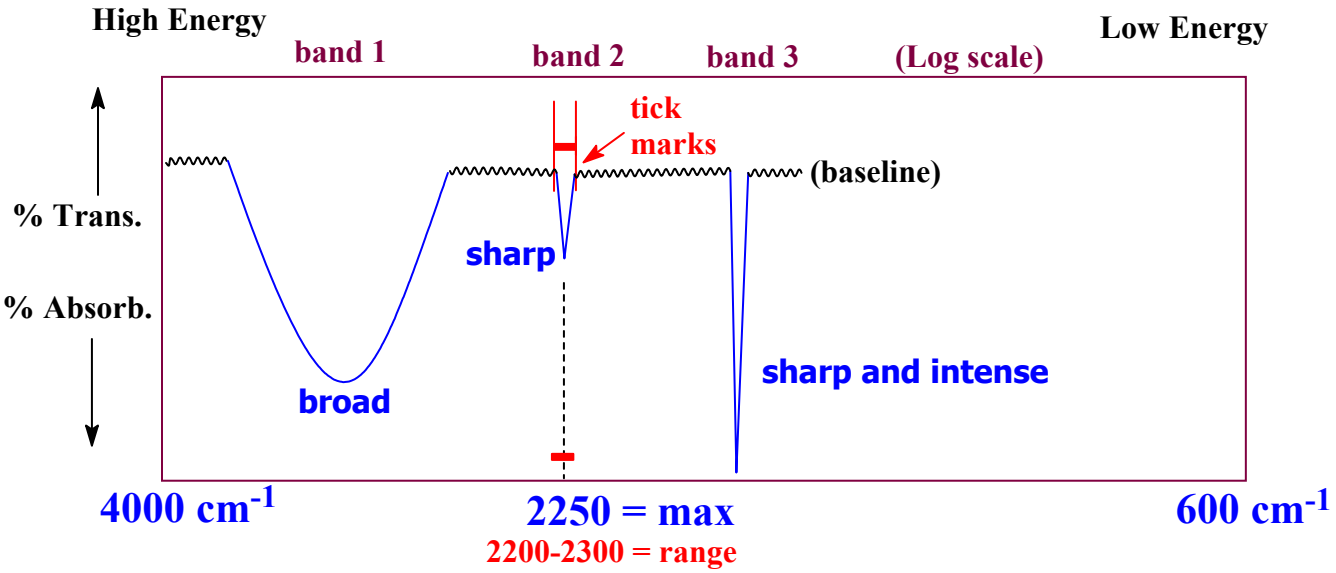


Table 2 Major IR Bands found in the Functional-Group Regions of the Spectra

Family	Wavenumber Range of Major Bands and the Shape of the Bands
Acid	
Alkane	
Alcohol	
Ketone	

3. Draw the structures of each of your four unknowns in the space provided above its family name.

Acid Alkane Alcohol Ketone

4. A structural feature produces the observed signal. That is a major tenet of spectroscopy. We want to learn as many structure-spectra correlations as possible. Table 3 shows partial structures at the top of each column. For each family identified in Table 3, place an X in the appropriate box. For example, the alkane contains a methylene (CH_2) group; therefore, an X is placed in the box in the Alkane row and the $–CH_2-$ column.

Table 3 Partial Structures Associated with Each Family

Partial Structure / Family	~OH	O ‖ C~	H – C~ – H
Acid			
Alkane			X
Alcohol			
Ketone			

5. From Table 3, what partial structure is common to an acid and an alcohol?
Answer_____ What is the shape of this band? Answer_____

6. Compare the spectrum of your acid with that of your alcohol. Find an IR band in Table 2 that is common to only the acid and alcohol. What is the wavenumber (range)?
Answer_____

7. From Table 3, what partial structure is common to an acid and a ketone?

Answer_____

8. Compare the spectrum of your acid with that of your ketone. Find an IR band in Table 2 that is common to only the acid and ketone. What is the wavenumber (range)?
Answer_____ What is the shape of this band? Answer_____

9. Find an IR Correlation Chart in a textbook or reference. What range of wavenumbers is shown for the common partial structure in an acid and alcohol (Review your X's in Table 3)?
Answer_____

10. From the IR Correlation Chart, what range of wavenumbers is shown for the partial structure that is common to an acid and ketone? Answer_____

5 Explorations in Infrared Spectrophotometry

(Identification of unknowns by IR)

A Summary of this Week's Lab Last week you prepared a table, showing the characteristic IR absorptions of four families. This week, you will use your table to identify the organic family of an unknown compound. You will prepare your unknown for analysis by dissolving it in chloroform, or you will conduct the analysis on a neat sample. The concentration will be near that of last week's samples. You will analyze the functional-group region of your unknown's IR spectrum and record the data in a new version of your table. You will then compare the data from your unknown against the data for each of the four families listed in your table. From the comparison, you will identify the family of your unknown. Your instructor will verify that you have correctly identified the family. You will then be given a list of 10 names of organic compounds from that family; one of the names is your unknown. You will find the IR spectrum for each of the 10 named compounds in the Sadtler reference set. You will compare the IR spectrum of your unknown with each of the 10 named spectra. You will identify your unknown on the basis of your comparison. You will prepare a report of your findings, which will be part 1 of the total report. For part 2, you will explore how the presence of chloroform as a solvent affects the IR spectra of two alcohols. You will take an IR spectrum of ethyl and isopropyl alcohols neat. ***Neat*** means a liquid is pure; no solvent or contaminate is present. You will then prepare or be given a chloroform solution of ethyl and isopropyl alcohols. You will record the spectra of these alcohols in chloroform and compare the spectra to those of the neat samples. You will prepare part 2 of your report on how the presence of chloroform affects the IR spectrum of the two alcohols. For home work, you will be given the IR spectra of four compounds, one from each of the four families we have studied. You will first analyze the functional-group region of each spectrum and identify the family to which the compound belongs. The compound will be one of 10 names that you will be given. You will look up the IR spectrum for each of the 10 named compounds in Sadtler's IR reference set and identify your unknown by comparing the finger-print regions. You will identify each of your unknown compounds from their spectrum in this manner from the 40 names, 10 for each family, that you are given. You will attach the solutions to your final report and turn it in at the beginning of the next lab period.

Introduction to Raman Spectroscopy

Imagine a beam of electromagnetic radiation passing through a transparent medium. When the beam interacts with the medium, a portion of the beam is scattered in all directions. Certain molecules cause a scattering that can be analyzed. Such an analysis is done by what is called Raman spectroscopy after the Indian physicist who discovered the phenomenon. A portion of the reflected beam is compared to the incident beam. The difference in wavelength between the incident and scattered visible radiation corresponds to mid-infrared wavelengths. The IR and Raman scattered spectra for a given substance are often quite similar. It turns out that Raman and normal IR are complementary; that is, normal IR is better for some applications, and Raman is better for others.

A major advantage of Raman spectroscopy is that water does not interfere with the technique. Thus, we can use glass or quartz cells instead of anhydrous sodium chloride salt disks for our samples.

Theory of Raman Spectroscopy

The theory of Raman Spectroscopy involves quantum chemistry that is beyond the scope of our analysis. The intention here is to provide a summary at the introductory level of how Raman spectra are produced and how they can by used by analysts.

How are Raman spectra produced? A sample is irradiated with a powerful laser source. The laser produces single-wavelength (monochromatic) light in the visible or near-infrared region of the electromagnetic spectrum. A small portion of the incident radiation bounces off the sample (is reflected) and is measured at some angle from the incident beam. The angle is typically 90^{o}. Quantum chemistry is necessary to fully explain the mechanism by which Raman spectra are generated. For our purposes, a new spectrum is produced that is characteristic of the sample. This Raman spectrum is complementary to a normal IR spectrum of the sample. Therefore, Raman spectroscopy simply offers the analyst a different way to acquire data on a sample that might be used to help identify the sample or to verify that the sample investigated is identical in all respects to another sample obtained at a crime scene.

Raman Instruments

The basic components of a Raman spectrometer are a laser, which provides the incident light beam, a sample illumination system such as a cuvette, and a spectrometer, which is similar to a normal IR instrument.

Laser Sources

In order for light to be reflected off a sample, a powerful light source is required. Accordingly, Raman instruments use a laser for the source. Lasers generate high intensity light. Laser is an acronym (a word made up of the first letters of several words) for **l**ight **a**mplification by **s**timulated **e**mission of **r**adiation. As the acronym suggests, a very intense beam of light (amplified light) is produced by stimulation (pumping) with the result that a beam of radiation is emitted. Substances with suitable electronic energy levels can be used in lasers. The substances that are used govern what the energy of the output is. For example, a ruby may be used as a laser source. A ruby contains Al_2O_3 as its major constituent, but it also contains some chromium(III) within the Al(III) lattice sites. The pump is a xenon lamp. Continuous pumping of the xenon produces more photons than are lost so that an intense energy beam can be developed. The wavelength of the beam is $\lambda = 694$ nm. Another laser that uses a solid source is called the Nd:YAG laser. Nd is the chemical symbol for the element neodymium, and YAG stands for yttrium aluminum garnet. Yttrium is also an element with the symbol Y, and garnet is a mineral. This laser produces a beam of $\lambda = 1064$ nm. Lasers are also produced that use gases instead of solids as the light source. Two of the kinds applicable to Raman spectroscopy are argon and krypton lasers. Argon and krypton are gaseous elements, symbols Ar and Kr respectively. The active species in argon and krypton lasers are the ions Ar^{+} and Kr^{+}. The argon laser produces light at $\lambda = 514$ nm, and the krypton laser produces light at $\lambda = 647$ nm. A dye laser is another kind of laser, which is tunable over a short range of wavelengths, typically 20-50 nm.

From the foregoing discussion, the argon and krypton ion sources have the advantage of producing lower wavelength, higher frequency light beams than the other sources. In general, the power of the Raman emission increases with the fourth power of the frequency of the source. The argon and krypton wavelengths are in the visible region of the electromagnetic spectrum. The Nd:YAG laser is a near-infrared source (light frequency is just below the visible). This laser has two major advantages over the higher frequency lasers. One is that it can be operated at a higher power (~50 Watts) and not

decompose the sample. The other advantage is that no competing fluorescence is produced by the Nd:YAG laser.

Two Types of Raman Spectrometers

One type of Raman instrument is a Fourier transform instrument equipped with cooled germanium transducers. Another type is a multichannel instrument based upon charge-coupled devices. When reviewing the literature, you should take note of which kind of Raman instrument is used by the investigators.

Applications of Raman Spectroscopy

There are literature citations for the use of Raman spectroscopy in both qualitative and quantitative analyses and in inorganic, organic, and biological systems. We'll limit our discussion to the Raman spectra of organic species.

Raman spectra are similar to IR spectra in that the bands are measured in cm^{-1}; however, Raman spectra tend to have fewer bands. Like IR, Raman is useful in the identification of functional groups. Likewise, they have fingerprint regions that are useful for the identification of specific compounds. The best way to learn about Raman spectra is to read articles in which Raman spectroscopy has been used by the authors for a specific purpose.

Two examples cited by Skoog, et al, in which Raman is superior to normal IR measurements are the identification of double bonds and the number of rings in a cycloalkane. Normal IR measurements give little information on these structural features.

Experimental:

1. Obtain an unknown from the instructor and record the unknown number in your notebook.

2. Prepare the sample by transferring it to a small Erlenmeyer flask and then adding 10-mL chloroform.

3. Fill the sample cell as in last week's experiment, obtain the spectrum, and print it.

4. Clean the sample cell thoroughly with chloroform, and obtain the spectra of ethanol and 2-propanol, respectively, in chloroform. Prepare the chloroform samples by dissolving 0.5 mL of alcohol in 10-mL chloroform.

5. Print the spectrum of each chloroform sample.

4. Next, obtain the IR spectra of neat ethanol and 2-propanol (isopropyl alcohol), one at a time, as follows. Place a few drops of the alcohol on a salt disk and carefully place a second disk on the first so that the liquid makes a thin film between the disks,

5. Insert the disk holder into the IR and place the two disks on the holder.

6. Obtain the spectrum and print it.

7. Carefully rinse the salt disks with acetone and replace them in the desiccator.

8. Clean are store all equipment.

9. Complete the lab report and turn it in at the beginning of the next lab period.

Lab Report #5
Identification of Unknowns by IR

Student Name_____

Part 1:

1. Complete Table 1 by filling in the characteristic wavelengths for the families indicated. Also add the wavelength of your unknown.

Table 1 The identification of Unknown

Family	Wavenumber of Characteristic Band(s)
Acid	
Alkane	
Alcohol	
Ketone	
The unknown compound Number _____	

2. What is the family of your unknown? _____
Show this answer to your instructor for verification.

3. After your unknown's family has been verified, you will be given a list of ten compounds. Your unknown is one of the ten compounds on the list. Go to the Sadtler references and find the IR spectra of the ten compounds and identify your unknown by comparing the bands in the fingerprint regions of the spectra. Enter the names of the ten potential unknowns in Table 2 below and indicate which one of them is your unknown.

Table 2 List of Compounds in the same family as my unknown

Compounds	Place a check (√) next to your unknown
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	

Part 2:

This part of your report deals with the IR spectra of ethanol (ethyl alcohol) and 2-propanol (isopropyl alcohol). You have two spectra of each compound, one neat and one in chloroform ($CHCl_3$). Attach these spectra to your lab report.

4. What bands in your spectra indicate that these compounds are alcohols?
_____

5. In the space below, write down the observations you made by comparing the neat spectrum of ethanol with the solution spectrum of ethanol.
_____
_____
_____
_____

6. In the space below, write down the observations you made by comparing the neat spectrum of isopropyl alcohol with the solution spectrum of ethanol.
_____
_____
_____
_____

7. Is it possible to make a generalization about how chloroform affects the spectrum of an alcohol?
_____

8. If so, write out the generalization.
_____
_____
_____
_____

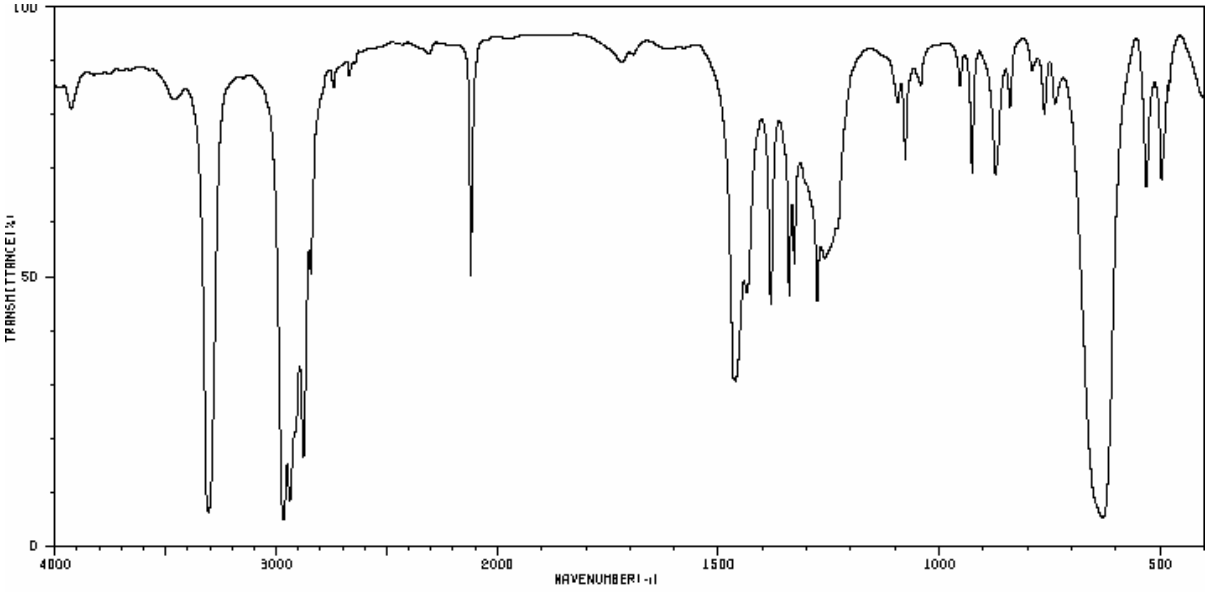
5 Homework

For each of the following groups, indicate the characteristic IR bands.

1. Alcohol _____
2. Ketone/Aldehyde _____
3. Ester _____
4. Carboxylic Acid _____
5. Alkane _____
6. Alkyne _____
7. Nitrile _____

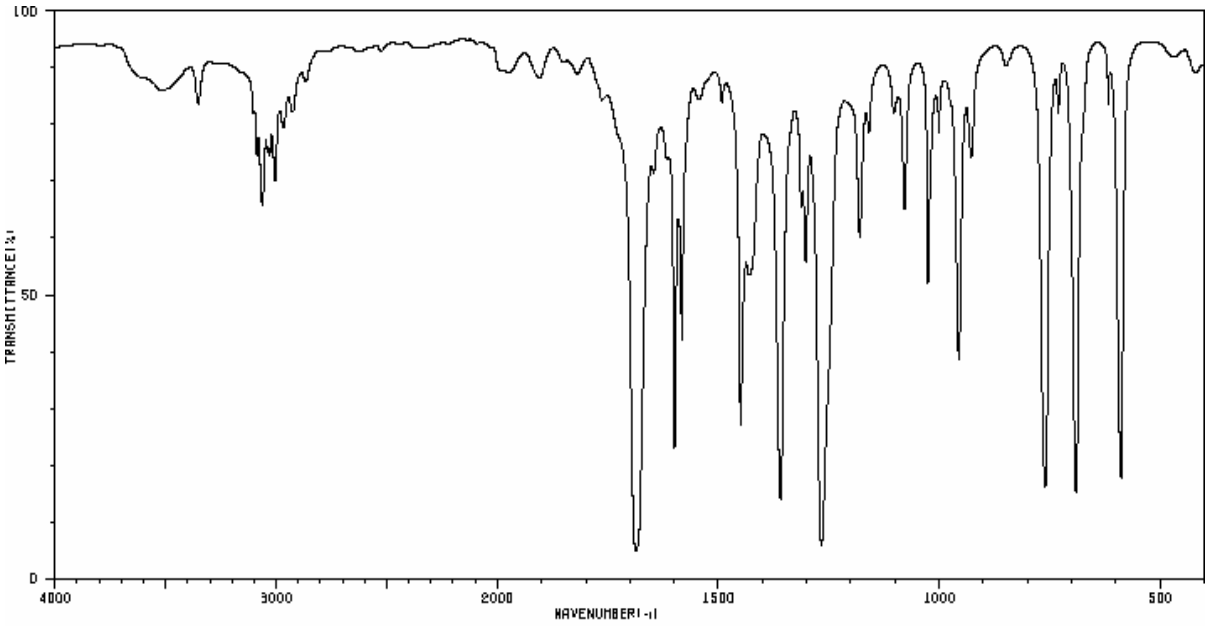
On the appropriate IR bands in the following IR spectra, label the functional groups that you identify.

8.[10]

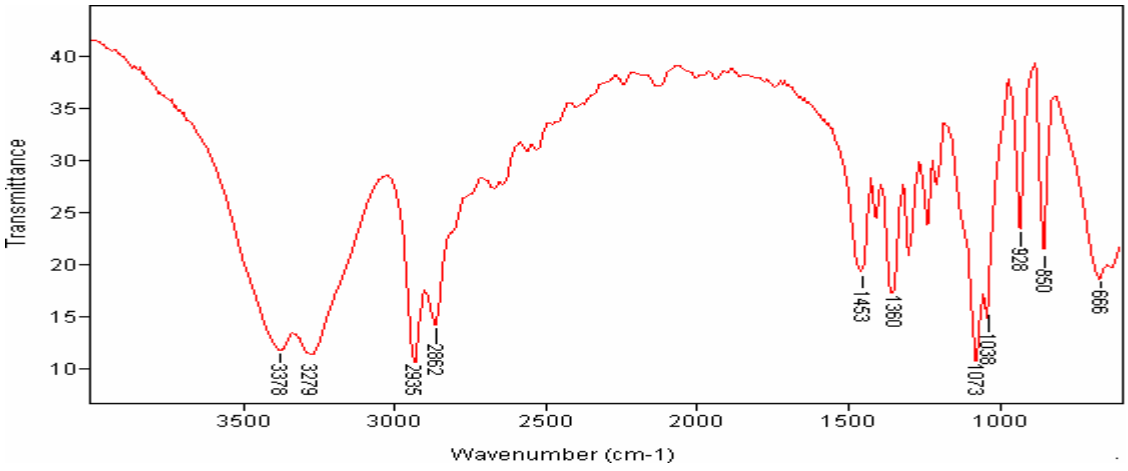


[10] Spectrum taken from SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).

9.[11]



10.[12]



[11] Spectrum taken from SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).
[12] Spectrum run at PGCC.

6 Forensic Analysis by Infrared Spectrophotometry

(Comparative Analysis of an Organic Liquid or Solid)

A Summary of this Week's Lab This week you will apply your training in infrared spectroscopy. You will make a qualitative analysis of a compound that belongs to one of the four families we studied. That is, you will identify the unknown by name. The compound will be pure, so a quantitative analysis will not be performed. However, you will record the mass of the sample if it's a solid or the volume if it's a liquid. The unknown will be a forensic sample, and you will handle it accordingly by maintaining a chain-of-custody. The sample is obtained hypothetically at a crime scene or from a suspect by a criminal investigator. The sample will be accompanied by a request from the investigator in the form of a question. The question might be "is this forensic sample acetophenone?" You will prepare the sample for analysis by dissolving it in chloroform if it is a solid. If it is a liquid, you may run the sample neat or in chloroform. You will first conduct a preliminary analysis of the spectrum of the unknown to determine to which family the unknown belongs. You will record whether or not the unknown belongs to the same family as the compound in the request. This will give you a preliminary answer to the request question of probably yes or probably no, depending upon your results. You will next ask the instructor for an authentic sample of the compound mentioned in the request. You will next obtain a spectrum of the authentic sample with exactly the same methodology you used for the unknown. A peak for peak match, particularly in the fingerprint region, will confirm the identity of the two compounds. A discernable mismatch of peaks will confirm the non-identity of the two compounds. If the two compounds are not identical, you will try to match the spectrum of your unknown with that of a compound from a list of 10 names provided by the instructor, using the Sadtler spectra. Once found, you will ask the instructor for an authentic sample of the prospective unknown. A peak-for-peak match will confirm the identity of the unknown. You will prepare a report of your findings, including a specific answer to the question that accompanies your sample. You will include other pertinent information such as the exact identity of the unknown.

Theory

It is possible to calculate approximate wavenumbers by use of the equation 1 shown below.

$$\text{nu bar} = 5.3 \times 10^{-12}\ \text{sec/cm}\ (k/\mu)^{1/2} \qquad (1)$$

In equation 1, nu bar is the wavenumber in reciprocal centimeters. The constant k is the force constant for the vibration in question. For a double bond, the value of the force constant is about 1000 Newtons per square meter. The Greek letter mu (μ) is used for a calculated value called the reduced mass. A reduced mass is a way of expressing the combined mass of two atoms joined by one or more covalent bonds. The combined mass is not a simple average mass but rather a weighted average of the two masses. The value of mu is given by equation 2.

$$\mu = m_1 m_2 / m_1 + m_2 \qquad (2)$$

In equation 2, m_1 represents the mass of one bonded atom, and m_2 represents the mass of the other. The reduced mass μ equals the product of the two masses divided by the sum of the two masses. When given a force constant k and a reduced mass μ, one can calculate nu bar by equation 1. For example, the reduced mass of a carbonyl group (C=O) is about 10^{-23} grams, and the force constant for the carbonyl group is about 10 Newtons per centimeter, so nu bar is about 1700 cm^{-1} for the carbonyl group.

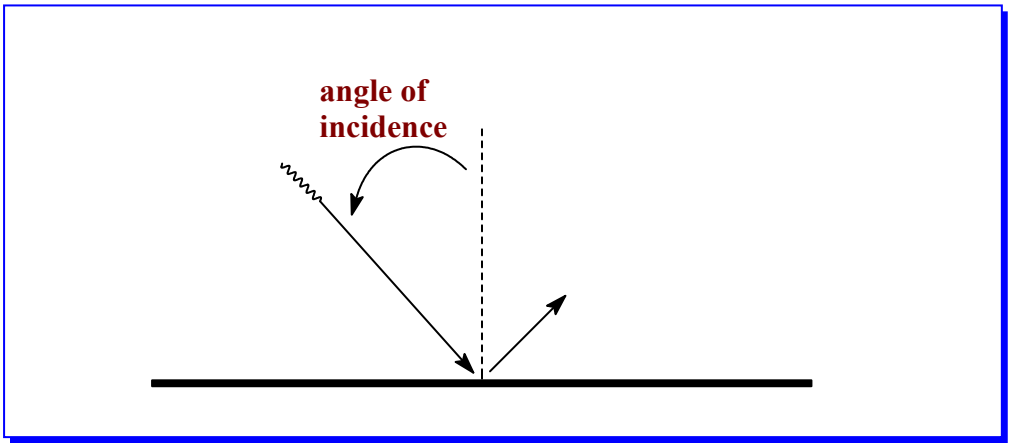
Reflection Spectrometry

Raman spectrometry is an example of reflection spectrometry. There are four kinds of reflection radiation; specular reflection, diffuse reflection, internal reflection, and attenuated total reflection (ATR). Of these four, we shall consider only attenuated total reflection. However, you should be aware of all four kinds and take note of them when you see them mentioned in scientific papers.

Attenuated Total Reflection (ATR)

ATR is an IR technique that is particularly suitable for samples for which it is difficult to obtain normal IR spectra. These samples include solids, which do not dissolve readily in common IR solvents, films, fibers, hairs, etc. ATR is especially useful in the forensic analysis of hairs for example.

Reflection occurs when a radiant beam passes through two layers, the first of which is more dense than the second. The portion of the incident wave that is reflected increases as the angle of incidence becomes larger.



The depth of penetration of a radiant beam depends on three variables: the wavelength of the incident radiation, the index of refraction of the surfaces, and the angle of incidence. The radiation that penetrates into the second layer and is absorbed by it is called the ***evanescent wave***. The reflected radiation is thereby attenuated at wavelengths of the incident radiation. This overall phenomenon is called ***attenuated total reflectance***.

The apparatus for obtaining an ATR spectrum includes a transparent crystalline surface, which might be a mixed crystal such as thallium bromide/thallium iodide, or a solid such as diamond, which has a high refractive index.

ATR spectra are similar to normal IR spectra but the intensities of the bands differ. Sample thickness is not a variable in ATR; whereas, it is a variable in normal IR spectra. In this lab, a former student tried to obtain normal IR spectra of two samples she prepared in an advanced research project. She found these samples to be insoluble in every solvent she tried. We were able to obtain ATR spectra for these solids and thereby characterize the two solids by IR. An ATR spectrum showing the ATR traces for both compounds is shown below.

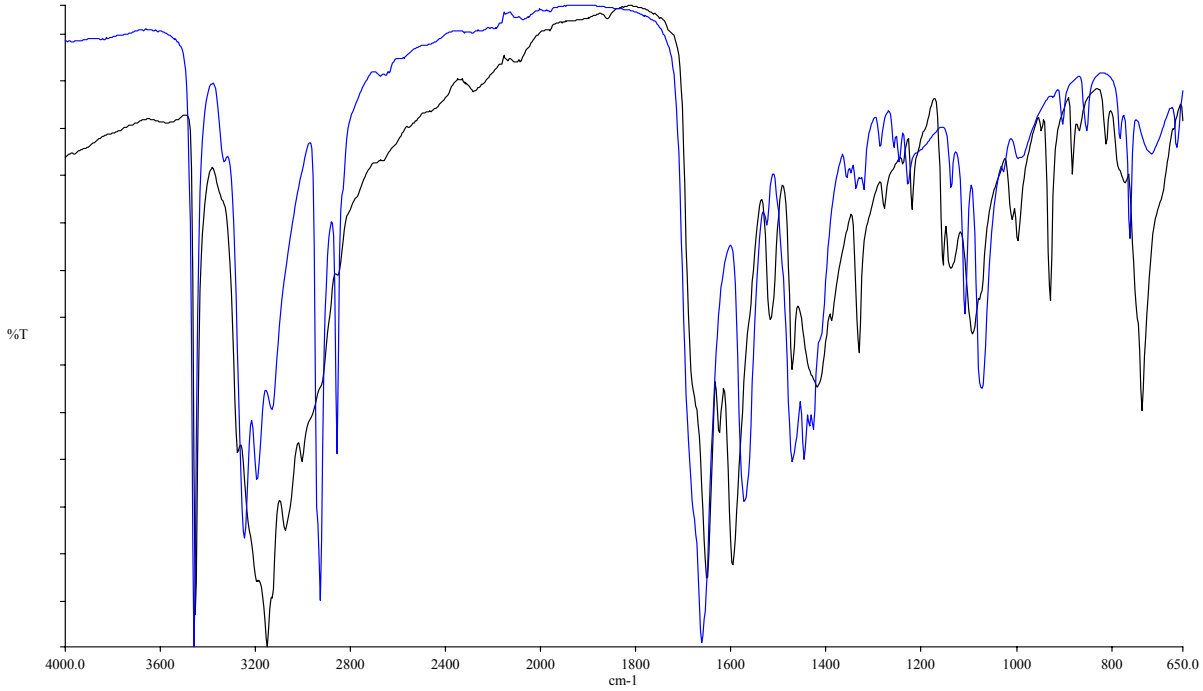


Figure 1. ATR spectra of two different solids taken on a diamond surface.

Lab Problem Scenario:

You are working at the ABC crime lab. Your supervisor gives you a forensic sample that was provided by a criminal investigation team that is working on a criminal investigation. During the course of the investigation, the team obtained several samples some liquid and some solid. These samples were all submitted to the ABC crime lab for analysis. Each sample has a question associated with it. Read the question asked by the crime team and answer it in your lab report.

Experimental:

Each sample will require a comparative analysis for an answer. That is, the IR spectrum of an authentic sample of the probable compound will be compared with the suspect compound to either prove or disprove their identity.

1. Prepare your sample for IR analysis per the directions of the previous two IR labs.
2. Collect the IR spectrum and print it.
3. Determine the functional group in the compound.
4. Obtain an authentic sample and run the IR on it in the same way you ran the IR of the unknown.

5. Compare the IR spectra peak for peak and determine whether identical or different compounds made the spectra.

Lab Report #6
Comparative Analysis of an Unknown Liquid or Solid by IR

Student Name _____

Label and attach the IR spectra you obtained in this experiment to this lab report.

1. Write the question posed by the crime team.

_____
_____
_____

2. What functional group did you determine to be present in the sample after your first IR?

_____

3. Did your preliminary analysis eliminate or include the suspect compound?

_____

4. Write your conclusions after running both IR's.

_____
_____
_____
_____
_____
_____

6 Homework

Define or explain what is meant by the following terms.

1. Raman spectrum

2. Attenuated Total Reflectance

3. Reduced mass

4. Force constant

5. Evanescent wave

6. What is the fundamental difference between an argon ion laser and a krypton ion laser?

7. Nd:YAG

8. What kind of sample would you submit to ATR analysis as opposed to a standard IR analysis?

9. Why do some alkynes show a strong IR absorption at 2200 cm^{-1}; whereas, others do not?

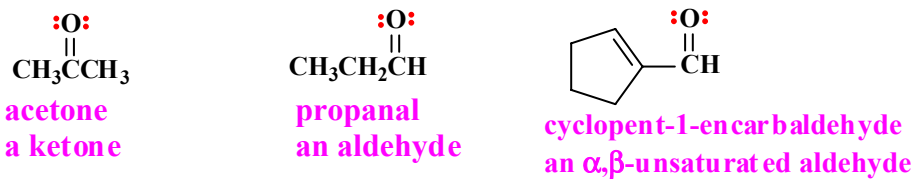
10. What is the fundamental difference between IR and UV absorption?

7 Introduction to Ultraviolet—Visible Spectrometry (UV-Vis)

(Identifying the wavelengths of maximum UV absorption for explosive components)

A Summary of this Week's Lab During this lab period, you will be introduced to ultraviolet spectroscopy. The prefixes ultra and infra mean above and below, respectively. Hence, the energy of ultraviolet radiation lies just above the visible violet light (i.e., ***ultraviolet means above visible violet light in energy***). Likewise, ***infrared means below visible red light in energy***. Because the energy range for a typical UV analysis is right next to the energy range of visible light, many instruments, including ours, operate in both the UV and visible regions. In this lab period, you will learn how to prepare samples for analysis and how to obtain data from the UV-Vis instrument. The samples are compounds that might be found in explosives. They are nitroaromatics or compounds that contain nitro groups ($-NO_2$) bonded to an aromatic ring. In this case, the aromatic ring is toluene. You will examine the output of the instrument in the wavelength region between 200 and 400 nm (nanometers). The range 200-400 nm is typical of UV instruments found in undergraduate labs. One nanometer is 1×10^{-9} meter. You will find the wavelength of maximum absorption (λ_{max}) for several nitroaromatics and record these values in a table. You will then correlate the λ_{max} values with structure. Thereafter, you should be able to predict the wavelength of maximum absorption for a given structure of a nitroaromatic. At the beginning of the next lab period, you will turn in a report of your laboratory findings.

A Simple Theory for UV Absorption[13] In terms of energy, the IR region lies below the visible region, which lies below the UV region. Thus, IR is strong enough to cause atoms within a molecule to vibrate, but not strong enough to cause electrons to change orbital locations. UV radiation between 200 and 400 nm is strong enough to cause loosely held electrons to change locations. These electrons can be either the non-bonding electrons (*n*-electrons) of aldehydes or ketones, or they can be the π-electrons of conjugated π-systems. Figure 1 shows typical structures of compounds that contain *n*-electrons.



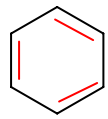
Non-bonding electrons are shown in red.

The oxygen atom of an aldehyde or ketone contains *n*-electrons that can be excited by UV radiation (200-400 nm) into a π* orbital, causing a UV abosorption.

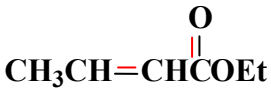
Figure 1

Figure 2 shows compounds that contain conjugated π systems.

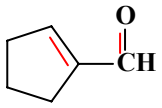
[13] McMurry, *Organic Chemistry*, 5th Edition, pp 543-548.



benzene
a conjugated
π syst.



ethyl 2-butenoate
a conjugated p syst.



cyclopent-1-encarbaldehyde
an α,β-unsaturated aldehyde

Conjugated π electrons are shown in red.

Conjugated p systems, shown by the red bonds above. display strong absorptions in their UV spectra between wavelengths of 200 and 400 nm.

Figure 2

Ultraviolet spectroscopy is one of several forms of spectroscopy that we will study this semester. Accordingly, it is important that you understand the capabilities and limitations of each of these forms of spectroscopy. The words ***spectroscopy*** and ***spectrometry*** have different meanings. A spectroscope is an instrument, and spectroscopy is the use of a spectroscope. Spectrometry means the measurement of a spectrum. One generally measures wavelengths or frequencies, or a spectrum of them. We will use the electromagnetic ***spectrum*** to gain information about organic molecules. The modifier ultraviolet means that the information will come from a specific region of the electromagnetic spectrum called the ultraviolet region. The electromagnetic spectrum includes all radiation that travels at the speed of light c (3×10^{10} cm/sec). The electromagnetic spectrum includes radio waves, which have long wavelengths, x-rays, which have short wavelengths, and visible light, which has wavelengths between those of radio waves and x-rays. All of these waves travel at the speed of light. We normally describe these waves in terms of their energy. Of the three kinds mentioned, x-rays are most energetic, visible light next, and radio waves least energetic. Thus, the shorter the wavelength is, the greater the energy of an electromagnetic wave.

Electromagnetic radiation (EMR) has a dual nature; it has the characteristics of both waves and particles. These particles are so tiny that they are indistinguishable from a wave. Both forms of EMR are important. From the wave nature of the waves we get the ***wavelength (λ) or distance between two crests***. The wavelength is related to the ***frequency (ν), how many wavelengths pass a given point in a given time***, by the velocity of the wave c. From the particulate nature of EMR, we get the energy *E* of a given wave, which is proportional to its frequency. Plank's constant h turns the proportionality into an equation. The mathematical relationships among these variables are shown below.

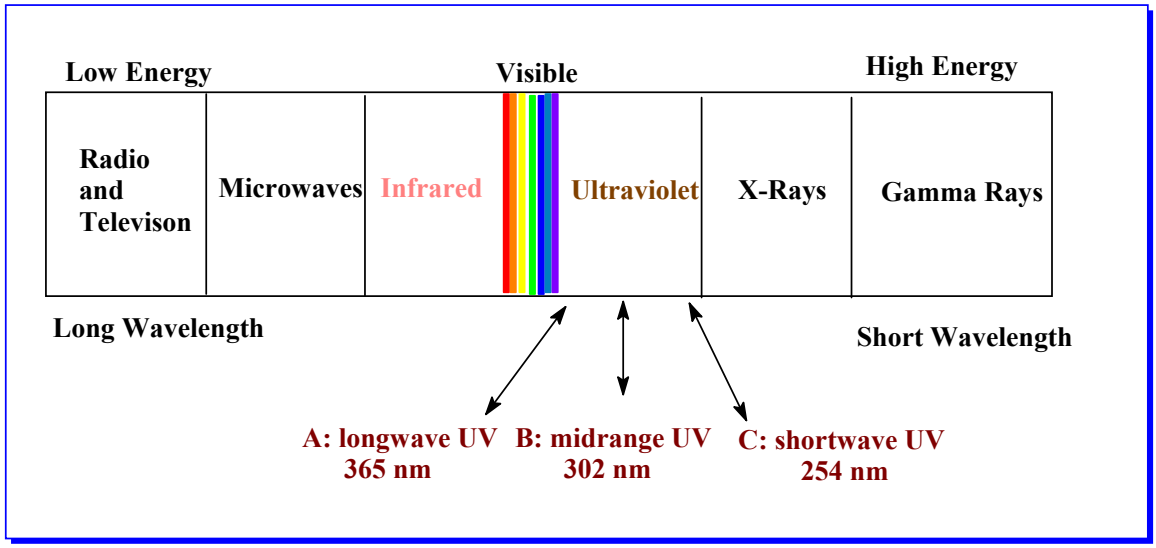
Frequency and Wavelength: $\nu = c/\lambda$ or $\lambda = c/\nu$ or $c = \nu\lambda$

Energy and Frequency: $E \propto \nu$ or $E = h\nu$

Energy and Wavelength: $E = hc/\lambda$

Visible light includes the rainbow colors red, orange, yellow, green, blue, indigo, and violet. A handy acronym for these colors is **ROY G BIV**, said like the name of a person called Roy. Note red is at the low-energy end of the visible spectrum and violet is at the high-energy end. These facts

allow us to quickly understand the terms infrared and ultraviolet. The prefix infra means below, and the prefix ultra means above. Thus, infrared radiation is outside the visible range and lies just below red on the energy scale. That is, infrared radiation is less energetic than visible light. Ultraviolet radiation is outside the visible range and is just above violet on the energy scale. Thus, infrared literally means "below" red (in terms of energy), and ultraviolet means "above" violet (in terms of energy). We know UV is more energetic than visible light or IR because UV light gives us sunburns.



We learned in general chemistry that visible yellow light is observed when sodium ions are heated in a Bunsen burner. The heat excites some ground-state electrons to higher energy levels, then when the electrons "fall" back to the ground state, they "emit" energy that corresponds to the energy difference between the energy states (orbitals) where the electrons are found. When this energy difference falls within the energy range of visible light, we can see it as a color. In the case of sodium, we see yellow light. Thus, the light results from the emission of energy by the electrons as they fall from higher to lower energy states (orbitals). Note that it takes the same amount of energy to make the electrons jump from the lower to higher states as the amount of energy the electrons emit when they fall from higher to lower states. We generally add more energy than is absolutely necessary for the transition to ensure that the transition occurs. When we add energy to a system, we give it a positive sign (***endoenergetic***). When a system gives off energy, we give it a negative sign (***exoenergetic***).

Just as heat causes some of sodium's electrons to move to higher energy states, ultraviolet radiation causes electrons in certain organic compounds to move from their ground state locations to orbitals of higher energy. The energy of the ultraviolet light acts just like the energy of the heat. In this case, the molecules are said to "absorb" ultraviolet radiation. A measurement of this phenomenon is called an absorption spectrum as opposed to an emission spectrum. When electrons move from lower to higher energy levels, we call the movement an ***electronic transition***. Thus, the basic interaction between UV light and organic compounds is that UV light causes electronic transitions in certain organic structures. That is, for a given molecule, an electron changes orbital locations because the energy of the UV light forces it to change locations.

The organic compound is dissolved in a solvent that does not absorb UV light. Such a solvent is said to be transparent to UV light. The sample (compound in its solvent) is placed in a cuvette. A cuvette is a sample holder that has very precise dimensions. The cuvette is placed in an ultraviolet spectrophotometer. The instrument produces ultraviolet light over a range of wavelengths between 200 and 400 nanometers (nm), and the UV light is split into two equal beams. One beam is directed through the solution of the organic compound (the sample) and the other beam is directed through the solvent (the reference). The two beams are called the ***sample beam*** and the ***reference beam***. A nanometer equals a millimicron (mμ), which is sometimes still used by chemists to report wavelengths. As the UV light passes through the sample, the instrument records a plot of absorbance (A) versus wavength (λ). In other words, the instrument measures how much UV light is absorbed (the absorbance A) and where the light is absorbed (the wavelength λ) for the specific sample. In this lab, we will obtain a UV spectrum (plot of A vs. λ) for a sample of the organic compound toluene (methylbenzene) dissolved in hexane. From the plot we will find the wavelength where the maximum absorbance occurs and record the wavelength as λ_{max} and the absorbance as a raw number. We call λ_{max} the wavelength of maximum absorbance. Thus, after you obtain your plot or printout of A vs. λ, all you will record on your data sheet from the printout is λ_{max} and A at λ_{max}.

We will then make use of certain relationships that govern how much UV light can be absorbed by a sample. Namely, that the amount of light absorbed (A) is proportional to how many molecules or the concentration (*c*) of molecules that are absorbing light, and how far the UV light must pass through this concentration or the path length *l*. These relationships are shown below.

$$A \propto c$$
$$A \propto l$$
$$A \propto c \times l$$

This last proportionality says that the absorbance A is directly proportional to the concentration of the sample and to the path length (width of the cuvette). This is why the dimensions of the cuvette must be precise. The proportionality is useful for one given concentration or sample. A far more useful form of the relationships above is the Beer-Lambert equation, which makes the proportionality into an equation by addition of the proportionality constant ε (pronounced epsilon).

$$A = \varepsilon cl$$

Beer-Lambert Equation

Like λ and ν, ε is a Greek letter. However, it is simply a constant that makes the above proportionality an equation. The constant ε does not vary. So various concentrations of toluene measured in different size cuvettes would give the same value of ε. Therefore, our laboratory exercise will include calculating ε. The constant ε is called the ***molar extinction coefficient***. Therefore, the concentration of the sample must be in moles per liter (mol/L). The standard path length is 1 cm. Thus, most cuvettes, including those in our lab, are exactly 1 cm wide where the light passes through. These conventions ensure that everyone calculates ε the same way.

Figure 3 shows two kinds of cuvettes.

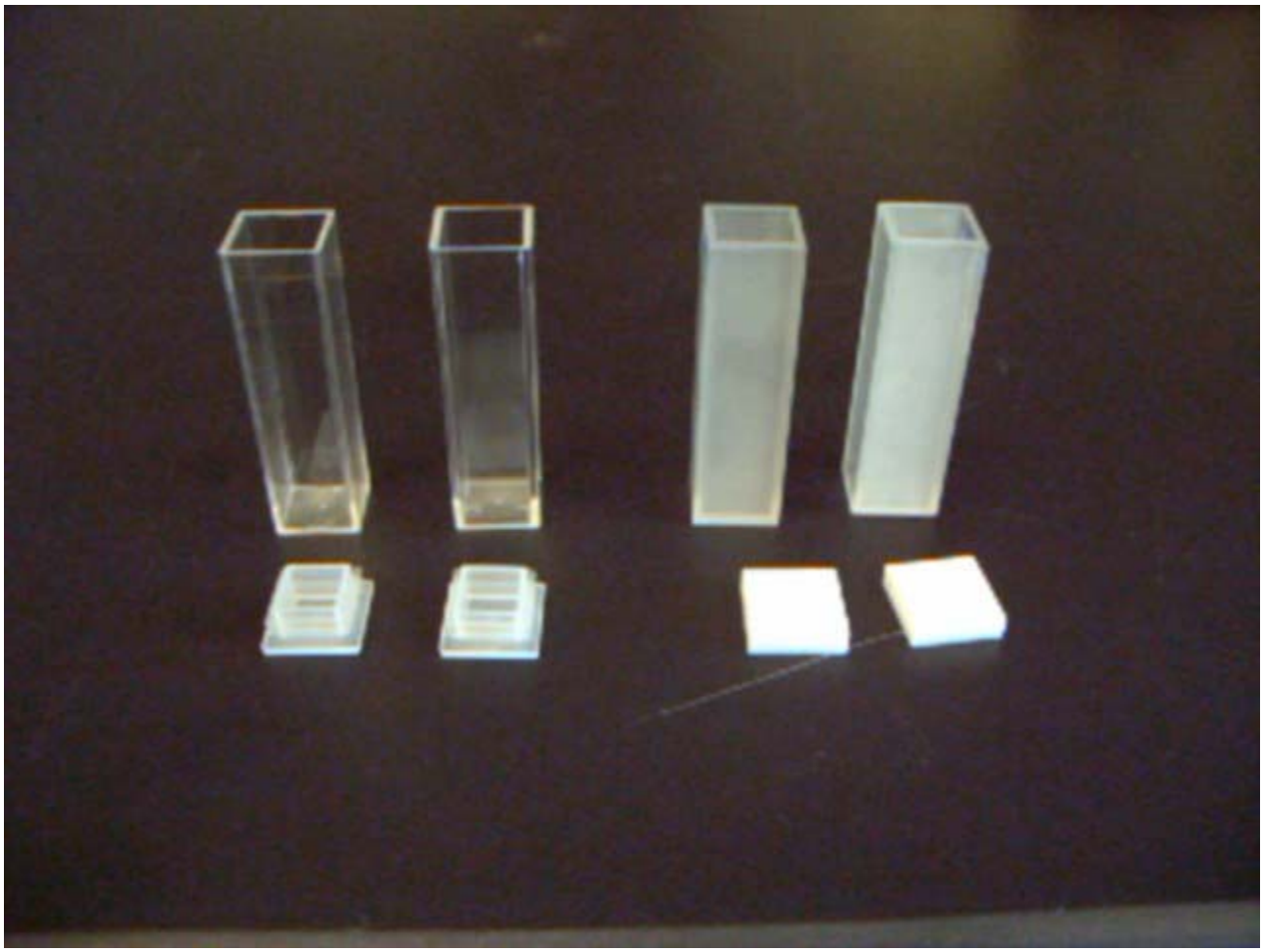


Figure 3. Plastic cuvettes for water soluble compounds (two cuvettes on the left), and quartz cuvettes for organic solvents (two cuvettes on the right).

We will learn that compounds such as benzene that contain conjugated π systems absorb UV light very strongly (i.e., ε is typically 5,000 to 30,000). Whereas aldehyde and ketones, which contain isolated carbonyl groups, absorb UV light weakly (i.e., ε is typically less than 100). The spectrum may be run on a very small sample, but small amounts of impurities that absorb UV must be avoided.

Example problem: One milligram of a compound of molecular weight 140 is dissolved in 20 mL of ethanol. The UV of the sample is measured in a 1-cm cuvette. The maximum absorption is 0.50 recorded at 248 nm. Calculate the value of ε.
Solution: The concentration in mol/L = [(0.001)/140]mol/.012 L = 0.0060 mol/L ; ε = A/cl = 0.50/(0.0060)(1.00) = 83.

Acids and acid derivatives, which contain a heteroatom next to the carbonyl might absorb UV radiation, but not measurably in the 200-400 nm range. Therefore, except for aldehydes and ketones,

compounds that contain carbonyl groups will not be considered to absorb UV radiation. Thus, UV spectroscopy enables us to identify a conjugated π system or the carbonyl group of an aldehyde or ketone by the value we find for ε. A strong absorption corresponds to ε > 1,000, and a weak absorption to ε < 100. If log ε = 5, is the UV absorption strong or weak? Does it represent an aldehyde or a conjugated system? Figure 4 shows the simulated UV spectrum of a conjugated ketone. Note that there are two maxima in the curve. There is a strong absoption (ε > 1,000) and a weak absorption (ε < 100). The strong absorption is due to a conjugated π system (i.e., C=C-C=O, alternating double bond, single bond, double bond). The weak absorption is due to the presence of a ketone carbonyl group in the compound. The carbonyl groups contain non-bonding electrons. Hence, the strong absorption is due to a π to π^* electronic transition and the weak absorption is due to an n to π^* transition.

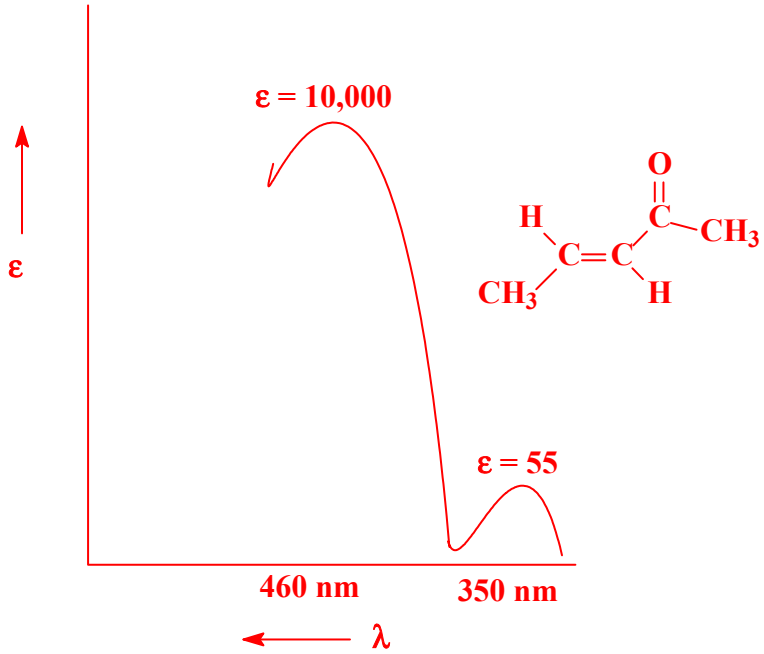


Figure 4. UV spectrum of an α,β-unsaturated ketone.

π Electrons and Conjugated π Systems

UV radiation has more energy than IR radiation; therefore, UV radiation interacts with compounds differently than does IR radiation. IR radiation makes molecules vibrate (i.e., twist, bend, scissors, etc.), whereas UV radiation causes a loosely held electron within a molecule to change locations. Because electrons can only exist in orbitals, the change in location is from one orbital to another orbital. The process of changing orbital locations is called a transition. We could say that when we walk from the first floor to the lab on the third floor that we transition to the lab, but the word transition is usually reserved for the movement of a particle such as an electron from one orbital location to another. When an electron changes orbital locations, we call the process an ***electronic transition***. Electrons do not change location spontaneously; they need help. The help appears in the form of UV radiation. UV radiation is just energetic enough to cause certain loosely held electrons to move from one orbital to another orbital but not energetic enough to cause an electron to be ejected from the molecule. Radiation of higher energy than UV radiation such as X-ray or Gamma radiation

is sufficiently energetic to eject electrons. When a negatively-charged electron is ejected, a cation or positively-charged particle is left. Therefore, high-energy radiation is called ***ionizing radiation***. Of course, if a human is subjected to ionizing radiation, the result can be a radiation injury. Thus, when you get a dental x-ray, a lead-containing protective apron is draped over you to protect your body from the ionizing radiation.

The Instrument: See Skoog, et. al., *Instrumental Analysis*, 5th edition, pages 320-325.

Sample Preparation:
1. Obtain a pair of quartz cuvettes from their storage location. Caution: Handle the cuvettes by touching only the opaque sides (i.e., the sides that light does not pass though.)
2. Rinse them with ethanol, fill one with ethanol and cap it, allow the other to dry.
3. The capped cuvette will serve as the reference. Carefully wipe the two clear sides of the cuvette with a tissue before inserting it into the instrument.
4. Rinse the dry cuvette and then fill it with the sample to be analyzed.

Instrument Startup: Start up the instrument according to the local procedure.

Obtain Spectrum:
1. Place the reference and sample cuvettes in the instrument in the correct locations.
2. Obtain the spectrum from 800-200 nm and print it out. Note that our instrument scans both visible and ultraviolet regions of the electromagnetic spectrum.
3. Repeat the procedure for each of your samples of known structure.

Instrument Shutdown: Shut down the instrument according to local procedures.

Lab Report #7
UV Spectra of Nitroaromatics

Student Name_____

Data: The path length for all analyses was _____ cm. Analyze your UV spectra and enter the appropriate data in Tables 1 and 2.

1. **Table 1. Wavelengths of Maximum Absorption for Nitroaromatic Compounds**

Compound	**λ_{max} (nm)**
1.	
2.	
3.	
4.	
5.	

2. **Table 2. Concentrations of Nitroaromatics**

Compound	**Concentration (*c*) mol/L**
1.	
2.	
3.	
4.	
5.	

.

3. **Table 3. Absorbance (*A*) of Nitroaromatics**

Compound	**Absobance (*A*)**
1.	
2.	
3.	
4.	
5.	

Calculate the molar extinction coefficients for each of your nitroaromatics.

4. **Table 4. Molar Extinction Coefficient (ε) for Nitroaromatics**

Compound	**Molar extinction coefficient (ε)**
1.	
2.	
3.	
4.	
5.	

5. For each of your nitroaromatics, write out the appropriate UV data in the format you would expect to see in a journal (e.g., nitroaromatic: λ_{max} 250 nm, ε 12,000).

7 Homework

1. Explain what is meant by lambda max (λ_{max}).

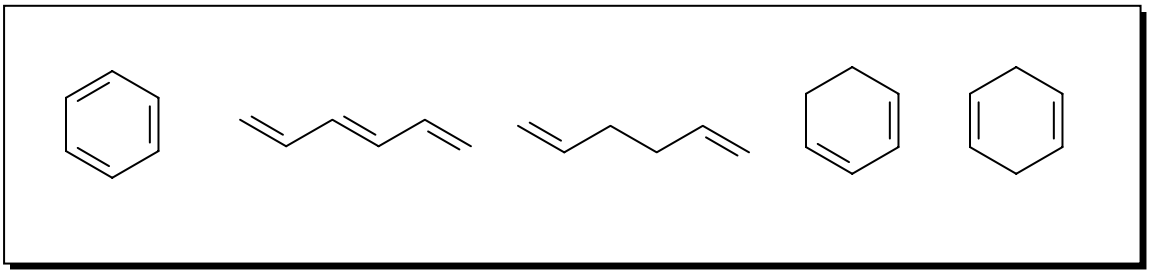
2. What information do you get from a molar extinction coefficient?

3. Draw the structure of cyclohexanone and show its non-bonding electrons. What value of ε do you expect for cyclohexanone?

4. Draw a bond-line structure of toluene. What value of ε do you expect for toluene?

5. Why do the two examples in Figure 4 both have two maxima in their UV spectra?

6. Circle the compounds below that display a strong UV.



7. A student dissolves 1.00 mg of a solid (140 g/mol) in 10.00 mL of ethanol. What is the concentration of the solid in mol/L in the ethanolic solution?

8. One milligram of a compound of molecular weight 160 is dissolved in 10 mL of ethanol. The UV of the sample is measured in a 1-cm cuvette. The maximum absorption is 0.60 recorded at 240 nm. Calculate the value of ε.

9. The UV data in Table 1 is for the purpose of preparing a Beer-Lambert calibration curve for a nitroaromatic. Standard solutions of a nitroaromatic were analyzed in a UV. The data in Table 1 were recorded at the wavelength of maximum absorption. The concentration of each solution was recorded and the absorbance read from the spectrum.

Table 1. Data for a Calibration Curve

Concentration (mol/L *x* 10^{-4})	**Absorbance (*A*)**
30.0	
40.0	
50.0	
60.0	
70.0	
80.0	
90.0	
100.0	
120.0	
140.0	
160.0	

Enter the data from Table 1 into an Excel spreadsheet. Determine the best equation for a straight line calibration curve with *c* and *A* as the variables for the data and record your answer below.

10. Given a sample of acetophenone, predict what kind of UV spectrum you expect.

8 Explorations in Ultraviolet Spectrometry (UV)

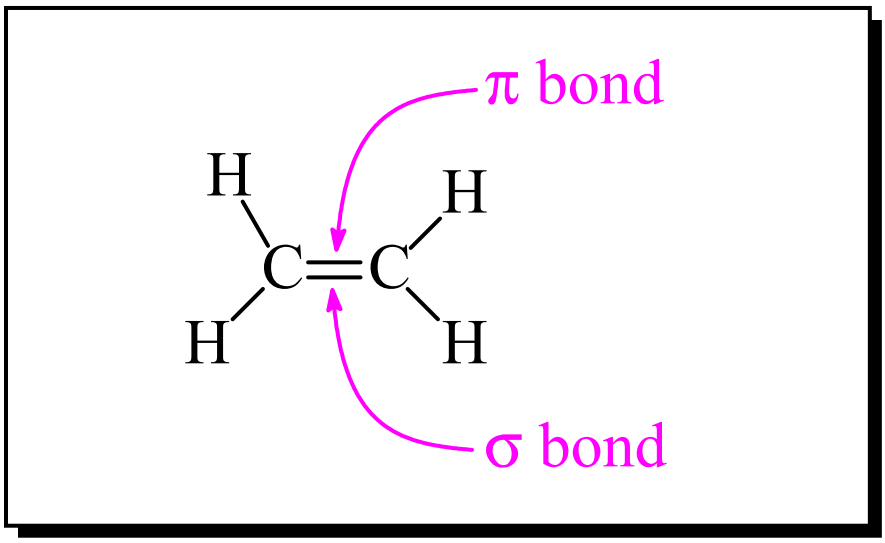
(The effect of a solvent on the position of maximum absorbance)

A Summary of this Week's Lab During this lab period, you will examine how a solvent affects the wavelength of maximum absorbance for the same set of compounds you studied in the last lab period. You will collect UV data for the compounds in three different solvents and determine whether or not the solvent affects the wavelength of maximum absorption. A recent paper[14] reported the UV data for a newly discovered compound as follows: UV λ_{max} (CH_2Cl_2) 232 and 322 (ε 8800 and 6900, respectively). Does this reported UV suggest that solvent is important or unimportant for this form of analysis?

Molecular Orbital Theory of Electronic Transitions

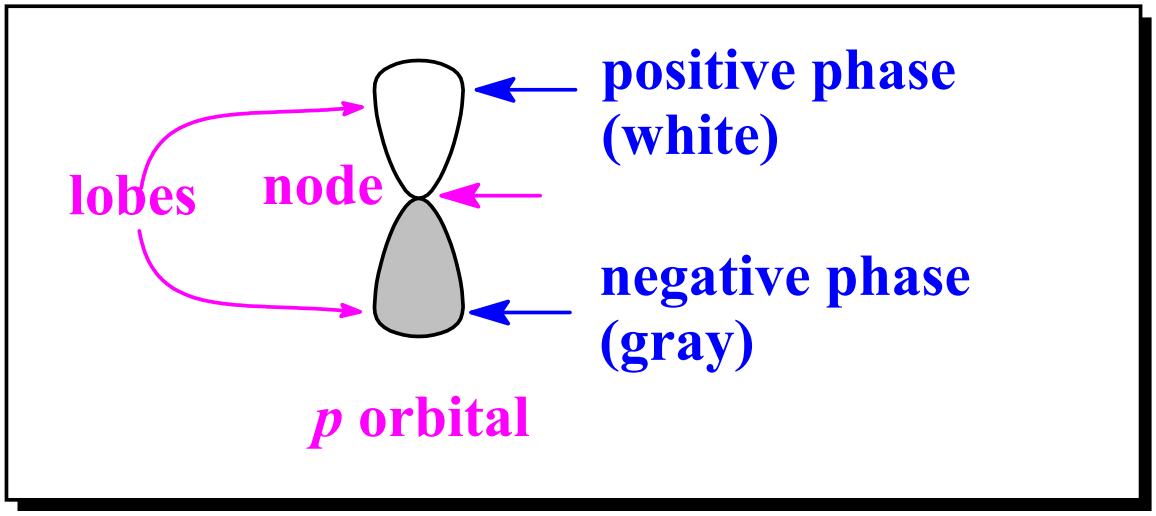
The electrons in atoms are found in orbitals. In atoms, which are not bonded to other atoms, these orbitals are called ***atomic orbitals***. When atoms join or bond together to form molecules, the electrons are still found in orbitals, but in the case of molecules the orbitals are called ***molecular orbitals***. You are familiar with the **VSEPR** model of molecular bonding. VESPR is an acronym for valence shell electron pair repulsion. The VESPR model helps us predict molecular geometries around a central atom by use of the concept of hybridization. The VSEPR model does not predict why certain compounds absorb UV radiation. An alternative theory of bonding is called the **MO or molecular orbital theory** of bonding. An introduction to MO theory will be presented in the following paragraphs.

Let's consider ethene (common name ethylene). It is made up of two carbon atoms joined by a double bond with all of the open valences filled in with hydrogen atoms. The structure of ethene is shown below.



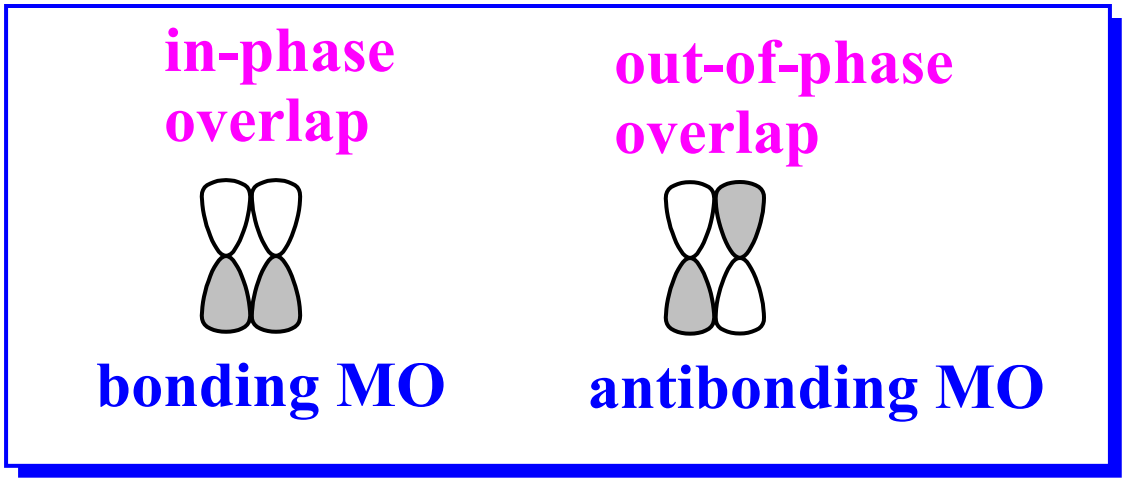
[14] I. C. Piña, M. L. Sanders, and P. Crews; *J. Nat. Prod.*, **2003**, *66*, 2-6.

The π bond is made up of the overlap of four orbitals, two σ orbitals and two *p* orbitals. Interestingly, the *p* orbitals are unhybridized. That means they are the same *p* orbitals that existed as atomic orbitals in atomic carbon before the two carbon atoms joined to make molecular ethene. A *p* orbital has a figure 8 or dumbbell shape. The point where the two halves of a *p* orbital come together is called a ***node***. The maximum number of electrons that may occupy any orbital is two. Thus, a *p* orbital, like all other orbitals, may hold zero, one or two valence electrons. Electrons have both particle and wave properties. An electron is so small, that it is pure energy spread through the orbital space. Thus, if we consider a single *p* orbital that holds one electron, that electron is spread throughout the orbital. We refer to an electron spread out in a wave or energy as electron density. Regions can have high electron density or low electron density, meaning that more or less of the total is found in a given region. For example, there is absolutely no electron density at a node of a *p* orbital, although there is an equal amount of the density in the two lobes of the *p* orbital. A picture of a p orbital is shown below.

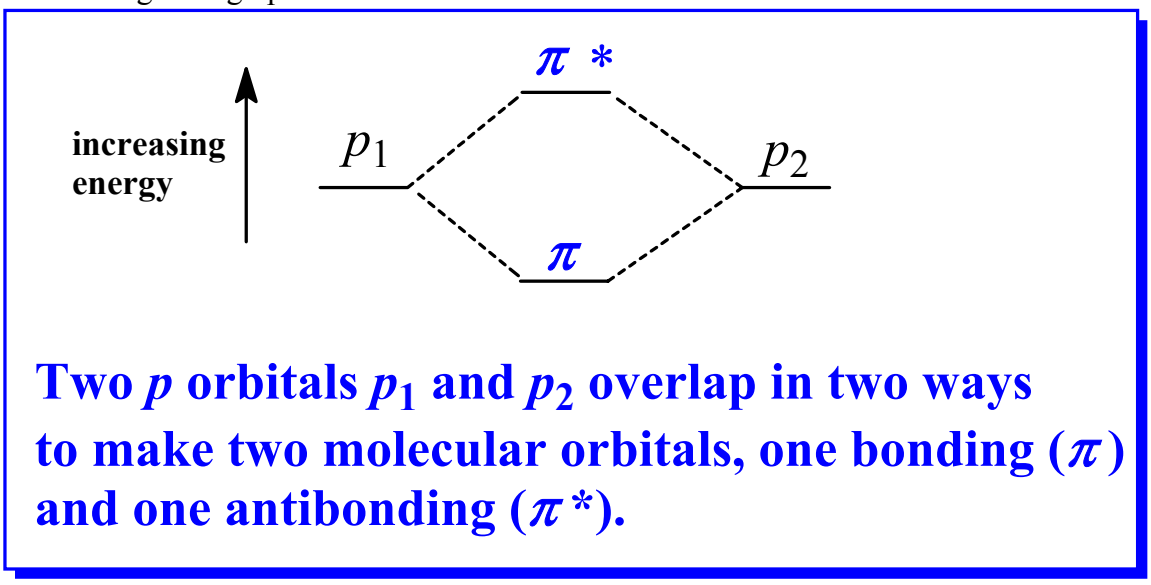


Molecular-orbital theory starts with atomic orbitals such as the one pictured above, and considers molecules to be formed by a linear combination of atomic orbitals. For many applications, the underlying sigma-bond network of atoms is ignored and only the π bonds are considered, specifically, the *p* orbitals that make up the π bonds. If we consider two *p* orbitals such as those in ethene, we can see that there are two ways a pair of *p* orbitals can overlap. One way is in-phase overlap in which like phases overlap. In our picture, the phases are shown in white and gray. A white-white, gray-gray overlap is an in-phase overlap, whereas a white-gray, gray-white overlap is an out-of-phase overlap.

The graphic below shows the two possibilities. The pictures show the two possible linear combinations of two *p* orbitals. One of the possibilities results in a bonding MO. Note that a bonding MO consists of two *p* orbitals overlapping in phase. The other possibility is the two *p* orbitals overlapping out of phase. The out-of-phase overlap results in an antibonding MO.

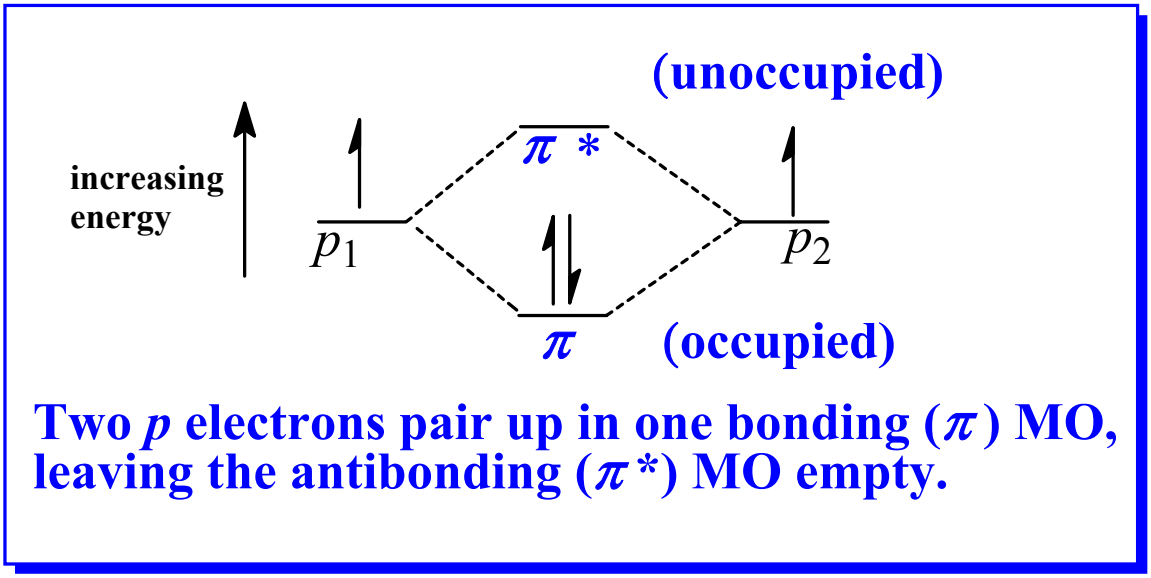


From this example, we can see the general principles that govern MO theory. When two atomic orbitals overlap, two molecular orbitals are formed. This is the principle of conservation of orbitals. We always make the same number of new molecular orbitals that we start with as atomic orbitals. In the case of ethene, we start with two *p* orbitals and make two new molecular orbitals. The orbital that results when two *p* orbitals overlap in phase is called a bonding MO and it is designated a π MO. The orbital that results when two *p* orbitals overlap out of phase is called an antibonding MO. Antibonding MOs are distinguished from bonding MOs by an asterisk. Thus, the antibonding MO of ethene is a π^* MO. Instead of drawing pictures of the orbitals, we can simply use the names of the orbitals to show how the new MOs are formed. We start with two unhybridized p orbitals and overlap them in the two possible ways to make two new molecular orbitals, one bonding and one antibonding. The graphic below shows the formation of MOs in ethene.

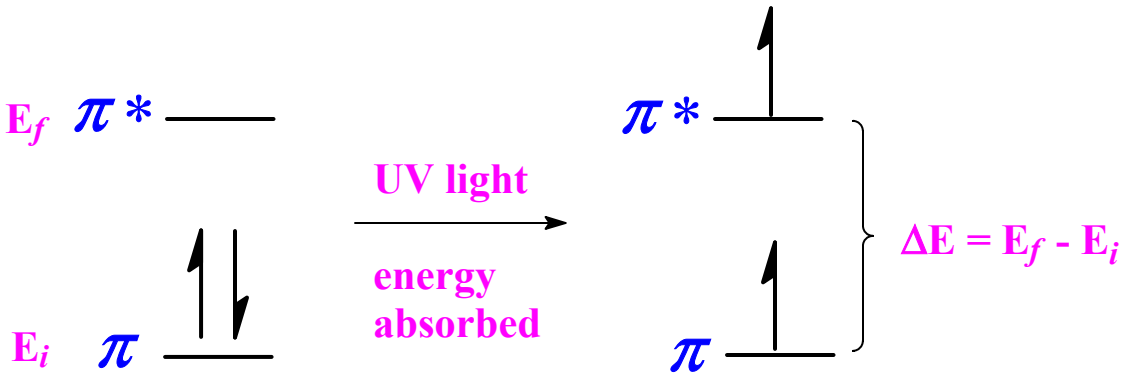


The diagram above shows how two *p* orbitals may overlap in two ways and make two molecular orbitals. This is the case when ethene forms. Thus, the above diagram represents the formation of the π bond in a typical alkene. In the following diagram, the orbitals are replaced by the electrons they contain. Each *p* orbital contains one electron, which is shown as a half-headed arrow. When these

electrons pair up to form a π bond they are found in the π bonding molecular orbital, and the newly formed antibonding (π*) molecular orbital is empty.



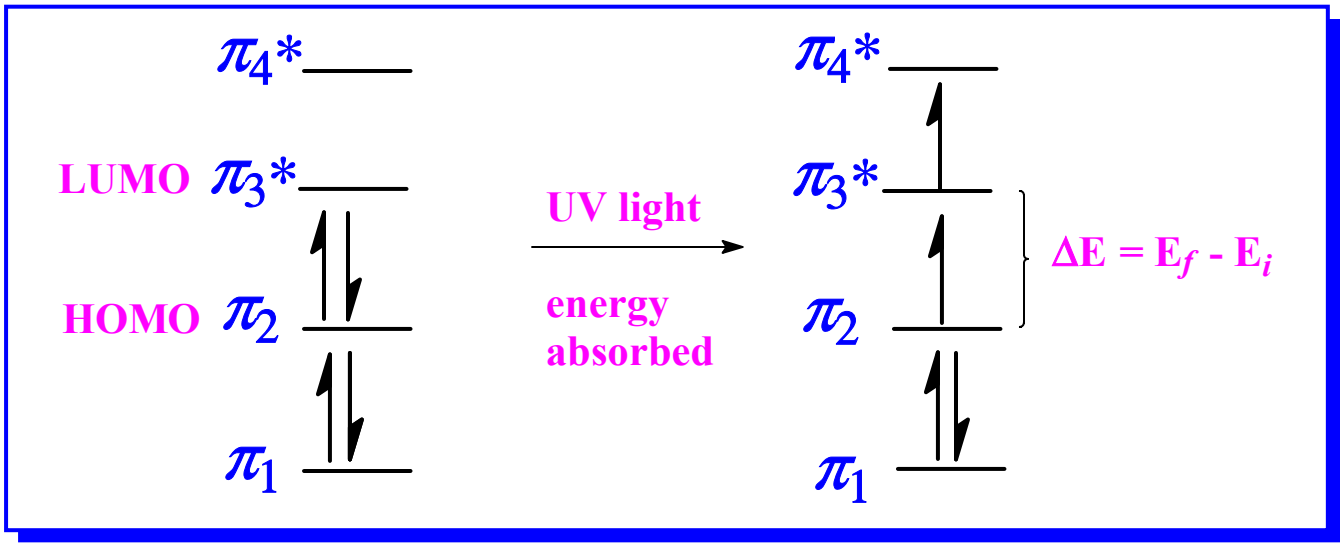
The molecular orbitals are described as occupied, meaning the orbital contains electrons, and unoccupied, meaning the orbital contains zero electrons. We see from the diagram that the occupied bonding MO is lower in energy (more stable) than the unoccupied antibonding MO (less stable). The energy diagram above is typical of all MO diagrams. There is a region in the diagram that separates an occupied orbital from an unoccupied orbital. These boundary orbitals have specific names. The highest-energy orbital that is occupied by electrons is called a HOMO, which stands for **highest occupied molecular orbital**. The lowest-energy orbital that is unoccupied by electrons is called a LUMO, which stands for **lowest unoccupied molecular orbital**. In MO theory the HOMO and LUMO orbitals are important because they define what is called the frontier or boundary between occupied and unoccupied orbitals. Accordingly, the HOMO and LUMO orbitals are referred to as ***frontier orbitals***. The most loosely held electron is found in a HOMO. For ethene, the HOMO is the π orbital and the LUMO is the π* orbital. When an electron moves from a HOMO to a LUMO in the case of ethene, it moves from the π orbital to the π* orbital. The transition of the electron from the π to π* orbital is called a π to π* transition. When UV light equal in energy to the difference in energy between the LUMO and HOMO is absorbed by ethene, one of its π electrons is excited from the π to π* orbital. Consider the absorption process for ethene. For a given molecule, two electrons are found in the same π bonding MO. Energy from UV light is required to move one of the electrons to a higher energy state, the π* MO. The diagram below shows the process.



The energy difference ΔE is the amount of energy from UV light that is necessary to cause a π to π* electronic transition.

In the case of ethene, the energy difference ΔE corresponds to a wavelength of 193 nm, which is outside the normal detection range of UV-Vis spectrophotometers. Thus, UV is not a good technique for detecting the presence of one isolated double bond under normal lab conditions. Because molecular oxygen also absorbs in the same region, samples must be degassed before a UV spectrum can be run even on machines that measure in the alkene region. The bottom line is that we shall use UV only in the 200-400 nm region. **A general principle is that conjugation of π bonds causes a shift to higher wavelengths or lower energies.** A shift to a longer wavelength because of a change in structure or change in solvent is called a ***bathochromic shift***, whereas a shift to shorter wavelengths is called a ***hypsochromic shift***. Hence, the bathochromic shift from alkene to conjugated diene makes the region 200-400 nm suitable for observing the presence of conjugated π systems in organic molecules.

Consider 1,3-butadiene or simply butadiene, which contains two double bonds. The two double bonds contain four p orbitals, which combine to make four MOs, two bonding and two antibonding, as shown below.

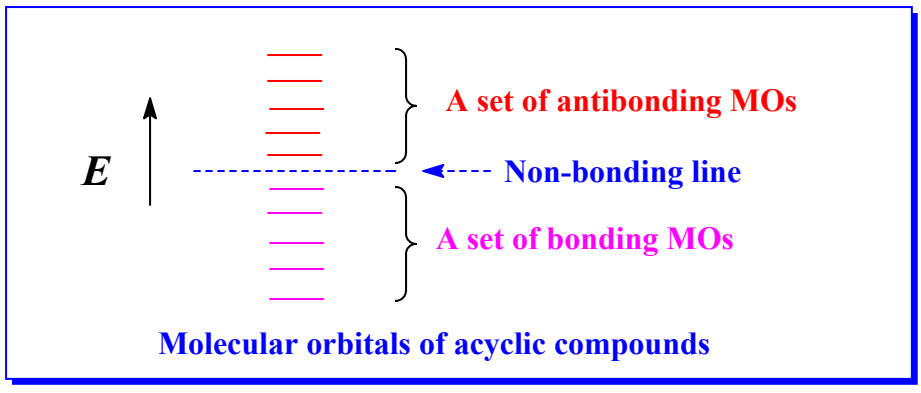


The energy required for the π to π* (π_2 to π_3*) transition is less for butadiene than for ethene, and the wavelength of maximum UV absorption is longer for butadiene than it is for ethene. The actual wavelength of maximum absorption for butadiene is 217 nm.

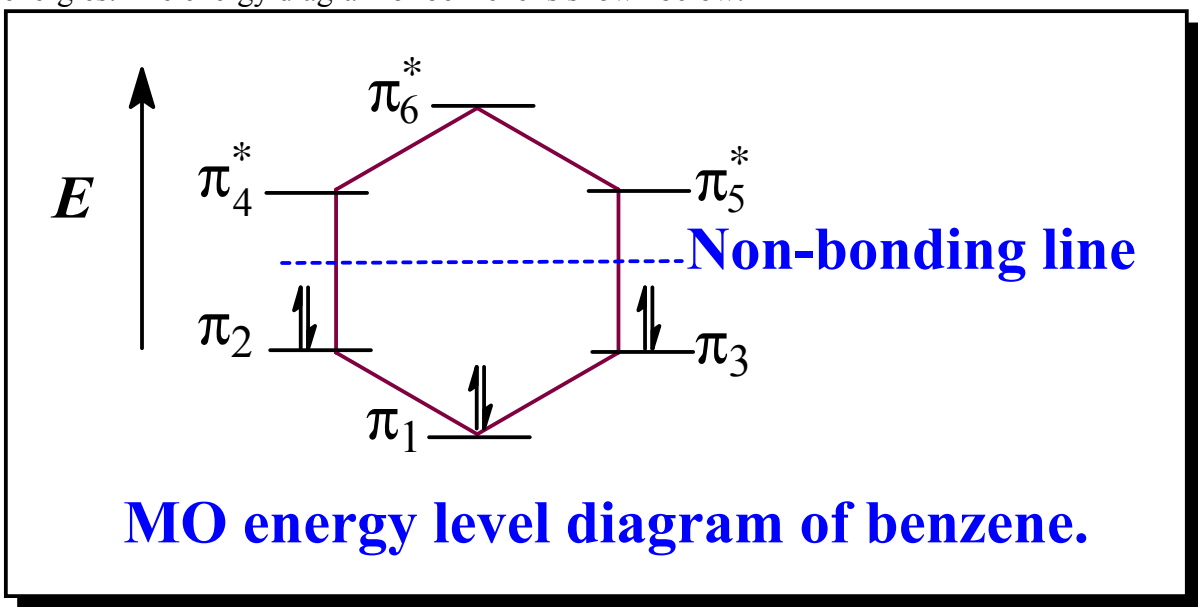
Example problem 1. Calculate ΔE for an electromagnetic wave of 217 nm wavelength.

Solution: The equation $\Delta E = h\nu = hc/\lambda$ gives $\Delta E = [(6.63 \times 10^{-34}\ \text{J sec})(3 \times 10^{10}\ \text{cm/sec})]/[(217\ \text{nm})(1\ \text{m}/10^{9}\ \text{nm})(100\ \text{cm}/1\ \text{m})] = 9.17 \times 10^{-19}$ J. This result is for one photon which equates to one molecule; this is a very small amount of energy indeed. To find the energy required to excite one mole of electrons (i.e., a sample of 6.02×10^{23} molecules), the above result must be multiplied by Avogadro's number.

Other acyclic compounds follow the pattern of ethene and butadiene. The number of *p* orbitals in the acyclic molecule determines the number of molecular orbitals, half of which are bonding MO's, starting with π_1, and half are antibonding. The energy of the orbitals increases with each additional orbital. The diagram below is a generalized set of MO's for an acyclic conjugated π system.



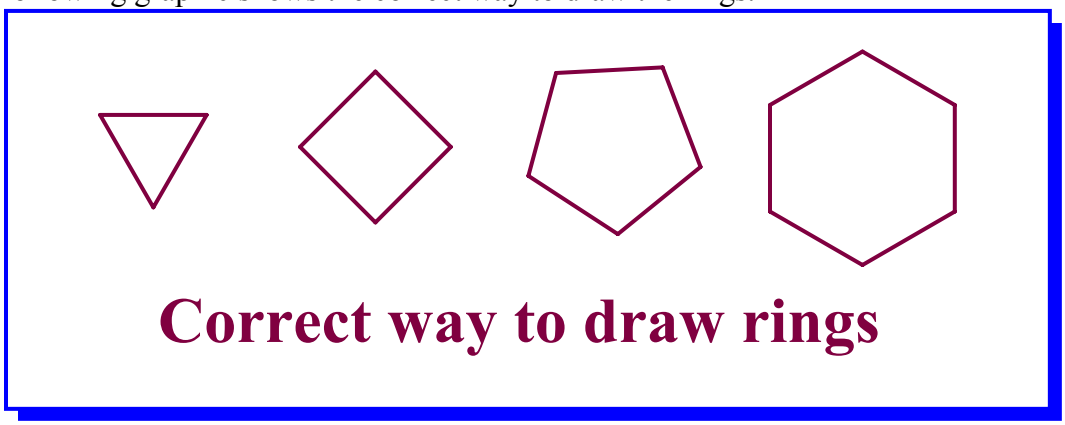
Cyclic Compounds The molecular orbitals in benzene are typical of cyclic conjugated π systems. As expected there are six molecular orbitals owing to the six *p* orbitals in benzene. However, the MO diagram for benzene and other cyclic compounds differ from those of acyclic compounds. Benzene has two sets of degenerate MOs. ***Degenerate*** means that two orbitals have identical energies. The energy diagram of benzene is shown below.



MO energy level diagram of benzene.

A π to π* transition in benzene would correspond to an electron going from π_2 to π_4^*. UV spectra are more complicated than indicated above, because molecules in a sample exist in a myriad of vibrational states so there is not one single discrete transition but rather a band of absorption, which produces an observed maximum.

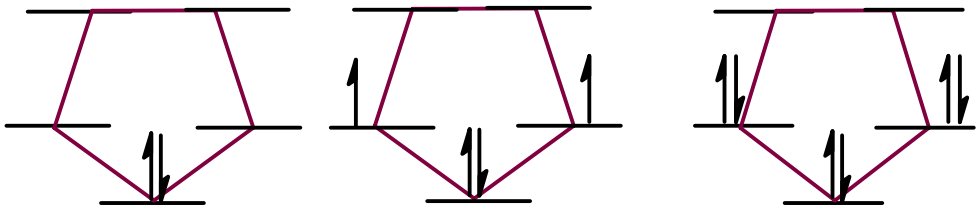
The energy-level diagrams of other annulenes can be drawn qualitatively in similar fashion to that of benzene by following a few simple rules. The ring is drawn with one carbon atom at the bottom. Each carbon in the ring corresponds to a MO. The MO's increase in energy from bottom to top. The following graphic shows the correct way to draw the rings.



Correct way to draw rings

Each corner represents a MO. The *p* electrons are placed in the MOs according to the ***aufbau principle***. That is, electrons go into the lowest energy MO first and fill orbitals by increasing energy. The order of filling also follows Hund's rule, one electron goes into each degenerate orbital of a

given energy before electrons pair up. Cyclopentadienyl carbanion, which contains six π electrons, is filled as shown below.

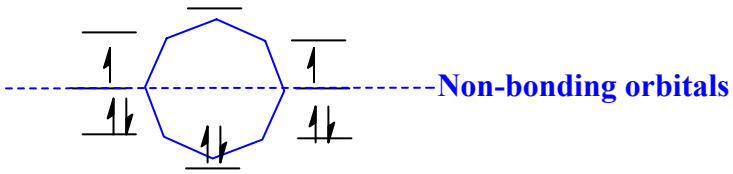


Degenerate orbitals fill by Hund's Rule

The use of a regular polygon to generate a MO diagram gives us a ***polygon rule***, which predicts the number and kinds of degenerate orbitals when the electrons are placed into orbitals at the corners of the polygon following the aufbau principle and Hund's rule. The polygon rule is particularly helpful in determining whether a cyclic compound is aromatic or antiaromatic. A compound with a filled set of bonding molecular orbitals is aromatic and a compound that contains electrons in non-bonding or antibonding molecular orbitals is anti-aromatic. Cyclic compounds that do not have a *p* orbital at each ring atom are non-aromatic.

Example Problem: Is cyclooctatetraene aromatic, non-aromatic or antiaromatic.

Solution: Cyclooctatetraene has 8 carbon atoms and 4 double bonds or 8 π electrons. The compound is either aromatic or anti-aromatic because it has a *p* orbital on every ring atom. The corners of an octahedron give us 8 molecular orbitals, which are filled by the aufbau principle and Hund's rule to give the diagram shown below.

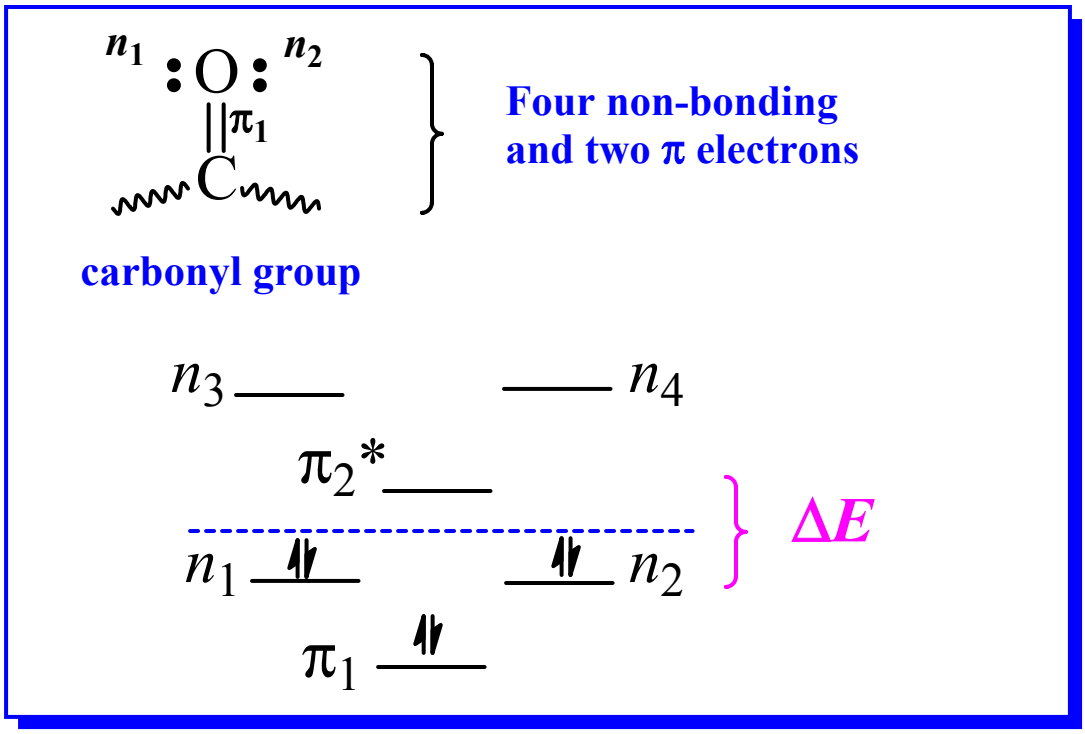


The unpaired electrons are found in non-bonding orbitals, making cyclooctatetraene anti-aromatic.

It is clear from the diagram that two unpaired electrons are found in non-bonding MOs; therefore, cyclooctatetraene is anti-aromatic.

Aldehydes and Ketones

The electronic transition in the UV observed for aldehydes and ketones arises from an *n* to π^* transition. The energy-level diagram below is a generalized one in which the orbitals for nonbonding and π electrons of a carbonyl group are shown.



The ΔE for an n to π^* electronic transition is less than a ΔE for a π to π^* transition, thus the molar absorptivity for aldehydes and ketones is less than that for conjugated systems. The insertion of a heteroatom next to the carbonyl such as the insertion of oxygen next to the carbonyl to make an ester or acid causes a hypsochromic shift in the UV. The resulting maximum absorption lies below 200 nm, making that absorption non-detectable by ordinary instruments. In many cases, the UV curve starts upward just at the tail end of the run. Such a curve, which goes up but forms no discernible maximum, is called ***end absorption***. Because most instruments scan from high to low wavelength, end absorption occurs near 200 nm and indicates the presence of a heteroatom next to a carbonyl. In most instances, end absorption indicates the presence of ester functionality in the compound.

Procedure:
Using the same techniques you used in the first UV lab period, collect UV spectra for five compounds in three different organic solvents. Note: use the quartz sample cells (cuvettes).

Lab Report # 8
UV Spectra of Nitroaromatics in Various Solvents

Student Name_____

Data: The path length for all analyses were _____ cm. Analyze your UV spectra and enter the appropriate data in Tables 1 through 3. Solvent # 1 = _____, solvent # 2 = _____, solvent # 3 = _____ .

1. **Table 1. Wavelengths of Maximum Absorption for Nitroaromatic Compounds in solvent # 1.**

Compound	**λ_{max} (nm)**
1.	
2.	
3.	
4.	
5.	

2. **Table 2. Wavelengths of Maximum Absorption for Nitroaromatic Compounds in solvent # 2.**

Compound	**λ_{max} (nm)**
1.	
2.	
3.	
4.	
5.	

3. **Table 3. Wavelengths of Maximum Absorption for Nitroaromatic Compounds in solvent # 3.**

Compound	**λ_{max} (nm)**
1.	
2.	
3.	
4.	
5.	

4. Using solvent 1 as the reference solvent, determine for each compound in Tables 1 and 2 whether the change in solvent resulted in a bathochromic, hypsochromic or no shift.

Table 4. Wavelength Shifts in Solvents 2 and 3.

Compound	**Solvent 2**	**Solvent 3**
1.		
2.		
3.		
4.		
5.		

5. What conclusion(s) can you draw from your data?

6. Report your data for compound 3 in solvent 3. See the "Summary of this Week's Lab" for the proper way to report UV data.

8 Homework

1. What is the ΔE in J/mol for UV radiation of 250 nm?

2. Under the name of each family listed below that gives a measurable UV spectrum, indicate the kind of transition that gives rise to the absorption (e.g., $n \rightarrow \pi^*$).

Aldehyde Ketone Alkene Arene

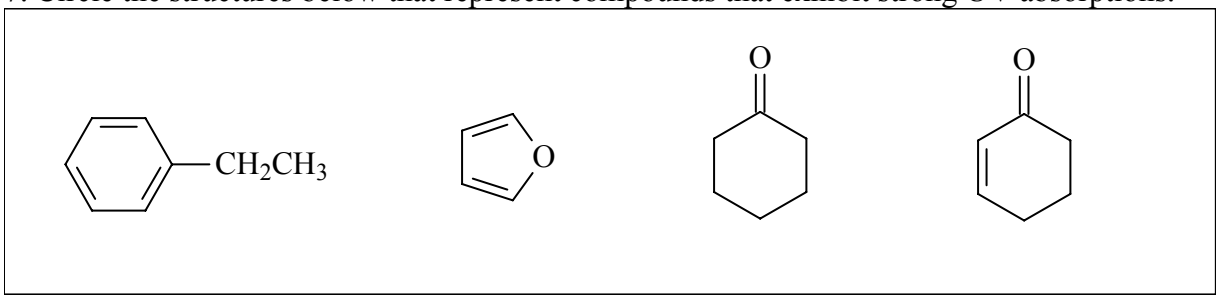
3. Draw a MO energy-level diagram for 1,3,5-hexatriene.

4. Draw a MO energy-level diagram for a cycloheptatrienyl carbonation.

5. Is either 1,3,5-hexatriene or the cycloheptatrienyl carbocation aromatic? If so, which one(s)?

6. Lycopene is the compound in tomatoes that causes them to be red. What kind of UV shift would account for the fact that a highly conjugated compound such as lycopene is colored?

7. Circle the structures below that represent compounds that exhibit strong UV absorptions.



8. A compound has a molar absorptivity of 22000. What concentration of the compound is required to give an absorbance of 0.50?

9. Two monocyclic compounds A and B of formula C_6H_{10} are constitutional isomers. One of them absorbs strongly in the UV and the other is transparent in the UV. Draw structures of the two compounds and indicate which one absorbs in the UV.

10. According to Hückel's rule, benzene is aromatic because it contains 6 π electrons which satisfy the $4n + 2$ constraints. Is Hückel's rule compatible with the MO theory?

9 Forensic Analysis by Visible Spectrometry

(A quantitative analysis of Salicylates in Blood by the Beer-Lambert equation[15])

A Summary of this Week's Lab During this lab period, you will conduct a quantitative analysis for salicylates (aspirin) in blood. Again, water will be used as a surrogate for blood, and aspirin will be determined as sodium salicylate. You will prepare a calibration curve as a plot of % transmittance (% T) on the vertical axis and concentration (C) in mg/dL on the horizontal axis, using the Excel program. You will the % T for an unknown and use the calibration curve to find the concentration of the unknown. Salicylic acid is a white solid, which reacts with dilute iron(III) nitrate (also called ferric nitrate) to produce a violet color. The intensity of the violet color is a function of the concentration of the salicylate. Therefore, the % T of the solution is directly proportional to the violet color or concentration of the salicylate. Because violet is in the visible region of the electromagnetic spectrum, we will be using the visible portion of the UV-Vis instrument. The first step is to determine the wavelength of maximum absorption. For the violet color, this wavelength is known to be 540 nm, which will be used throughout for this experiment. In the following paragraphs, we will conclude our study of the interpretation of ultraviolet spectra by studying the rules formulated by R. B. Woodward in the 1940's.

The Interpretation of UV Spectra

Historically, UV predates IR, NMR, and mass spectrometry as instrumental techniques. In general, these latter instruments give more information than UV. Hence, UV is not studied to the extent it once was by chemists. However, there are some interesting structure-absorption properties that are worthy of study by sophomore students. These are known collectively as Woodward's rules. They are empirically derived rules. That is they are based on the observartions of Professor Woodward. These rules will be developed, starting with the absorption observed for the simple diene system present in 1,3-butadiene.

Types of groups and the effect of substituents on the UV_{max}.

Two groups that are commonly encountered are called ***chromophores***—groups that absorb in the UV region of the electromagnetic spectrum and ***auxochromes***—groups that do not themselves absorb but do alter either λ_{max} or ε for a chromophore with which it is bonded in a molecule. As noted earlier, a shift in λ_{max} to a longer wavelength is called a *bathochromic shift*, and a shift to a shorter wavelength is called a *hypsochromic shift*.

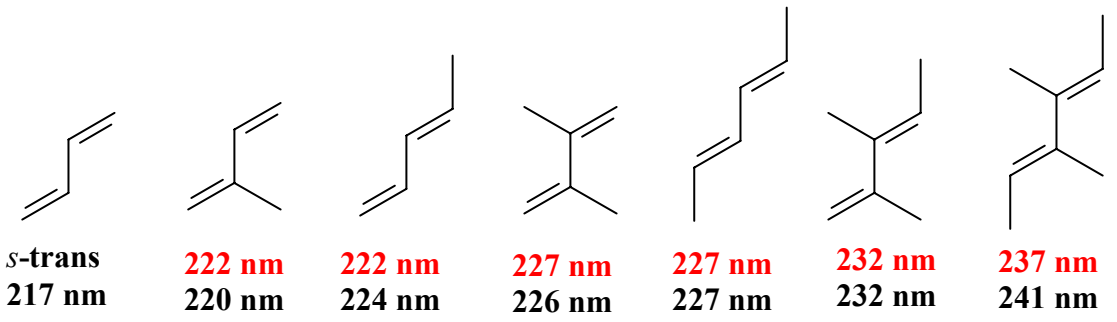
Dienes and Polyenes

A conjugated diene might exist in one of two major conformations, an *s*-cis conformation or an *s*-trans conformation. Recall that an *s*-cis conformation is required for the Diels-Alder reaction. A

[15] This experiment was modified from: Meloan, C.E, James, R.E, and Saferstein, R., Lab Manual for *Criminalistics: An Introduction to Forensic Science*, 7th ed.

compound that is free to rotate is considered to adopt the *s*-trans or more stable conformation for these calculations. In general, we start with a given structure and assign a base value for λ_{max}. Then, adjustments are made to the base value for each substituent we add to the conjugated system. The base value for 1,3-butadiene in the *s*-trans conformation is 217 nm. For each alkyl group bonded to 1,3-butadiene, we add 5 nm to λ_{max}. Figure 1 shows butadiene and six derivatives with the predicted and actual values of λ_{max}.

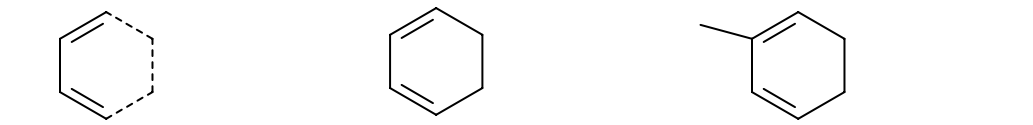
Figure 1 Acyclic 1,3-Butadienes



Actual λ_{max} in black, predicted value in red for conjugated dienes in *s*-trans conformations.

In the examples of Figure 1, all of the compounds can freely rotate to attain the *s*-trans conformation shown. Some compounds are locked into an s-cis conformation as shown in Figure 2. For six-membered conjugated cyclohexadienes the base value is 253 nm, plus 5 nm for each alkyl substituent.

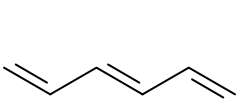
Figure 2 Conjugated Cycohexadienes



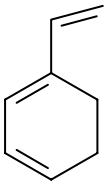
In most instances, a ring is needed to lock a diene into an *s*-cis conformation.

The presence of an additional double bond in conjugation with an existing diene causes a bathochromic shift of 30 nm for either conformation.

Figure 3 The Bathochromic Shift of Adding a New Conjugated Double Bond



base = 217 nm
extra db = 30 nm
predicted 247 nm
actual = 258 nm



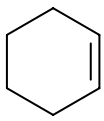
base = 253 nm
extra db = 30 nm
two alkyl = 10 nm
predicted = 293 nm

An extra conjugated db adds 30 nm (bathochromic shift).

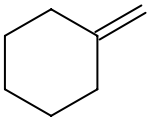
Exocyclic vs Endocyclic Double Bonds

Figure 4 shows two kinds of double bonds. In one double bond, both C atoms of the double bond are part of the ring; it is called an ***endocyclic*** double bond. In the other double bond only one C atom is part of the ring; it is called an ***exocyclic*** double bond.

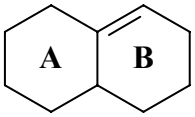
Figure 4 Endocyclic and Exocyclic Double Bonds



endocyclic double bond



exocyclic double bond



double bond exocyclic to ring A
endocylcic to ring B

The bathochromic shifts caused by various auxochromes are shown in Table 1 below.

Table 1 Bathochromic Shift of Various Groups

Group added to Base	Correction in nm
Conjugated double bond	30 nm
Alkyl group	5 nm
Exocyclic double bond	5 nm

Conjugated Aldehydes and Ketones

The same kind of empirical rules apply to conjugated aldehydes and ketones. The base value for an α,β-unsaturated aldehyde is 210 nm and for an α,β-unsaturated ketone is 215 nm. The general structures for a,b-unsaturated aldehydes and ketones are shown in Figure 5.

Figure 5 Conjugated Aldehydes and Ketones

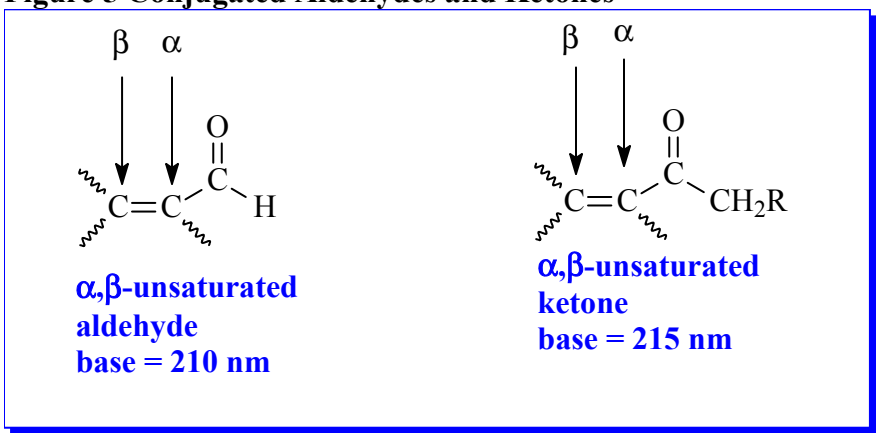


Table 2 shows the bathochromic shifts caused by the addition of alkyl groups at the α or β carbon atoms, an exocyclic double bond at the β carbon and an additional conjugated π bond.

Table 2 Bathochromic Effects in α,β-Unsaturated Carbonyl Compounds

Group	Correction (add)
α-alkyl substituent	10 nm
β-alkyl substituent	12 nm
Exocyclic double bond, β carbon	5 nm
Extended conjugation	30 nm

The strong UV absorptions in α,β-unsaturated aldehydes and ketones are due to π to π* transitions that start at 210 and 215 nm, respectively. For the *n* to π* or weak transitions that are also present, the absorption occurs between 280 and 300 nm.

Lab Preparations:

Stockroom Prepartions:
Stock solution of sodium salicylate: Add 5 drops of chloroform, a preservative, to a 1-L volumetric flask. Then, add 1.16 g of sodium salicylate. Dissolve the solid by the addition of water. Continue adding water to the 1-L mark on the volumetric flask.
Ferric Nitrate: Dissolve 1.0 g ferric nitrate in 99 mL of water to make a 1% solution of ferric nitrate. (Total volume = 100 mL of 1% ferric nitrate)
Nitric Acid I: Make 100 mL of 0.07 M nitric acid.
Dilute Ferric Nitrate: Mix 5 mL of 1% ferric nitrate with 4 mL of 0.07 M HNO_3 (nitric acid I) and label the container "dilute ferric nitrate."
Nitric Acid II: Make 100 mL of 0.039 M nitric acid.

Student Preparations:
1. Transfer 5.00 mL of stock solution to a 150 erlenmeyer flask and add 95.00 mL of water. This is your ***standard solution***. Its concentration is 5.0 mg/dL.

Procedure for Spectronic 20 instrument in the visible range.

1. Turn on the instrument and allow it to warm up for about 20 min.

2. Set the wavelength to 540 nm.

3. Depress the "mode" control key and set the mode to "transmittance."

4. Fill six cuvettes with varying amounts of your standard solution and adjust the volumes of each to 2.0 mL as shown in Table 1 below.

Sample	**Standard (5.0 mg/dL)**	**Distilled Water**	**Dilute Ferric Nitrate**	**Nitric Acid II (0.039 M)**
Blank	0.0	1.0	0.0	1.0
# 1	0.2	0.8	1.0	0.0
# 2	0.4	0.6	1.0	0.0
# 3	0.6	0.4	1.0	0.0
# 4	0.8	0.2	1.0	0.0
# 5	1.0	0.0	1.0	0.0

5. The zero transmittance will be determined with the sample holder empty. With the sample compartment empty and the lid closed, set the transmittance to zero by adjusting the on-off knob.

6. Determine 100 % transmittance (% T) for the blank. Place the cuvette all the way into the sample holder. Make sure the marking on the cuvette is aligned with the mark on the instrument. Adjust the on-off knob until the % T is 100%.

7. Replace the blank in turn with each of the numbered samples and determine the %T for each. Carefully record the % T as you go into your lab notebook.

8. Prepare your unknown for analysis by mixing 1.0 mL of the unknown with 1.0 mL of the dilute ferric nitrate solution.

9. Determine the % T for your unknown and record it in your notebook.

Lab Report #9
Quantitative Determination of Salicylate by Visible Spectrometry

Student Name_____

1. Prepare a calibration curve of % transmittance (% T) on the vertical axis and concentration (C) on the horizontal axis. Use the excel program for this purpose. Print out your calibration curve and append it to your lab report.

2. Use your calibration curve to determine the concentration of your unknown. The concentration of my unknown is _____ .
(Show on your calibration curve how you determined the concentration you found.)

9 Homework

You are given the following set of data.

Sample	**Standard (5.0 mg/dL)**	**% T**
Blank	0.0	00
# 1	0.1	20
# 2	0.3	30
# 3	0.5	40
# 4	0.7	50
# 5	0.9	60

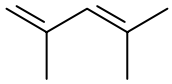
1. Determine the concentration of each sample.

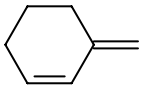
Sample	**Concentration (mg/dL)**
Blank	
# 1	
# 2	
# 3	
# 4	
# 5	

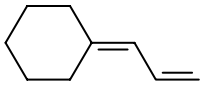
2. Prepare a calibration curve for the data set using excel and attach the curve to your homework.

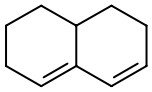
3. What is the concentration of a sample for which the % T is 35%?

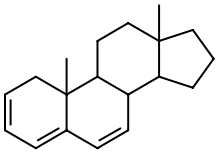
For each of the following compounds, estimate the wavelength of maximum absorbance in its UV spectrum. Apply the corrections shown in Tables 1 and 2 above to the base values for each compound.

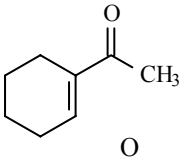
4. 

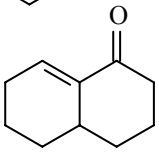
5. 

6. 

7. 

8. 

9. 

10. 

10 Introduction to High Performance Liquid Chromatography

(The Preparation of a Calibration Curve for Cocaine nee Caffeine[16])

A Summary of this Week's Lab During this lab period, you will be introduced to high performance liquid chromatography and its application to forensic analysis. You will learn how the instrument operates, what data you can get from it, and how to analyze the data to get qualitative and quantitative results. As your introduction to the instrument, you will be allowed to prepare and inject four samples. Each of these samples will contain a known but different concentration of cocaine (i.e., caffeine, which serves as a surrogate for cocaine). You will share your data with others so that you have four different concentrations. You will prepare a calibration curve using the data you get from the eight samples. Next week you will repeat these injections, only at that time you will inject a given concentration five times. Each student will inject a different concentration five times. The data will be tabulated and a precision and accuracy determination will be made so that plus and minus limits can be placed on your data. The third week you will be given a sample that contains an unknown concentration of cocaine (i.e., caffeine). You will determine the concentration by using the calibration curve from the previous week. Each student will have a different unknown sample.

Introduction to HPLC

In a typical column chromatography, a glass column is packed with a solid such as silica gel in the form of a slurry and is maintained wet (i.e., a liquid permeates the solid). The sample in solution is placed on the column and gravity flow causes the sample to traverse the length of the glass column. The sample is separated into its components according to the components' affinity for the solid in the column and the solvent in which they are dissolved. High performance refers to the use of a very small diameter solid in the column, which in this case is a stainless steel tube instead of glass. Because of the small-diameter solid, an applied pressure is required in order to get the liquid to flow through the column. Thus, as we shall use it, HPLC refers to a chromatographic separation in which a stainless steel tube is packed with a small-diameter solid, and a pressure is applied to force a sample dissolved in a solvent to pass through the solid and be separated into its components in the process. HPLC essentially refers to newer forms of chromatography that were made possible by the development of small-diameter particles for use in stainless steel columns.

Liquid Reservoirs

A given separation may use only a single solvent of constant concentration or a constantly changing concentration. An elution with a constant concentration solvent is called an ***isocratic separation***; whereas, a variable concentration separation is a called a ***gradient elution***. Bubbles of oxygen or nitrogen interfere with the procedure. Hence, the liquids used in HPLC must be degassed prior to use. The effect of gaseous bubbles is to cause the baseline to vary. The following picture shows how the four glass container reservoirs are arrayed on our HPLC.

[16] Gross, Shah and Jasarevic, 36th Annual Meeting of the Middle-Atlantic Region of the American Chemical Society, Princeton University, June 8-11, 2003, Paper Number 342.

Figure 1. Array of glass reservoirs.

A schematic of an HPLC is at the following web site. http://elchem.kaist.ac.kr/vt/chem-ed/sep/lc/hplc.htm

A schematic of an HPLC separation of venomous proteins is at the following web site.

http://ntri.tamuk.edu/hplc/hplc-animation.gif

Pumps

HPLC systems require pressures up to 6000 psi (pounds per square inch). Thus, pumps are required which can generate these pressures. Most HPLC systems use reciprocating pumps (i.e., one in which a back and forth motion is involved). Other types of pumps are displacement and pneumatic. Displacements pumps depend on a plunger to expel the liquid, and pneumatic pumps depend on a collapsible container that is activated by compressed gas.

Injection Ports

In most HPLC systems, including ours, the limiting factor for the precision of the instrument depends upon the reproducibility of the injection. That is, can you put the same amount of sample on the column each time? The reason this is difficult is because the sample sizes are very small. The volume injected on the column is about 500 µL. A syringe injection leads to about a 5% variability in precision. Thus, precision and accuracy studies should be part of any method involving HPLC.

The instrument

Figure 2 shows a picture of the HPLC in our lab. The instrument is controlled in much the same way as the gas chromatograph is controlled. The procedure is opened from the computer terminal and the parameters are adjusted.

Figure 2. HPLC system (HP-1100)

Startup procedure for the HPLC 1100

1. Turn on the diode array detector (DAD), the quaternary pump, and the vacuum degasser in the order listed by pressing the on/off button located in the lower left corner of each component.

2. Turn on the computer station, click on start, programs, HP ChemStations and select Instrument 1 Online. Wait until the configuration window appears, select ok and wait until the next window with the heading "Instrument 1 (online): Method and Run Control" appears.

3. At this point we have to set the parameters (flow rate, pressure, start-and stop time…) since each application requires different conditions. The various parameter settings for a given application (determination of cocaine in blood) make up the method. If a method exists for the desired application, we have to load the specific method.

4. Load the specific method by choosing method, and then load method. The new window "Load Method: Instrument 1" lists all methods available. Select the desired one and click ok.

5. Looking at the window we can observe small colored boxes indicating the status of the Quaternary Pump and the DAD. A red or yellow field means either that the component is not ready (red) or that it is turned off (yellow). Once all fields are green, we can proceed with the data collection.

6. In order to turn on the pump go to Instrument, More Pump, and click on the field next to on and ok. Now, all fields should turn green, including the green field in the upper-left corner that turns green after all other components are ready.

7. Check your parameters by looking at the window in which "LC Parameters" is selected. After all parameters are according to the application and, if no changes are necessary, move on with the data collection.

8. Changes in flow rate, stop time, solvents and pressure limits are made by selecting Instrument, and then Set up Pump. Changes in spectrum, time, signals and peak width are made by clicking on Instruments, and then Set up DAD signals. Ultraviolet and visible light lamps are turned off by selecting Instrument, and then More DAD. As you become familiar with the instrument you will discover that most of all of the parameters are accessible by clicking on the icons in the window.

9. In order to obtain accurate and precise results it is essential that the baseline (blue) is exactly on zero of the coordinate system. Once the parameters and the baseline are correct, we can proceed with the data collection.

Procedure:

Open the instrument as shown in the open and shut down procedures. Select the Caffeine (Surrogate Cocaine) Procedure. Adjust the parameters to the following conditions:

Column: 4 x 125 ODS Hypersil 5 µm, C_{18} column

Mobile Phase: A = HPLC grade acetonitrile 40%; B = HPLC grade water and methanol (1:1) 60%

Flow Rate: 1.0 mL/min

Elution: Isocratic

UV Detector: 270/20 nm; Reference: 360/80 nm standard cell

Run Time: 3 min

Injection Volume: 1.0 µL

After the instrument has been running approximately 15 min, and the pump has been primed inject a reference sample.

The reference sample will be 100, 200, 400, or 600 µg/mL

After the run, print out the results. Find the area (mAU's). Obtain the values for three other student runs and plot a calibration curve (linear regression); y = area (mAU) and x = concentration (ppm). Determine the values for y and R^2.

Lab Report #10
Calibration Curve for Cocaine

Student Name_____

Concentration of Sample_____

Area of Sample Elution _____

Data: Complete Table 1 by inserting your experimentally observed area for the concentration you ran. Then enter data from other students.

1. **Table 1 Concentrations vs Areas**

Concentration (µg/mL) (*x* variable)	**Area (mAU) (*y* variable)**
Remarks: Instrument Parameters:	

2. Use your graphing calculator to prepare a calibration curve from the data in Table 1. You will do this by running a linear regression on the data. Attach your curve to this report and turn it in with the homework problems.

3. In the space below, record your values for y and R^2.

y = _____

R^2 = _____

10 Homework

1. The HPLC data in Table 1 is for the purpose of preparing a calibration curve for cocaine assays. Standard solutions of cocaine were injected into an HPLC and the peak area of each standard solution was recorded. The concentration of cocaine is expressed as the number of milligrams (µg) of cocaine in 1.00 milliliter (mL) of water. Each area is expressed in area units (AU).

Table 1 Cocaine (i.e., caffeine) Standards

Area (AU)	Concentration (µg cocaine/mL)
200	200
340	400
520	800
760	1200

Enter the data from Table 1 into a TI-83 calculator. A manual titled "Data Handling and Analysis on the TI-82 and TI-83/83 Plus Graphing Calculators: A Resource for Science and Mathematics Students" is available in room 100, Chesapeake Hall. Enter the peaks areas in L_1 and the concentrations in L_2 of the calculator. Observe your data in the trace format on the calculator's screen. Then, conduct a linear regression analysis with diagnostic on and find the value of a, b, r^2 and r. Record the values of a, b, r^2 and r in the spaces indicated below.

a = _____ and b = _____

r^2 = _____ and r = _____

2. Using the values you obtained for a and b, convert the equation $y = ax + b$ from your calculator into an equation in which AU (peak area) replaces y, and C (concentration) replaces x. Round off the values of a and b to the corresponding number of significant figures in your data. The new equation is:

_____

3. Use your equation that shows concentration C as a linear function of peak height h to calculate values for the concentration of ethanol corresponding to each peak height recorded in Table 2. The GC operator must be approved/certified by the state, the machine must be calibrated on a frequent basis and a record of the calibration maintained for review by inspectors. The instrument must be maintained and a maintenance log kept. The analyst should participate in frequent blind tests to demonstrate and maintain proficiency.

Table 2 Date: 10/26/02; Operator: RAG; GC Number: 02; Other:

Run	Time	Lab Number	Forensic Id. Number	Experiment Peak Area (AU)	Calculated Concentration (µg/100 mL)	Remarks
1	0815	BA-2144	CID-5R-02544	420		PGCC Campus Police
2	0830	BA-2145	FBI-DC-13	860		

4. Define isocratic elution:

5. Define gradient elution:

6. List two advantages of HPLC over a gravity chromatographic separation.

7. What happens if bubbles of oxygen or nitrogen get into an HPLC sample?

8. How did your HPLC detect the sample as it emerged from the column?

9. Give a literature reference in which experimenters used HPLC in a forensic application.

10. What is the difference between a displacement and a pneumatic pump?

11 Explorations in High Performance Liquid Chromatography

(Precision and Accuracy Studies)

A Summary of this Week's Lab You learned last week how to run a sample on a HPLC instrument and how to prepare a calibration curve. In this week's experiment, you will learn how precision and accuracy come into play. In brief, accuracy is how well your results compare to a value that is known or assumed to be correct. Precision refers to how tight your data is; that is, how reproducible it is. To establish the precision and accuracy of your method, it will be necessary to make several runs. The more runs you make, the better your results will be. That is because the precision and accuracy equations that we use are based on probabilities or statistical performance. Thus, the more times you flip a coin, the more likely it is that you will get half heads and half tails. Likewise, the more times you run a sample, the more likely it is that your average value will reflect a true average, etc. In lab, you will make as many runs as you can in the allotted time. You will then apply the statistical equations that we cover in the lecture to your data. The result will be that a plus (+) and minus (–) can then bracket your results. For example, a previous result of 3.0 might be written 3.0 ± 0.1.

Introduction

Many times in chemical analyses, statistics enter into a calculation. For example, when a compound contains bromine, we automatically use the weighted average of the ^{79}Br and ^{81}Br isotopes that make up what we call bromine's atomic weight. Since these two isotopes occur in a near 1:1 ratio, the average mass is near 80 grams/mole, which is what we use in our calculations. We can use 80 because on a statistical basis the averages will prevail. That is, when we have Avogadro's number of bromine atoms, we can be certain that the averages hold. If we flip a coin a million times, the same statistical advantage will accrue. However, it is quite possible to flip two or three heads consecutively. Therefore, the same kinds of statistics do not govern a limited number of trials that govern an almost unlimited number of trials. For this reason, scientists have developed methods for taking a small number of trials into account. These considerations lead to two important questions, especially for forensic analyses.

(1) How certain are you that your results are true (accurate)?
(2) How reproducible are your results (can someone else repeat your procedure and get the same results--precision)?

Why are these questions important? A small mistake can lead to an innocent person being convicted of a crime, which the person did not commit. The inability of someone else being able to repeat your procedure could lead to a guilty person being freed from jail because the defense's team could not repeat your examination of the evidence. Thus, it is particularly important in crime labs that procedures are well documented and validated. Validation is important for individual analysts, for units of people within a given laboratory, and for groups of laboratories. Sometimes labs will participate in blind studies in which various labs get samples from the same bulk sample for analysis and report their results to an authenticating agency.

Error Analysis

For every procedure you carry out, you should identify where errors can occur and evaluate the magnitude of these errors. Each step in an analysis is a potential source of error. ***Sources of error*** include each and every measurement or procedure the analyst uses. A special source of error is cross contamination. ***Cross contamination*** occurs when any part of one's analytic procedure comes in contact with some part of a distinctly different procedure or analysis, and unwanted material is transferred into the analytical procedure. The result of cross contamination is that the true result is altered by the contaminant from the other procedure. One way to build one's confidence that a procedure works is to repeat the procedure a number of times and get the same result each time. The more times you repeat the procedure with the same results, the more confident you become that the procedure works. When you repeat a procedure exactly the way you ran it the first time, you replicate the procedure, or you run ***replicate samples***. A replicate always comes from the original sample. Thus, if you are given a sample of cocaine and analyze the sample three times by the same procedure, you have run three replicates.

Arithmetic Mean

Let's say you run an HPLC assay for cocaine (i.e., caffeine) and you run the procedure five times and get the results shown in Table 1 below.

Table 1. Concentration of Cocaine in a Forensic Sample

Sample	Concentration (μg/mL)
1	201
2	198
3	205
4	196
5	202

What value do we report for the five replicate samples? Over many years, statisticians have learned how to analyze data of this kind in order to report the most meaningful data. A series of statistical tests must be performed on data in order to arrive at the best way to report the results. The arithmetic mean is a more meaningful result that an isolated value. The arithmetic mean for a set of *N* test runs is the sum of all test results divided by *N*. For the set of five runs in Table 1, the arithmetic mean, also called simply the mean or average, is given by (201 + 198 + 205 + 196 + 202) divided by 5 or 200.4, which rounded to three significant figures gives 200 μg/mL.

Range of Values

The ***range*** of measurements is the highest value minus the lowest value. For the data in Table 1, the range is 205 -196 = 9 μg/mL.

Deviation from the Mean

Once a mean value is established (e.g., 200 μg/mL in the example), then the deviation from this value may be obtained for each value in the data set. The ***deviation from the mean*** is found by subtracting the mean value from the individual value. Table 2 shows the deviation from the mean for each value in Table 1.

Table 2. Deviation from the Mean for Concentrations of Cocaine in a Forensic Sample

Sample	Concentration (μg/mL)	Deviation from the mean (*d*)
1	201	201 – 200 = + 1

2	198	198 – 200 = - 2
3	205	205 – 200 = + 5
4	196	196 – 200 = - 4
5	202	202 – 200 = + 2
$N = 5$	Sum = 1002 Mean = Sum/N = 200	Average deviation = d_{ave} d_{ave} = 14/5 = 2.8

Please note that calculations of the deviation from the mean give positive values when the measured value is more than the mean value (200) and is negative when the measured value is less than the mean value. Analytical chemists typically use capital letters to refer to measured values and lower case letters to refer to calculated values. Since the deviation from the mean is a calculated value, it is represented by the lower-case letter *d*.

Variance

The ***variance*** of a set of numbers is found by squaring each deviation from the mean, adding the squares, and dividing the sum of the squares by N minus one.

Table3. Variance

Sample	Deviation from the mean (d)	Square
1	201 – 200 = + 1	1
2	198 – 200 = - 2	4
3	205 – 200 = + 5	25
4	196 – 200 = - 4	16
5	202 – 200 = + 2	4
$N = 5$	$N - 1 = 5$	Sum = 50 Variance = 50/4 =12.5

Standard Deviation

The ***standard deviation*** is simply the square root of the variance. For the above example, the variance is 12.5 $(\mu g/mL)^2$, so the standard deviation is $(12.5)^{1/2}$ or 3.5 µg/mL. The standard deviation has the same units as the values in the set of numbers. In this case, the units are micrograms per milliliter.

Relative Standard Deviation

The ***relative standard deviation*** is the fraction defined by the standard deviation divided by the mean. The fraction for the example above is 3.5/200 or 0.018. Because the standard deviation and mean have the same units, their quotient is unitless. Thus, relative standard deviations have no units. Relative measures are ratios and are unitless; whereas, absolute measures have units.

Percent Relative Standard Deviation

When the relative standard deviation is expressed as a percentage instead of a fraction, the result is called the ***percent relative standard deviation***. Thus, the percent relative standard deviation for the example is 1.8%.

Precision

The ***precision*** of a set of measurements relates to the scatter or spread of the values. When the set of values is tightly bunched, the precision is high; when the set of values is spread over a wide range of values, the precision is low. Precision is distinctly different from accuracy.
Figure 1 shows two sets of data, one set of high precision and one set of low precision.

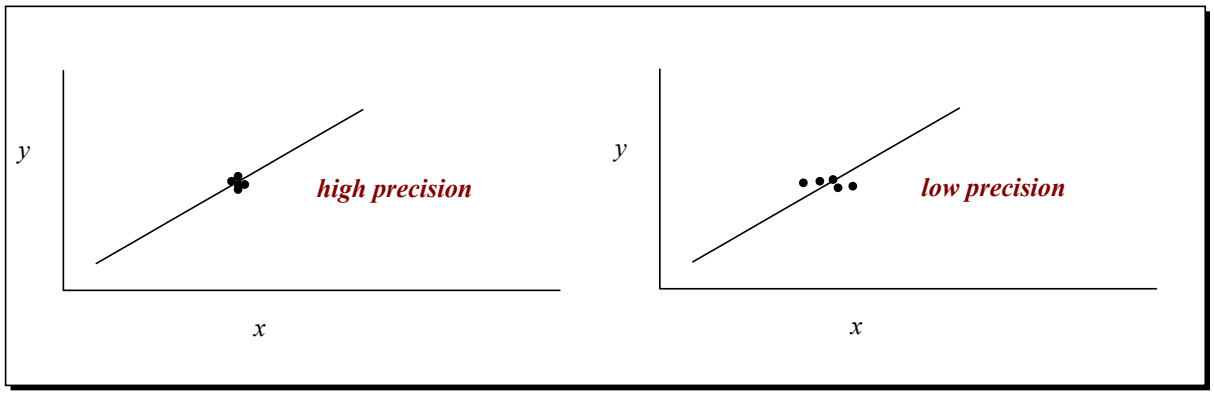


Figure 1. Two plots of data.

Accuracy

The ***accuracy*** of a series of measurements is a comparison of the average value to the true value. When the mean value is near the true value, the accuracy is high. When the mean value is remote from the true value, the accuracy is low.

Two Kinds of Errors

A ***systematic error*** (determinate error) is constant for every analysis because it is inherent in the procedure. A ***random error*** is different every time. The standard deviation is a measure of random errors.

Confidence

The word confidence implies assuredness. How sure are we that our analysis is in the ball park? The confidence limit is a quantitative measure of this assuredness. We shall use the relationship shown in Equation 1 as our definition of confidence for a 95% probability of being correct. When applied to a set of data, Equation 1 gives us the plus and minus values alluded to in the summary of this week's experiment.

$$\text{Meaurement} = \text{Mean} \pm (t/N^{1/2}) \text{ times standard deviation} \qquad (1)$$

The term $(t/N^{1/2})$ is a statistical factor that relates to the fact that confidence improves with the square of the number of trials. Thus, to double our confidence for any number of runs, we must quadruple the number of runs. We shall generally make four or five runs and use the associated value of (t/N^{12}) for four or five runs. When $N = 4$, then $(t/N^{1/2}) = 1.59$ and when $N = 5$, then $(t/N^{1/2}) = 1.24$. For the example, the mean was 200 µg/mL, and the standard deviation was 3.5 µg/mL. Therefore, our 95% confidence limit for four runs is $200 \pm (1.59)(3.5) = 200 \pm 6$ µg/mL. For five runs, the 95% confidence limit is $200 \pm (1.24)(3.5) = 200 \pm 4$ µg/mL. This gives us a feeling of how increasing the number of runs or trials lowers the uncertainty of the result. A table of *t* values is given on page 809 of your text (Rubinson and Rubinson) for various probability percentages.

For you experiment, you will make four or five runs, depending upon the time available. Then you will calculate the confidence limit at the 95% probability level for your data.

Procedure

1. Startup the HPLC using the local procedures.

2. Run four or five trials with the standard sample of cocaine (i.e., caffeine) you are given.

3. Use Equation 1 to calculate the 95% confidence limit for your number of runs.

4. Complete the lab report and homework and turn them in next week.

Lab Report #11
Confidence Limits for Cocaine Determination

Student Name_____

Data: Complete Table 1 by inserting your experimentally observed area for the concentration you ran. Use the equation from last week's experiment to calculate the concentration of each sample. Use the value given to you by the instructor as the true value and make the statistical calculations shown below, ending with the 95% confidence limits.

1. **Table 1 Concentrations vs Areas**

Concentration (µg/mL) (*x* variable)	**Area (mAU) (*y* variable)**

2. What is *N* for your data? *N* = _____

3. What is the mean value for your concentration, including units? _____

4. What is the range of values for your data? _____

5. In Table 2 below find the deviation from the mean for each of your concentration values.

Table 2 Deviation from the Mean

Concentration (µg/mL)	**Deviation from the Mean (*d*)**

6. Find the variance for your data in Table 2.
Variance = _____

7. Find the standard deviation from your variance.
Standard Deviation = _____

8. and 9. Find the relative standard deviation from your mean and standard deviation.
Relative Standard Deviation = _____
Percent Relative Standard Deviation = _____

10. According to Equation 1, what is your concentration to the 95% confidence level?
Concentration = _____

11 Homework

Table 1 contains data for the HPLC analysis of a forensic cocaine (i.e., caffeine) sample.

Table 1. Concentration of Cocaine in a Forensic Sample

Sample	Concentration (µg/mL)
1	206
2	200
3	205
4	198
5	207

1. Calculate the mean or average value for the set of data in Table 1, including units.
Average = _____

2. What is *N* for these data? *N* = _____

3. What is the mean value for the concentration, including units? _____

4. What is the range of values in the data? _____

5. In Table 2 below calculate the deviation from the mean for each concentration.

Table 2 Deviation from the Mean

Concentration (µg/mL)	**Deviation from the Mean (*d*)**

6. What is the variance for the data?
Variance = _____

7. What is the standard deviation?
Standard Deviation = _____

8. and 9. Find the relative standard deviation from your mean and standard deviation.
Relative Standard Deviation = _____
Percent Relative Standard Deviation = _____

10. According to Equation 1, what is your concentration to the 95% confidence level?
Concentration = _____

12 A Forensic Application of HPLC

(The Determination of Cocaine nee Caffeine in a Cola Sample)

A Summary of this Week's Lab In the first experiment in this series, you prepared a calibration curve. In the second experiment, you put precision and accuracy bands on your calibration curve. Now, you are ready to conduct an experiment in which you use your calibration curve, and report the results with the precision and accuracy bands allowed by the relevant data. You will be given a forensic sample, which is suspected to contain cocaine. The sample is a cola. You will perform an HPLC analysis on the sample in the same way you performed analyses in the previous two labs. You will find the area generated by your unknown and calculate its concentration from your calibration curve or the linear equation you get from the linear regression analysis of your calibration-curve data. You will report your answer in µg/mL of cocaine with the confidence levels found at the appropriate concentration in the previous lab.

Procedure:

1. Obtain a sample that is suspected of containing cocaine (i.e., caffeine) from the instructor.

2. Run the sample in exactly the same way you ran the standard samples during the previous two laboratory periods. Run as many replicate samples as allowed by the available time.

3. Complete your lab report and homework.

Lab Report #12
Determination of Cocaine (i.e., caffeine) in a Cola

Student Name_____

Record your data in Table 1 and then make the statistical calculations necessary to report the concentration to the 95% confidence level.

1. **Table 1 Concentrations vs Areas**

Concentration (µg/mL) (*x* variable)	**Area (mAU) (*y* variable)**

2. What is *N* for your data? *N* = _____

3. What is the mean value for your concentration, including units? _____

4. What is the range of values for your data? _____

5. In Table 2 below find the deviation from the mean for each of your concentration values.

Table 2 Deviation from the Mean

Concentration (µg/mL)	**Deviation from the Mean (*d*)**

6. Find the variance for your data in Table 2.
Variance = _____

7. Find the standard deviation from your variance.
Standard Deviation = _____

8. and 9. Find the relative standard deviation from your mean and standard deviation.
Relative Standard Deviation = _____
Percent Relative Standard Deviation = _____

10. According to Equation 1, what is your concentration to the 95% confidence level?
Concentration = _____

12 Homework

Define or explain what is meant by each of the following terms.

1. Cross contamination

2. Mean value of a data set

3. Determinate error

4. Range of values

5. Deviation from the mean

6. Variance

7. Standard deviation

8. Relative vs absolute standard deviation

9. Precision

10. Accuracy

11. Confidence

13 Introduction to Gas Chromatography-Mass Spectrometry (GC-MS)

(Identification of Alkanes Commonly Found in Gasoline)

A **Summary of this Week's Lab** During this lab period, you will be introduced to a method of analysis in which two instruments are linked. You will inject various compounds that might be found in gasoline into a gas chromatograph. The samples will be both pure hydrocarbons and also mixtures of hydrocarbons. The GC is a HP 6890 series instrument just like the one you used for the blood-alcohol analysis. Thus, the sample handling and injection will be done with a syringe, except in this case an auto-injector will be used. The function of the GC is to separate the hydrocarbon mixtures into their component compounds, so they can be analyzed as pure compounds by the mass spectrometer. Because gasoline contains many hydrocarbons in the C_6-C_{10} range, we will focus on analyzing hydrocarbons whose numbers of carbon atoms fall within this range. As each compound emerges from the GC column, it will be split into two samples. The GC will detect one sample, and the other sample will be directed into the mass spectrometer where it will be ionized and excited. As the excited molecular ions lose energy, they will fragment into pieces. The positive-ion fragments will be detected by the MS's transducer and sent electronically to the data station. The output from each compound will be a mass spectrum. You will analyze the spectra of pure compounds and then compare the spectra of the pure compounds with those found in the mixture. You will determine the composition of your mixture by comparing the mass spectra of each compound with a reference spectrum obtained on the same instrument and under the same conditions as you used to obtain your spectra. Once you have correlated actual structures with mass spectra, you will also attempt to correlate the compounds with their order of emergence from the GC. You will complete the report for this lab period and turn it in at the beginning of the next lab period.

Forensics Uses of Gas Chromatography/Mass Spectroscopy (GC/MS)

The number of applications of GC-MS is vast and growing every day. Therefore, only a few important forensics applications will be mentioned. The application of most interest to us is in the area of arson investigations. Because the technique lends itself to the identification of small quantities of compounds in the gaseous state, it is one of the best techniques for identifying volatile remnants from a fire. Another important application is the identification of drugs (and their metabolites) of abuse in blood, urine, and saliva. In addition, any information this technique offers to a general chemist might also be of interest to a forensics chemist. For example, the determination of the structure of an organic compound, the identity of components of a thin-layer chromatogram, and pesticide analyses are applications that might be of forensic interest.

General Considerations of Mass Spectrometry

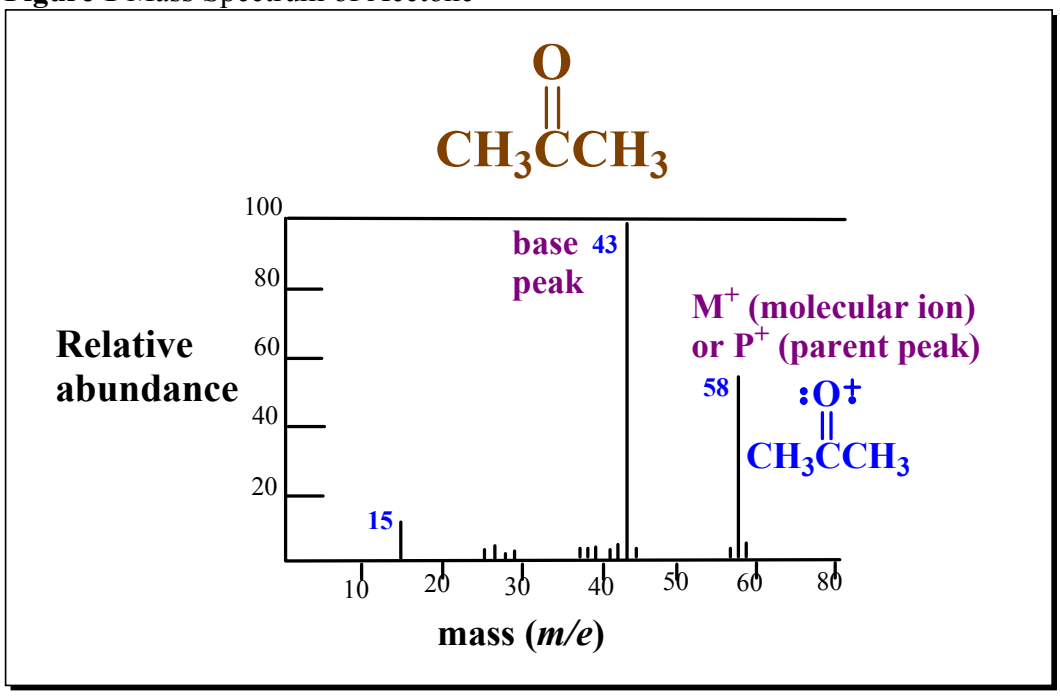
There are two types of mass spectrometry, atomic and molecular. We will consider only molecular mass spectrometry. As the name implies, mass spectrometry is a technique that measures masses. In molecular mass spectroscopy, molecules from a sample are ionized. The ions break apart into fragments. If a fragment is a charged particle or ion, it will have a mass to charge ratio (m/z). When the charged fragment passes through a magnetic field, the field causes the ion's path to bend with a radius of curvature that is a function of m/z. The fragment impinges on a transducer that converts the m/z into an electronic signal that is amplified, sent to a data station and plotted along with the m/z's of other fragments to give a spectrum of m/z's. If z =1, then m/z = the mass of the fragment. Hence,

the technique can give us valuable information about the structure of the molecule by the initial ionization and subsequent fragmentation. The fragmentation pattern is somewhat like a fingerprint of the molecule's structure. In this laboratory period we will focus on the interpretation of mass spectra.

Important Terms

Carbon-12 is the reference standard for all atomic masses; one mole of carbon-12 has a mass of exactly 12.0000 grams. Carbon-12 has one isotope, carbon-13. ***Isotopes*** differ by the number of neutrons in their nuclei. Hence, isotopes are chemically the same because their electronic structures are the same. A mass spectrometer distinguishes fragments of differing isotopic composition by their different mass to charge ratios. A mass spectrum reflects the natural isotopic abundance of atoms in a sample of molecules. Since carbon-13 constitutes about $1/100^{th}$ of all carbon atoms, fragments containing one ^{13}C atom will show up one *m*/*z* unit higher than the corresponding fragment containing only ^{12}C atoms. The intensity of the peak for the ^{13}C-containing fragment will be tiny compared to the peak for the ^{12}C-containing fragment. The ***intensity*** of a given peak reflects the number of fragments having that *m*/*z*. High-resolution mass spectrometers measure masses very precisely, up to many decimal places. The presence of isotopes gives rise to several different molecular weights. The ***integral molecular weight*** is the molecular weight of a compound comprised of the lowest-mass isotopes of all elements in the molecule. The average or ***chemical molecular weight*** is a weighted average that includes all isotopes, according to their natural abundance. The ***nominal mass*** is the whole number one gets by rounding a decimal. The mass spectrum of acetone in Figure 1 will be used to describe other terms.

Figure 1 Mass Spectrum of Acetone



Relative abundance refers to the number of ions with a given mass/charge ratio. The peak in the spectrum of the ion with the largest relative abundance is called the ***base peak***. The base peak serves as the reference peak. The intensity of the base peak is arbitrarily set at 100, and the intensity of

every other peak in the spectrum is found as a percentage of the base peak. In Figure 1, the base peak is the peak at *m*/*e* 43 (the electronic charge may be represented by *e* or *z*). Likewise, the peak at *m/e* 58 has a relative abundance of approximately 50%, because its height is approximately 50% that of the base peak. The ***relative abundance*** is read as a percent in terms of the base peak. Thus, the peak at *m/e* 15 has a relative abundance of about 15%.

It is generally true that modern mass spectrometers are designed so that only singly charged ions are recorded. When the charge in the mass/charge ratio is one, the *m/e* simply becomes the mass of the fragment. Thus, we often just refer to the fragments by their mass when we really mean mass/charge. We can do that because the numbers are the same, and it simplifies the discussion. With some exceptions, the largest significant mass that we find in a spectrum is called the molecular ion. The ***molecular ion*** is the radical-cation that forms when an electron is ejected from an intact molecule. The molecular ion for acetone is shown in Figure 1; it has a mass of 58. For our purposes, the mass of the molecular ion is the molecular weight (molar mass) of the molecule. The terms molecular ion and parent ion may be used interchangeably; they both refer to the mass of the radical-cation formed from the initial ionization of the molecule. The base peak at *m/e* 43 arises from the fragmentation between one of the methyl groups and the carbonyl group in acetone. Often, by subtracting the mass of a fragment from the mass of the molecular ion, one gets the *m/e* of a significant peak in the spectrum. In the case of acetone, the subtraction of the mass of a methyl group (15) from M^+ (58) gives the base peak (43).

Tips on the Interpretation of Mass Spectra
Like any other property of a compound, a mass spectrum is a result of the structure of the compound's molecules. Thus, various partial structures reveal themselves in a mass spectrum in various ways. Some of the obvious correlations between mass spectral features and structural features are presented below.

The Nitrogen Rule
If a compound has an odd number of N atoms (1, 3, 5, etc.) in its structure, it will have an odd-number molecular weight. Thus, ***if a molecular ion M^+ is an odd number, the compound must contain an odd number of N atoms.***

Isotopes of Chlorine and Bromine
Chlorine has two isotopes Cl-35 and Cl-37 in a three to one ratio. Thus, in a sample of a compound that contains one chlorine atom, such as chloromethane, 75% of the molecules contain Cl-35 and the other 25% contain Cl-37. This distribution is reflected in the mass spectrum in the ratio of peak heights for the respective molecules. Chloromethane has the formula CH_3Cl, so molecules that contain Cl = 35 have a mass $M^+ = 50$, and the molecules with Cl-37 have a mass $M^+ = 52$. The $M^+ = 50$ peak will be three times more intense than the $M^+ = 52$ peak, reflecting the isotopic distribution in the molecules. Thus, there are actually two M^+ peaks; they are two mass units apart in a 3:1 ratio of peak heights. ***When a mass spectrum displays two M^+ peaks, in a 3:1 ratio and two mass units apart, the compound must contain one chlorine atom.***

Bromine has two isotopes (Br-79 and Br-81) in a near 1:1 ratio. ***When a mass spectrum displays two M^+ peaks, in a 1:1 ratio and two mass units apart, the compound must contain one bromine atom.***

Compounds containing multiple chlorine and/or bromine atoms.
When a compound contains more than one chlorine and/or bromine atoms, it is possible to determine how many atoms of each kind are present in the molecule from the number of M^+ peaks and their relative intensities, providing all of the molecular-ion peaks are visible. For example, if we encounter a spectrum with three M^+ peaks in a 1:2:1 ratio and two mass units apart, the compound contains two bromine atoms. Likewise, if there are three M^+ peaks in 9:6:1 ratio and two mass units apart, the compound contains two chlorine atoms. Thus, the key to the presence of isotopes is multiple M^+ peaks that are two mass units apart. The ratio of the peak heights tells us how many of each multi-isotope atom we have. A methodology for finding the isotopic distribution from the shape and intensity of the M^+ ions is given below.

M^+ for Br and Cl Isotopic Distributions

1. The isotopic abundances of Cl-35 and Cl-37 are 3:1, and of Br-79 and Br-81 are 1:1.

2. To find the ratio of peak heights for more than one of these atoms per molecule, set up a binomial expansion by letting a = the light isotope and b = the heavy isotope.

3. For example, for CH_2Cl_2, let a = Cl-35 and b = Cl-37. Then the binomial becomes 3a + 1b. There are two chlorine atoms, so square the binomial. $(3a + 1b)^2 = 9a^2 +$ 6ab $+ 1b^2$. These coefficients are the relative peak heights of M^+. That is, when you have two Cl atoms in a molecule, the peak heights are in the ratio 9:6:1 (two mass units apart).

4. For three Br's(Br_3): $(a + b)^3 = a^3 + 3a^2b + 3ab^2 + b^3$ or 1:3:3:1

5. For ClBr: $(3a+ b)(a + b) = 3:4:1$

6. For $ClBr_2$: $(3a + b)(a+ b)^2 = 3:7:5:1$

7. For Cl_2Br: $(3a + b)^2(a + b) = 9:15:7:1$

Note: The nominal molecular weight is the mass of the lightest M^+ in a spectrum.

Observations from the binomial expansions.
1. The number of M^+ ions is one more than the number of isotopic atoms in the sample. For example, a compound that contains one Br atom has two M^+ ions; a compound with one Br and two Cl atoms has four M^+ ions.
2. Because Br-79 and Br-81 occur in a 1:1 ratio, compounds that contain only Br atoms will display a mass spectrum in which the ratio of the lowest mass M^+ to the highest mass M^+ is always 1:1. Thus, when Br is the only multi-isotopic atom in the sample, the number of Br atoms is one less than the number of M^+ ions, and the ratio of the first and last M^+ is one to one. The following patterns are found for one, two, three, and four bromine atoms (1:1, 1:2:1, 1:3:3:1, and 1:4:6:4:1).

3. Because Cl-35 and Cl-37 occur in a 3:1 ratio, the ratio of the lowest mass M^+ to the highest mass M^+ gives the number of chlorine atoms in the sample. When the ratio of the lowest mass M^+ to highest mass M^+ is 3:1, there is one chlorine atom in the sample. When the ratio is 9:1, there are two

Cl atoms, etc. Thus, a mass spectrum with four M^+ ions in the ratio of 3:7:5:1 means that we have three Br and Cl atoms and one is Cl (3:1) ratio—thus the other two are Br. Likewise, a mass spectrum with four M^+ ions in the ratio of 9:15:7:1 means that we have two Cl atoms (9:1) = Cl_2—thus the other one is Br.

The Chlorine Rule[17]

The chlorine rule applies to the mass spectra of compounds that contain both Cl and Br atoms. The chlorine-rule equation is $I_{L/R} = 3^n$. L = the leftmost molecular-ion cluster peak and R = the rightmost peak in the cluster. The ratio L/R gives 1:1 when the number of chlorine atoms *n* is zero (i.e., $n = 0$), 3:1 ($n = 1$), 9:1 ($n = 2$), 27:1 ($n + 3$). The number of molecular ions in the cluster is the sum of the number of chlorine atoms n plus the number of bromine atoms m plus one. So a five-peak pattern for which L/R = 9:1 represents a compound that contains two chlorine atoms and two bromine atoms (i.e., 2 + 2 + 1 = 5).

Molecular Weight Calculations

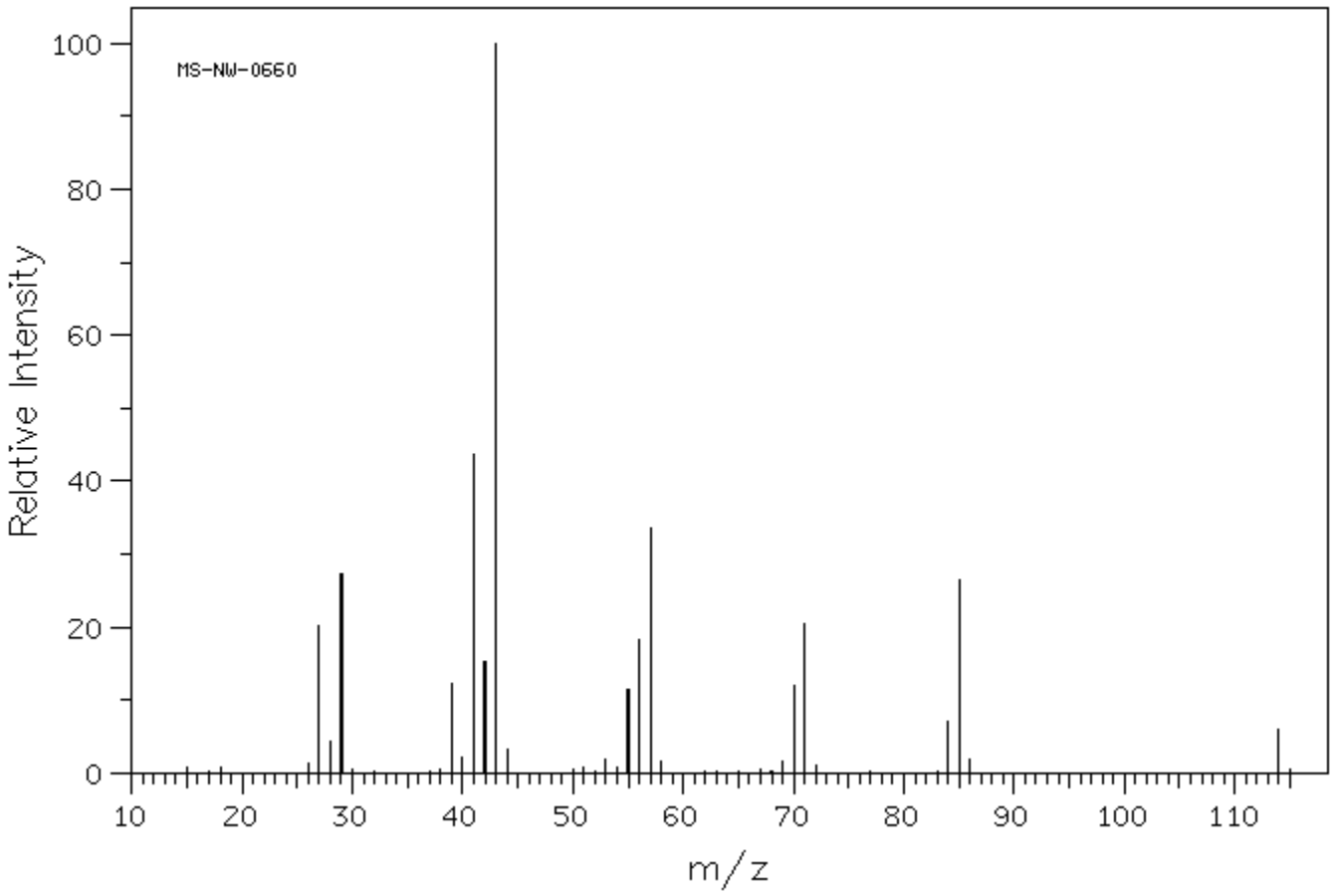
Because compounds that contain isotopes of significant natural abundance display multiple M^+ ions, we select the one with the lowest mass as the nominal molecular weight for calculation purposes. Hence, we use H-1, C-12, N-14, O-16, Cl-35, Br-79, and I-127 in our calculations.

Spectra of Aliphatic Compounds

Consider the mass spectrum of octane (Figure 2). Initial ionization produces a radical-cation.

Figure 2 Mass Spectrum of Octane[18]

[17] Gross, Jr., R. A. *J. Chem. Educ*., in press.
[18] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).



The radical-cation fragments by the loss of a radical to form a set of even-electron ions. The lost radical is often a methyl group of mass 15. A typical fragmentation is shown in Figure 2.

Significant peaks are predicted to be at *m*/*z* 43, 57 and 71. These peaks are indeed found in Figure 2. The fragmentation pattern is explicable by the ***Even-Electron Rule***. Molecules contain an even number of electrons. When an electron is ejected from an intact molecule, the ensuing radical-cation contains an odd number of electrons. Hence, a molecular ion contains an odd number of electrons. Odd-electron ions decompose by the loss of either a radical or a molecule, as shown below.

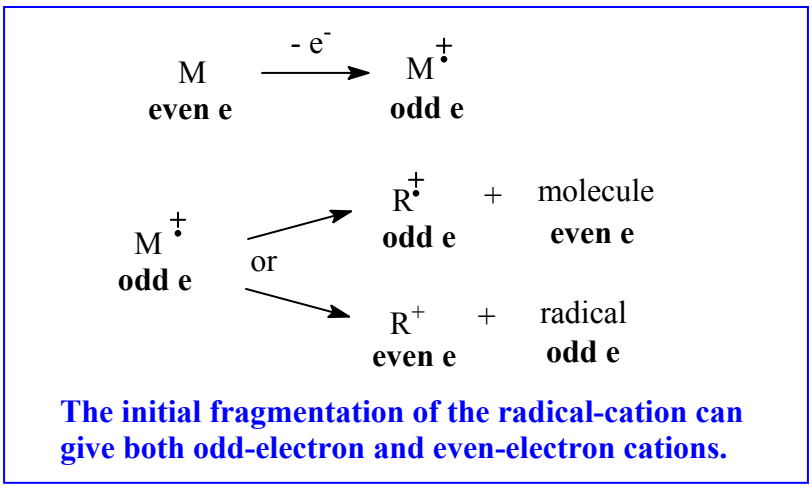


Figure 3 Initial Ions from a Hydrocarbon

Thus, a molecular ion may decompose into a carbocation R^+ (loss of odd-electron radical) that contains an even number of electrons, or into a new radical-cation (loss of a molecule) that has an odd number of electrons. The new odd-electron radical-cation produced by this process can also go on to decompose by the loss of either a radical or molecule to produce daughter ions, as shown in Figure 4.

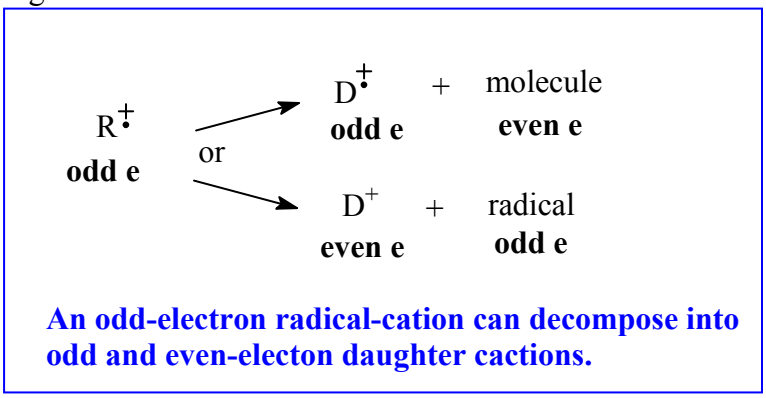


Figure 4 Fragmentation of Odd-electron Radical-cation

The new carbocation R^+ (Figure 5) contains an even number of electrons and decomposes only by the loss of a molecule. Thus, an even-electron carbocation R^+ produces a new even-electron daughter carbocation D^+.

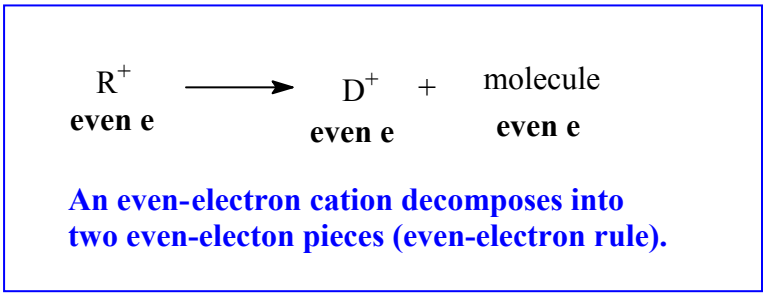


Figure 5 Fragmentation of Even-electron Cation

The Instrument

As noted earlier, our mass spectrometer is linked to a HP 6890 gas chromatograph. The eluents from the gas chromatograph enter a HP 5973 selective mass detector. Figure 2 shows both instruments. The mass chromatograph is on the left side of the gas chromatograph. The system is equipped with an auto-injector, which can be seen on top of the gas chromatograph.

Figure 2 Gas Chromatograph-Mass Spectrometer

The data station is shown in Figure 3.

Figure 3 Data Station

Experimental

Standard Procedure for GC-MS

Start up the instrument in accord with local procedures.

Hydrocarbon Samples

The instrument has been set to the standard conditions for hydrocarbons.

1. Obtain a set of hydrocarbons from the instructor. The set will contain five samples. Four of the samples are pure unbranched hydrocarbons each of which contains from 5-10 carbon atoms (i.e., they range from pentane to decane). You will be given the identity of the four pure hydrocarbons. The fifth sample is a mixture of hydrocarbons. The mixture contains from three to five hydrocarbons. These five hydrocarbons might be any of the C_5-C_{10} unbranced hydrocarbons.

2. In turn, inject each sample and print out the output in the form of a mass spectrum. For the mixture, you should obtain one mass spectrum for each component.

3. Analyze the spectra and fill in the boxes in the report form.

4. Complete the report and turn it in at the beginning of the next lab period.

Lab Report #13

Hydrocarbons in a Mixture by GC-MS

Unknown Mixture Number _____

The unknown mixture contains the following compounds:_____

_____

_____

1. Analyze your mass spectra and complete Table 1 by filling in the missing data. For hydrocarbons #1-#4, write in the name of the pure hydrocarbon in the sample. For the mixture, fill in the data for each compound but do not name the compound. You will name the unknowns after you have tabulated the data on them. If you have a three-component mixture, leave the data for Mixture #4 and Mixture #5 blank.

Table 1 Mass Spectra of Unbranched Hydrocarbons

Compound Name	Molecular Ion (M^+)	Base Peak (m/e)	Other Significant Peaks
Hydrocarbon #1			
Hydrocarbon #2			
Hydrocarbon #3			
Hydrocarbon #4			
Mixture #1			
Mixture #2			
Mixture #3			
Mixture #4			
Mixture #5			

2. Identify the components of your unknown mixture from your data in Table 1, and write the name next to its molecular weight (M^+) in Table 2.

Compound	M^+	Name of Hydrocarbon
Mixture #1		
Mixture #2		
Mixture #3		
Mixture #4		
Mixture #5		

3. Write the names you identified in step 2 in the space provided at the top of this report.

4. How many M^+ ions should you observe for a compound that contains two chlorine and one bromine atoms in its structure? Answer_____

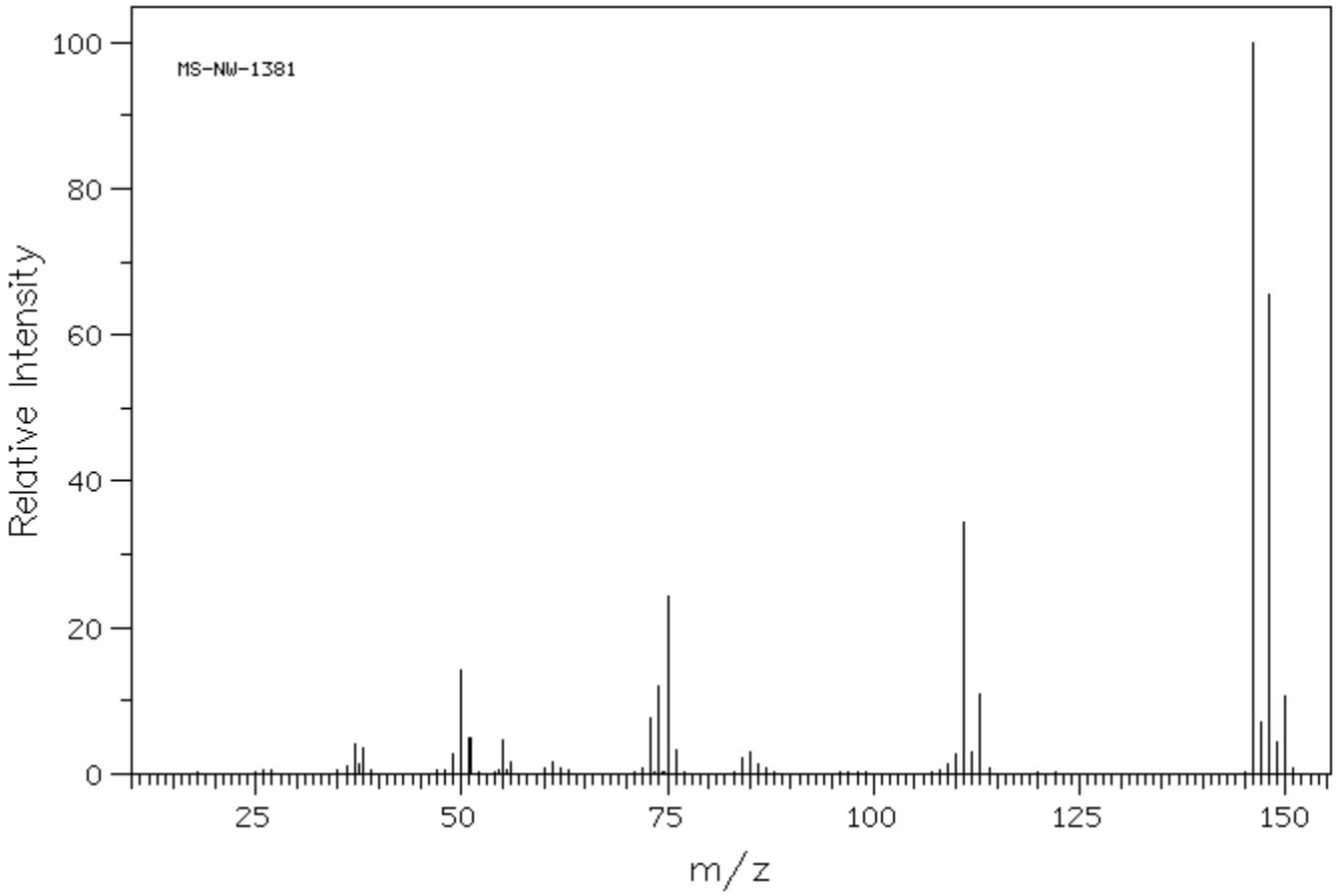
5. Calculate the ratio of peak heights you expect to see for the M^+ ions for problem 4. Answer_____

6. Write the formula for an organic compound that has a molecular weight of 31 Daltons. Answer _____

7. What M^+ ions and in what ratio do you expect to see them in the mass spectrum of CH_2Cl_2? Answer_____

8. What M^+ ions and in what ratio do you expect to see them in the mass spectrum of $C_6H_4Br_2$? Answer _____

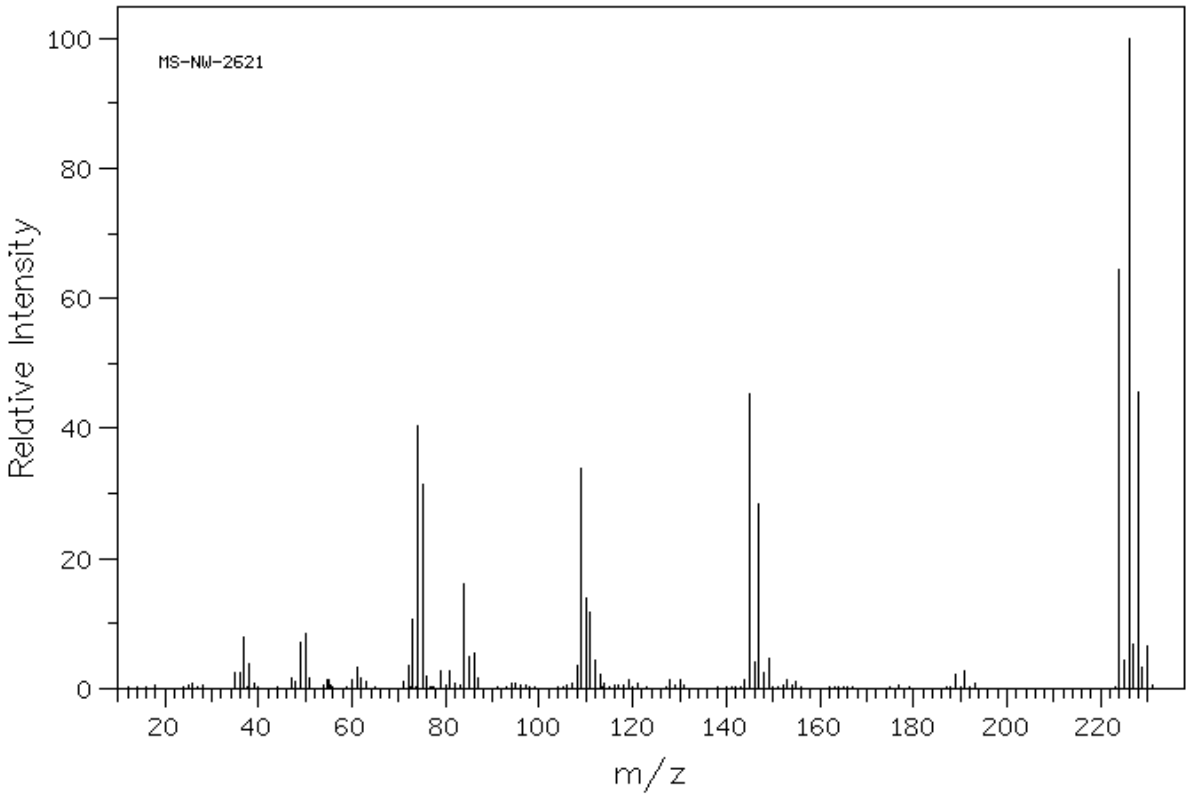
9. Analyze the mass spectrum[19] below.



a. What is the nominal mass of the sample? Answer _____
b. What is the total number of Br and Cl atoms present? Answer _____
c. How many Br atoms? ____ How many Cl atoms?_____

[19] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).

10. Analyze the mass spectrum[20] below.



a. What is the nominal mass of the sample? Answer _____
b. What is the total number of Br and Cl atoms? Answer _____
c. How many Cl atoms are present ? _____ How many Br? _____

[20] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).

14 Explorations in Gas Chromatography-Mass Spectrometry (GC-MS)

(Identification of Isomeric Compounds Commonly Found in Gasoline)

A **Summary of this Week's Lab** During this lab period, you will extend your study of hydrocarbons by analyzing the spectra of simple constitutional isomers of the hydrocarbons you analyzed in the last period. As before, these compounds might be found in gasoline. The samples will again be both pure hydrocarbons and mixtures of hydrocarbons. The previous sample set contained only unbranched or so-called straight-chain hydrocarbons. To study the effect on the mass spectrum of a structural change, only a small perturbation in the structures will be made to the compounds to be analyzed. The isomers are all 2-methylalkanes (i.e., they all contain an isopropyl group). Spectra will be obtained for the 2-methyl isomer of each of your four compounds from the previous experiment. You will analyze the spectra of the isomeric compounds with the view of finding the characteristic fragmentation patterns that distinguish the isomeric pairs of compounds. Then you will apply what you discover to the analysis of a mixture that contains from three to five compounds, which might be either branched or unbranched hydrocarbons. You will also attempt to correlate the structures of the compounds with their order of emergence from the GC. You will complete the report for this lab period and turn it in at the beginning of the next lab period.

Fundamentals of Mass Spectroscopy

The following discussion illustrates the basic principles of the simplest of instruments.
A compound is introduced as a vapor into a system that is under a very high vacuum. See Figure 1. In the inlet system, the molecules become widely dispersed. A portion of the sample is channeled into a beam of electrons. The energy of the electron beam is (~70 eV = 7620 kJ/mol) is sufficient to ionize the sample (Ion Source). The ions are accelerated and analyzed (Mass Analyzer). Inside the analyzer, the ions are accelerated in a magnetic field ***H***. The magnetic field deflects the ions in a circular path of radius *r*. The radius is given by Equation 1, which is the fundamental equation of mass spectroscopy.

$$r = \left(\frac{2V(m/z)}{\boldsymbol{H}^2} \right)^{1/2}$$

Equation 1

From the form of Equation 1, we see that the radius of curvature is a function of both the applied potential *V* and the field strength ***H***. The detector converts the beam of ions into an electrical signal that is processed (Signal Processor) and either stored as a mass spectrum or printed out. The requirement for a high vacuum distinguishes the technique of mass spectroscopy from most of the other commonly used techniques by chemists. The care and maintenance of the high-vacuum system is a laboratory necessity that comes with experience and training. Our primary focus is to learn how these instruments can be used in chemical analysis generally and specifically in forensic analysis.

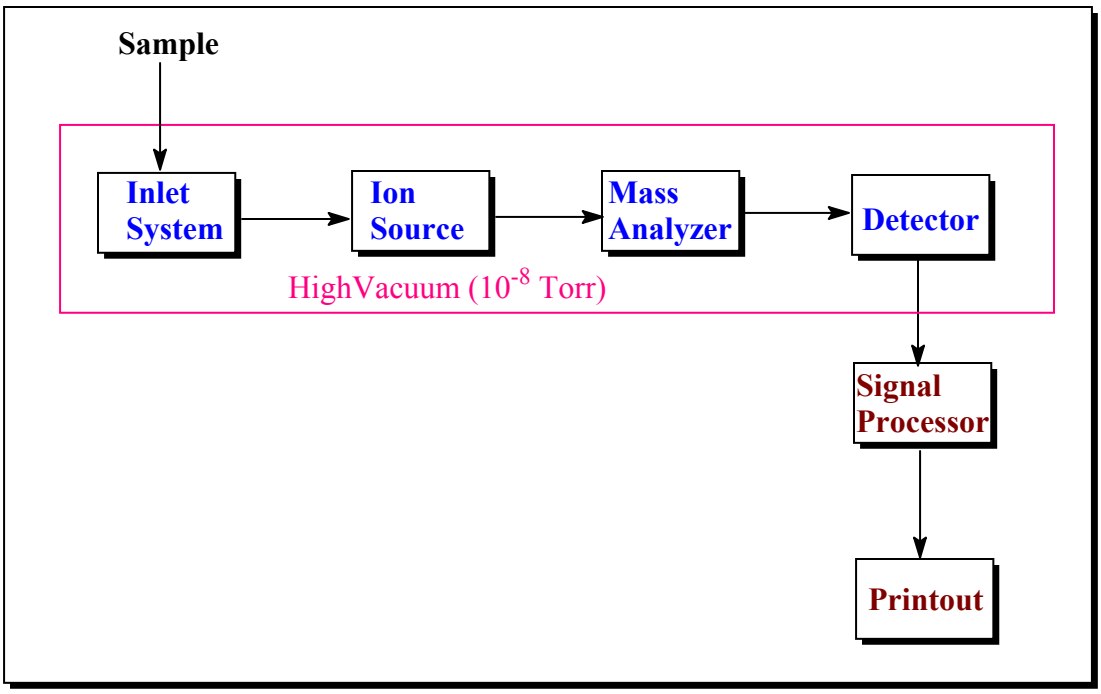


Figure 1 Basic Components of a Mass Spectrometer

To view another schematic of a mass spectrum, click on the following link.
http://www.oceta.on.ca/profiles/sciex/elan6000.html

Ion Sources
Some compounds are thermally stable, and some are not. Likewise, some molecules are volatile, easily vaporized, and others are not. Accordingly, a variety of methods has been developed to ionize various compounds, so they can be subjected to mass spectral analysis. The two major categories of ion sources are gas-phase and desorption sources, and examples of both types will be described below. Sources may also be described as hard or soft. A hard source imparts more energy to a sample than does a soft source. The more energy that is imparted, the more the sample fragments to produce daughter ions. Thus, soft sources produce a limited number of peaks, perhaps only a molecular ion peak; whereas, hard sources produce many peaks. Whether to use a hard source or soft source for an application depends upon the level of detail needed from the spectrum. If the analyst is certain of the compound in question and only wants to confirm its presence, a soft source might be sufficient. If the analyst is trying to prove the structure of a compound, a hard source is required.

Gas-Phase Sources
Gas-phase sources are generally useful for compounds that boil below 500^{o}C and are stable up to that temperature. Three kinds of gas-phase sources, electron impact (EI), chemical ionization (CI), and field ionization (FI) are described below.

Electron Impact (Gas-Phase Source)
The description of ionization in the introduction to this block of instruction (Lab # 13) was that of an electron impact source. Figure 2 is a very simplified version of an electron impact source.

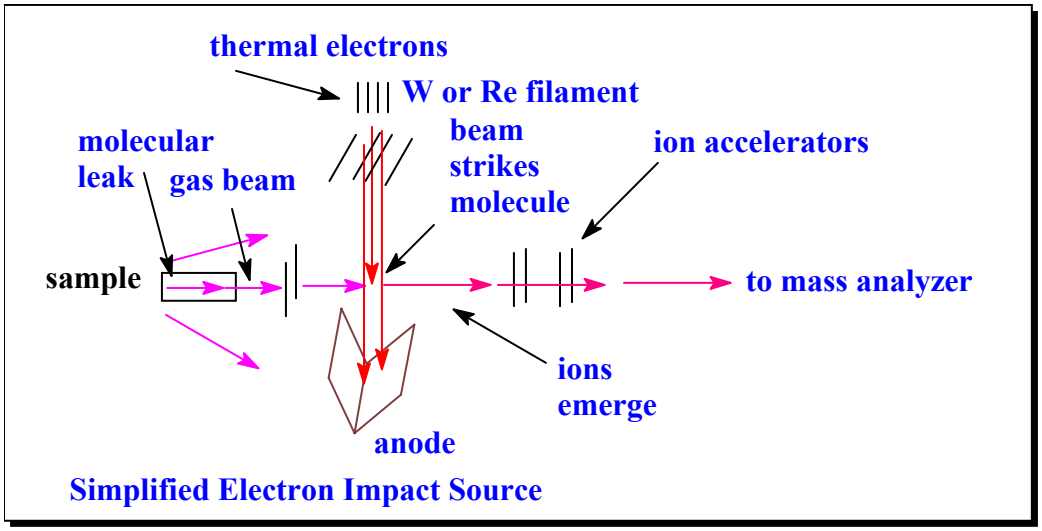


Figure 2

The sample (magenta arrows in Figure 2) is introduced into the system via a so-called molecular leak, meaning that only a small portion of the incident ions is allowed into the chamber. Electrons (red arrows) are emitted from a heated tungsten (W) or rhenium (Re) filament. They pass through a potential of about 70*V*, giving them an energy of 70 e*V*. The electron beam and sample beam cross paths at right angles. Radical-cations are produced when the high-energy electrons come close enough to eject an electron from a sample molecule by electrostatic repulsion (i.e., this is the ***electron impact***). The initial reaction or chemical transformation is shown in Equation 2.

$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$

Equation 2

Although the ionization of the analyte molecule produces a radical-cation, it will be written as M^+ in this manual and M^+ will mean molecular ion (i.e., radical-ion). The emergent ions are accelerated into the analyzer. Because the ionizing electrons have a small mass but high kinetic energy, they eject electrons from M and create M^+ ions that are at higher vibrational and rotational energy levels but not at a substantially higher translational energy level. Therefore, the M^+ ions lose energy by fragmenting into lower-mass daughter ions. The presence of these daughter ions makes electron impact sources very useful when identification of the analyte is important. Click the following link to see a more detailed schematic and to read about an electron-impact source http://www-methods.ch.cam.ac.uk/meth/ms/theory/eims.html.

Chemical Ionization (Gas-Phase Source)

With certain instrument modifications, a reagent gas can be co-introduced with a sample into the beam of ionizing electrons. The reagent-gas molecules outnumber the sample molecules by 1-to-10 thousand-fold. Thus, the high-energy electrons ionize the reagent-gas molecules in a manner described above under electron impact. The reagent-gas ions then interact with the sample molecules, generally by the transfer of a hydrogen atom as either a proton or a hydride. Various

gases such as methane, propane or ammonia might serve as the reagent gas. The basic concept is that a secondary chemical causes the ionization of the analyte. The chemical is an ion produced in the spectrometer. If we let RH be the reagent gas and M be the sample, then the equations in Figure 3 show the ionization process whereby a proton is transferred from the reagent gas to the sample.

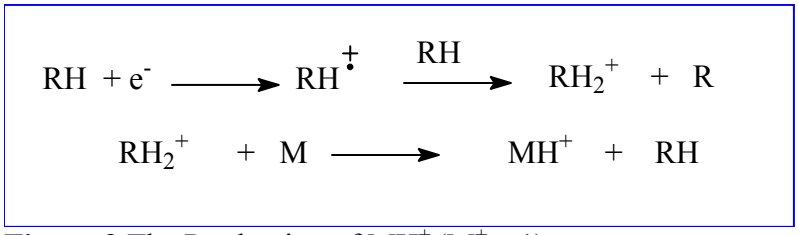


Figure 3 The Production of MH^+ ($M^+ + 1$)

As seen in the figure, the ion produced by the proton transfer has a mass one mass unit higher than the mass of the molecular ion. The transfer of a hydride leads to an ion one mass unit less than the mass of the molecular ion. Thus, for a compound such as decanol, which has a nominal mass of 158 Daltons, we could see significant mass peaks at m/z = 159 ($M^+ + 1$) or at 157 ($M^+ - 1$). These peaks result from the transfer of a proton or hydride, respectively. Ions produced in this manner have less energy than those produced by direct electron impact. Accordingly, they do not produce as many daughter ions, and the mass spectrum contains fewer peaks than a corresponding spectrum from an electron impact source.

Field Ionization (Gas-Phase Source)
This ionization technique involves the use of a very high-voltage electrical field that is generated by the use of specially constructed carbon micro-needles. The field itself ionizes the sample, making an $(M + H)^+$ ion, which is detected. A few daughter ions are observed in the spectrum. Hence, field ionization is a soft source.

Field Desorption (Desporption Source)
Desorption techniques are most applicable to nonvolatile compounds such as high-molecular weight bio-molecules or to thermally unstable molecules that fall apart before they reach a gas-phase source. Field desorption differs from field ionization in the manner in which the sample is subjected to the field. Instead of being introduced as a gas, the sample is applied directly to the electrode containing the micro-needles. Then the electrode is placed in the instrument, and a very high potential is applied to the micro-needles. The resulting electrical field ionizes the sample, giving only a protonated molecular ion peak $(M + 1)^+$ and its accompanying isotopic peak as the only peaks in the spectrum. This technique is applicable to samples in which all that is needed is a verification of the molecular species.
Electrospray Ionization (Desorption Source)
This technique allows for the production of ions having $z > 1$. Therefore, very high molecular weights can be determined at relatively low masses because of the high z values. Please click on the following link to learn the specifics of ESI-MS.
http://analytical.chem.wisc.edu/524class/Folders/Heiden/electrosprayB.html

Experimental
Standard Startup Procedure for GC-MS
Turn on the GC/MS system and follow the standard startup procedure.

Hydrocarbon Samples

1. Obtain a set of hydrocarbons, which are isomeric with the set you analyzed last time. The set will contain five samples. Four of the samples are pure branched hydrocarbons each of which contains

from 5-10 carbon atoms (i.e., they range from isopentane to isodecane). You will be given the identity of the four pure hydrocarbons. The fifth sample is a mixture of hydrocarbons. The mixture contains from three to five hydrocarbons. These five hydrocarbons might be any of the C_5-C_{10} branched or unbranched hydrocarbons.

2. In turn, inject each sample (or place them in the auto-inject sample holder and auto-inject them). Print out the mass spectrum for each sample. For the mixture, you should obtain one mass spectrum for each component.

3. Analyze the spectra and fill in the boxes in the report form.

4. Complete the report and turn it in at the beginning of the next lab period.

Lab Report #14 and Homework
Hydrocarbons in a Mixture by GC-MS

Unknown Mixture Number _____

The unknown mixture contains the following compounds:_____
_____
_____

1. Analyze your mass spectra and complete Table 1 by filling in the missing data. For hydrocarbons #1-#4, write in the name of the pure hydrocarbon in the sample. For the mixture, fill in the data for each compound but do not name the compound. You will name the unknowns in the mixture after you have tabulated the data on them. If you have a three-component mixture, leave the data for Mixture #4 and Mixture #5 blank.

Table 1 Mass Spectra of Unbranched Hydrocarbons

Compound Name	**Molecular Ion (M^+)**	**Base Peak (m/e)**	**Other Significant Peaks**
Hydrocarbon #1			
Hydrocarbon #2			
Hydrocarbon #3			
Hydrocarbon #4			
Mixture #1			
Mixture #2			
Mixture #3			
Mixture #4			

Mixture #5			

2. Compare the mass spectrum of your Hydrocarbon #1 from this experiment (Lab #14) with the mass spectrum of your Hydrocarbon #1 from the previous experiment (Lab #13). These compounds are isomers. Enter the names of the respective Hydrocarbon #1 in Table 2. Fill in the data from the respective spectra.

Table 2 Hydrocarbon # 1 and Isomer

Compound Name	**Molecular Ion (M^+)**	**Base Peak (m/e)**	**Other Significant Peaks**

3. Repeat the steps for Hydrocarbon #2 and its isomer.

Table 3 Hydrocarbon # 2 and Isomer

Compound Name	**Molecular Ion (M^+)**	**Base Peak (m/e)**	**Other Significant Peaks**

4. Repeat the steps for Hydrocarbon #3 and its isomer.

Table 4 Hydrocarbon # 3 and Isomer

Compound Name	**Molecular Ion (M^+)**	**Base Peak (m/e)**	**Other Significant Peaks**

5. Repeat the steps for Hydrocarbon #4 and its isomer.

Table 5 Hydrocarbon # 4 and Isomer

Compound Name	**Molecular Ion (M^+)**	**Base Peak (m/e)**	**Other Significant Peaks**

6. Summarize below the mass spectral characteristics that distinguish an unbranched hydrocarbon from its 2-methyl isomer.

7. Use your analysis in paragraph 6 to identify the components of your unknown mixture. Write the names next to their molecular weight (M^+) in Tables 6 and 1.

Table 6

Compound	M^+	Name of Hydrocarbon
Mixture #1		
Mixture #2		
Mixture #3		
Mixture #4		
Mixture #5		

8. Give examples and briefly describe a gas-phase source and a desorption source.

9. What is the difference between a hard source and a soft source?

10. What kind of source is appropriate for the molecular weight determination of a high-mass protein?

15 Forensic Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

(Identification of Compounds in Gasoline, or other Accelerants, by Headspace Analysis)

A **Summary of this Week's Lab** During your final lab period, you will sample the space above a small amount of gasoline or other potential accelerant and analyze the sample for volatile components. This technique, called ***headspace analysis***, is often employed to sample the volatiles from the charred remains of a fire as part of an arson investigation. You will collect the air sample on powdered carbon or charcoal. A gas has an electrostatic attraction to activated carbon and sticks to its surface by a process called ***adsorption***. The charcoal is activated by heating it in an oven. This drives off moisture and the immense surface area of the dry charcoal provides sites for vapors to adhere to the surface. The volatiles from the gasoline will be adsorped onto charcoal and then collected by dissolution into liquid carbon disulfide. The resulting carbon disulfide sample will be analyzed by gas chromatography—mass spectrometry. The volatile compounds from the gasoline sample will be separated by the gas chromatograph and their mass spectra obtained as in the previous two labs. You will compare the mass spectra from your previous two labs (eight spectra in all) with the mass spectra of the compounds found in the headspace of your sample. The object is to find how many of the known compounds are volatile components of your sample. Determining the commonality of mass spectral peaks in a suspect and known liquid is a standard forensics technique. You will expand your mass spectral interpretive skills by learning some of the common fragmentation patterns that recur in mass spectra. You will complete your lab report and turn it in when you report for the Final Exam.

Advanced Mass Spectral Analysis

Certain masses recur in mass spectra because, like all properties, a mass spectrum is a function of the structure of the molecules that produce it. Therefore, certain structural features beget specific m/z peaks. In the following sections, we will discuss some of the structural features that give easily recognizable ions.

Aldehydes, Ketones and the McLafferty Rearrangement

The molecular radical-cation of adehydes and ketones may decompose by homolytic cleavage of one of the carbonyl carbon's single bonds, producing an acyl carbocation (acylonium ion) and a hydrogen atom-radical or alkyl radical. The resulting acylonium ion can lose carbon monoxide and produce a daughter carbocation 28 mass units lower than the mass of the acylonium ion. In the case of ethanal, we would expect to see a molecular ion at $m/z = 44$; an M – 1 peak at 43 (acylonium ion); and an M – 29 peak at 15. The corresponding peaks in propanal are at $m/z = 58$, 57, and 29. Likewise for 2-butanone, the molecular radical-cation ($m/z = 72$) might lose either a methyl radical or an ethyl radical. The rule of thumb is that the larger mass group cleaves preferentially. Thus, cleavage of the ethyl group leaves an ethylonium ion with $m/z = 43$. The actual spectrum shows a base peak of 43 and a tiny peak at 57, which corresponds to loss of the methyl radical. Figure 1 shows the sequence of cleavages that start with an α-cleavage of an aldehyde.

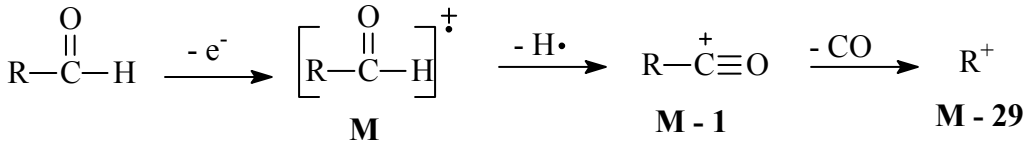


Figure 1

Figure 2 shows the sequence when fragmentation of a ketone begins with an α cleavage.

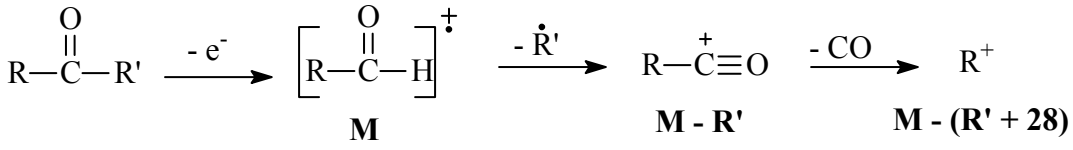


Figure 2 R' > R

When R is phenyl in either Figure 1 or Figure 2, significant peaks are observed at m/z 105 for the loss of an H atom or alkyl radical and at 77, corresponding to the loss of CO.

When an aldehyde or ketone contains an alkyl group so that a quasi-six-membered ring such as those shown in Figure 3 can form, the molecular radical-cation eliminates two alkenes. One of the alkenes is a neutral molecule and the other is a new radical-cation that is detectable in the mass spectrometer. For an aldehyde, the mass of the latter ion is 44. For a methyl ketone, the mass is 58. The rearrangement that produces the new radical-cation from the ring is called a McLafferty rearrangement after the chemist who discovered it.

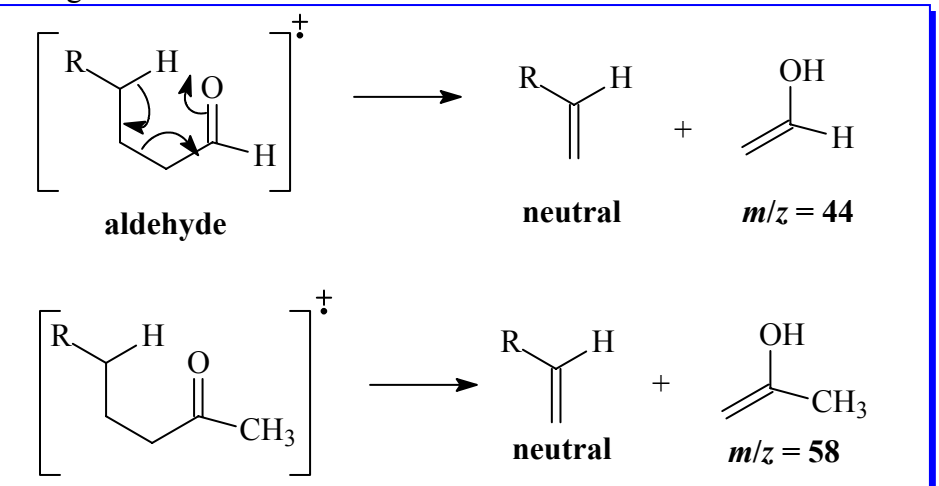


Figure 3

Alkenes, Alkynes and the Retro-Diels—Alder Reaction

The molecular ions of alkenes are relatively stable, so a strong M^+ peak is usually observed in the mass spectrum of an alkene. The molecular ions of terminal alkynes readily lose an H atom, giving a very strong M -1 peak in addition to the molecular ion peak.

Cyclic alkenes that contain six carbon atoms readily undergo a retro-Diels—Alder reaction, producing a neutral ethane molecule and a butadiene radical-ion, which is detectable. Figure 4 shows an example of the retro reaction.

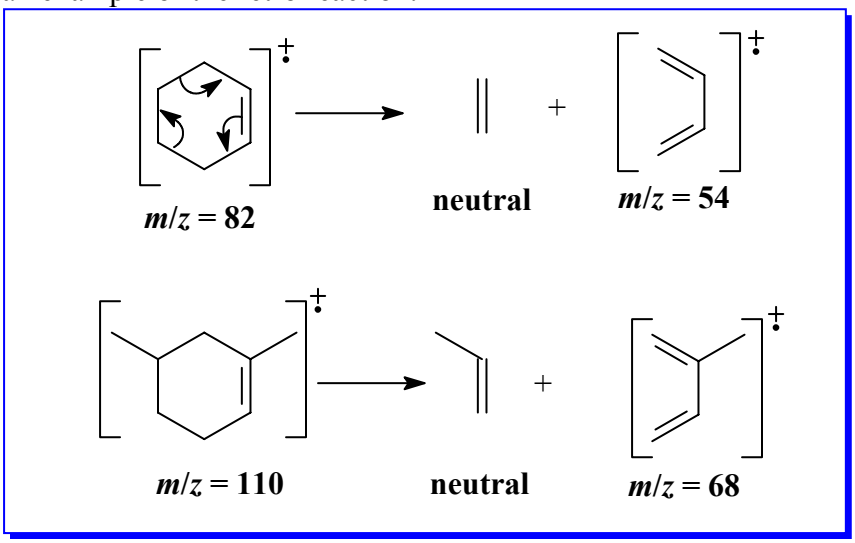


Figure 4 Examples of Retro-Diels—Alder Fragmentations

Aromatic Hydrocarbons and the Tropylium Ion

Like simple alkenes, benzene displays a strong molecular ion because of its stability by no other characteristic mass peaks. Alkylbenzenes fragment at the benzyl carbon, producing benzyl carbocations with $m/z = 91$. A benzyl carbocation rearranges into a more stable seven-membered ring carbocation called a tropylium ion that is aromatic (i.e., it contains $4n + 2$ π electrons) and hence very stable. The production of a tropylium ion from an alkylbenzene is shown in Figure 5.

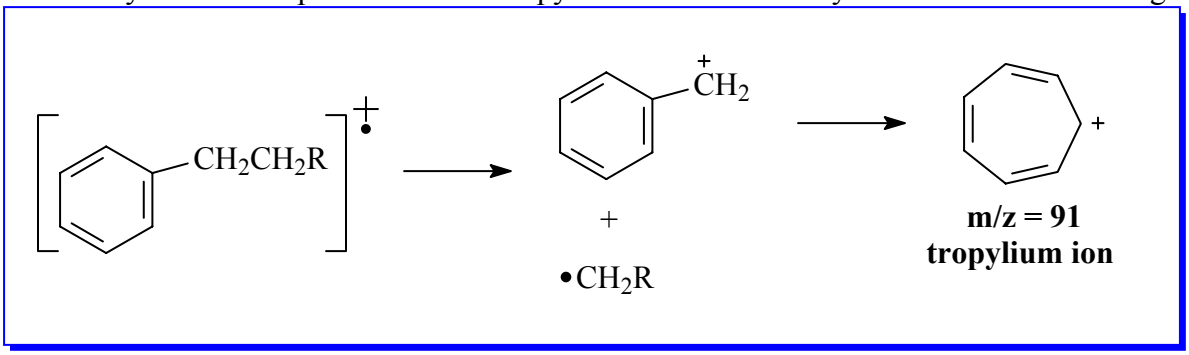


Figure 5 Formation of a Tropylium Ion from an Alkylbenzene

Acids and Esters

Esters readily undergo both α-cleavages and McLafferty rearrangements. Figure 6 shows these fragmentation patterns for methyl pentanoate.

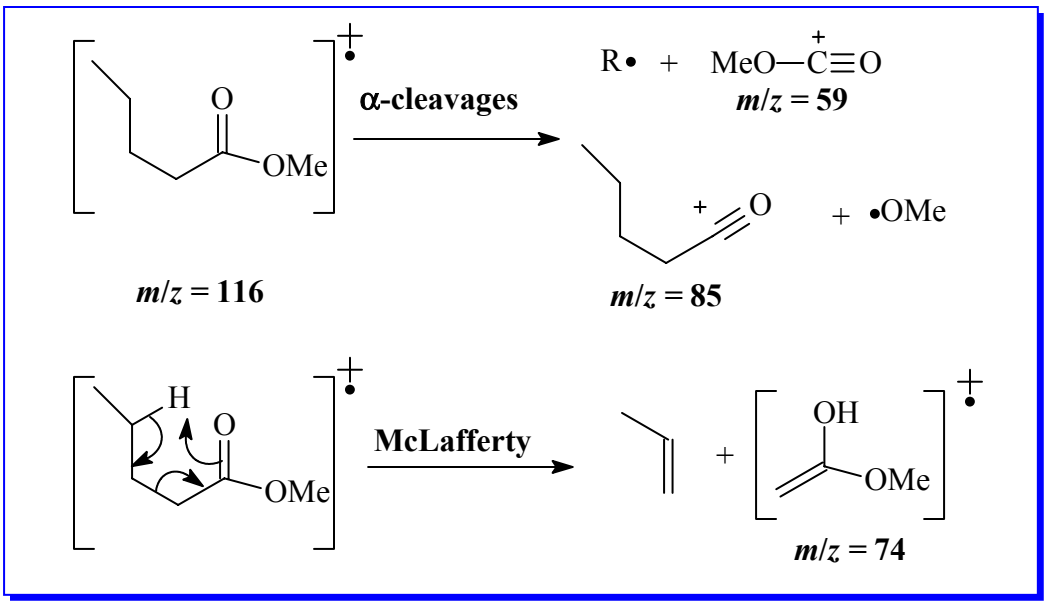


Figure 6 Fragmentation Pattern of a Methyl Ester (Methyl Pentanoate)

Thus, methyl esters commonly show peaks at m/z = 59 and 74, which are the result of an alpha-cleavage and a McLafferty rearrangement, respectively. Likewise, ethyl esters show significant peaks at 73 and 88, respectively.

Carboxylic acids readily form acylonium ions by the loss of a radical OH from the molecular ion. As we know, the acylium ion can go on to lose CO with the formation of a detectable R^+.

Alcohols and Phenols

Primary and secondary alcohols may dehydrate by a mechanism similar to the McLafferty rearrangement. They may also fragment by cleavage of a carbinol carbon—carbon bond with the expulsion of an alkyl radical and production of a new radical-cation. Figure 7 shows this latter kind of fragmentation for 2-penanol.

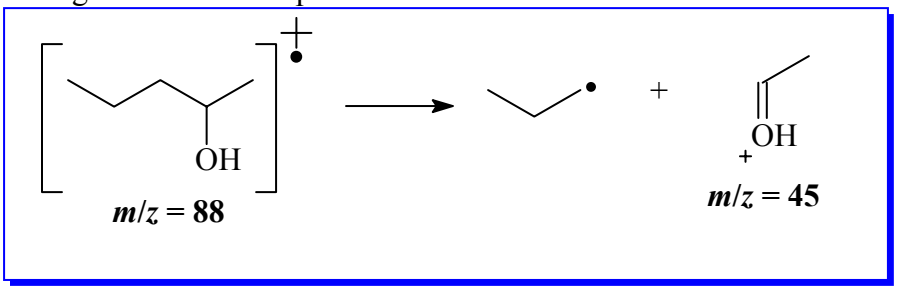


Figure 7 Cleavage in a Secondary Alcohol (2-Pentanol)

The dehydration mechanism also involves the expulsion of a neutral alkene molecule, as shown in Figure 8 for 2-pentanol.

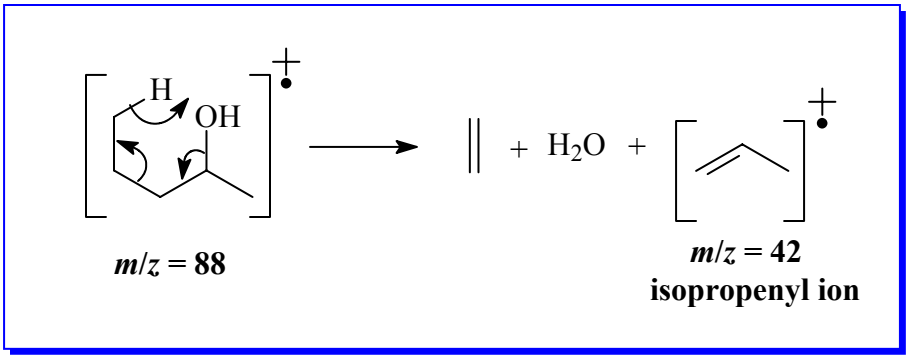


Figure 8 Dehydration Mechanism for a Secondary Alcohol (2-Pentanol)

Note that the mass of an isopropyl group is 43; whereas, the mass of an isopropenyl group is one mass unit less (42).

Unlike most primary and secondary alcohols, phenols display a strong molecular ion peak. Phenols also display intense peaks at M – 28 and M-29, which correspond to the loss of CO and CHO, respectively. Subtract the major peaks from the M^+ peak in a spectrum to find the 28/29 combination that indicates the presence of a phenol.

Summary
In the discussion, we have covered some of the well-known fragmentation patterns that you are likely to observe in mass spectra. Several important families have been omitted from this discussion, but the power of recognizing acylium ions, tropylium ions, retro-Diels—Alder reactions and McLafferty rearrangements will become obvious to you as you explore mass spectra.

Ensure the GC-MS is turned on and that the operating system is set to the procedure you used in the previous two experiments. The setup procedure for the instrument is described in the handout next to the instrument.

Procedure

1. Obtain a copper wire that is approximately 10 cm in length and is flattened on one end.

2. Obtain a sample of activated charcoal and grind it into a fine powder with a mortar and pestle.

3. Dip the flattened end of the copper wire into a sodium silicate solution.

4. Immediately dip the wet end of the wire into your powered charcoal, making a fine coating of carbon on the wire.

5. Rest the wire on a beaker inside an oven (70 ^{o}C) so that the coated end of the wire is not disturbed.

6. Leave the wire in the oven for 15 min. This reactivates the charcoal.

7. While waiting for the wire to dry, obtain a sample of an accelerant from the instructor. The sample is contained in a medium sized, septum-capped test tube.

8. Set up a water bath on a hot plate. The water bath is a 250-mL beaker that is about ¾ full of water.

9. Clamp your test tube to a ring stand. Place the clamped test tube in the water bath and adjust its depth in the bath so that the liquid in the test tube is below the water line of the water bath.

10. Make sure the test tube firmly clamped in place and heat the water bath to 80^{o}C but not to boiling, because we do not want to create an excess of water vapor.

11. Carefully remove the beaker and wire from the oven. Set the beaker on your bench top with the wire still in place and let the wire cool to room temperature

12. Carefully push the un-coated end of the wire through the center of a small piece of cardboard (6 cm x 6 cm square). Rest the cardboard on a ring stand with the coated end of the wire pointing downward.

13. Remove the septum from your accelerant sample and place the coated end of the copper wire into the headspace of the test tube. Adjust the position of the coated wire so that it is just above the surface of the accelerant. The cardboard square serves as a holder for the wire and rests on the top of the test tube.

14. Leave the wire in the headspace with the water bath at 80^{o}C for about 15 min.

15. Lift the wire out of the sample and quickly rinse the coated end of the wire with carbon disulfide. Catch the washing in a glass vial that can be capped and sampled by an auto-injector.

16. Cap your sample vial.

17. Make sure your vial has a sufficient amount of carbon disulfide solution for the needle of the auto-injector to sample it.

18. Place your sample vial, marked for identification, into the auto-injector's rack.

19. Inject the sample into the GC-MS and obtain mass spectra as in the previous experiments with the GC-MS.

Lab Report #15 and Homework

Volatile Components of an Accelerant by GC/MS

Student Name _____

Unknown Accelerant Number _____

1. Compare the mass spectra obtained from the headspace analysis of your accelerant with the eight mass spectra of pure hydrocarbons you obtained in the previous two experiments.
In the following space, list the hydrocarbons that you can clearly state are present in the accelerant.

_____

_____

_____

2. Draw the structure of a tropylium ion and enter its mass in the space provided.

Tropylium ion, mass = _____

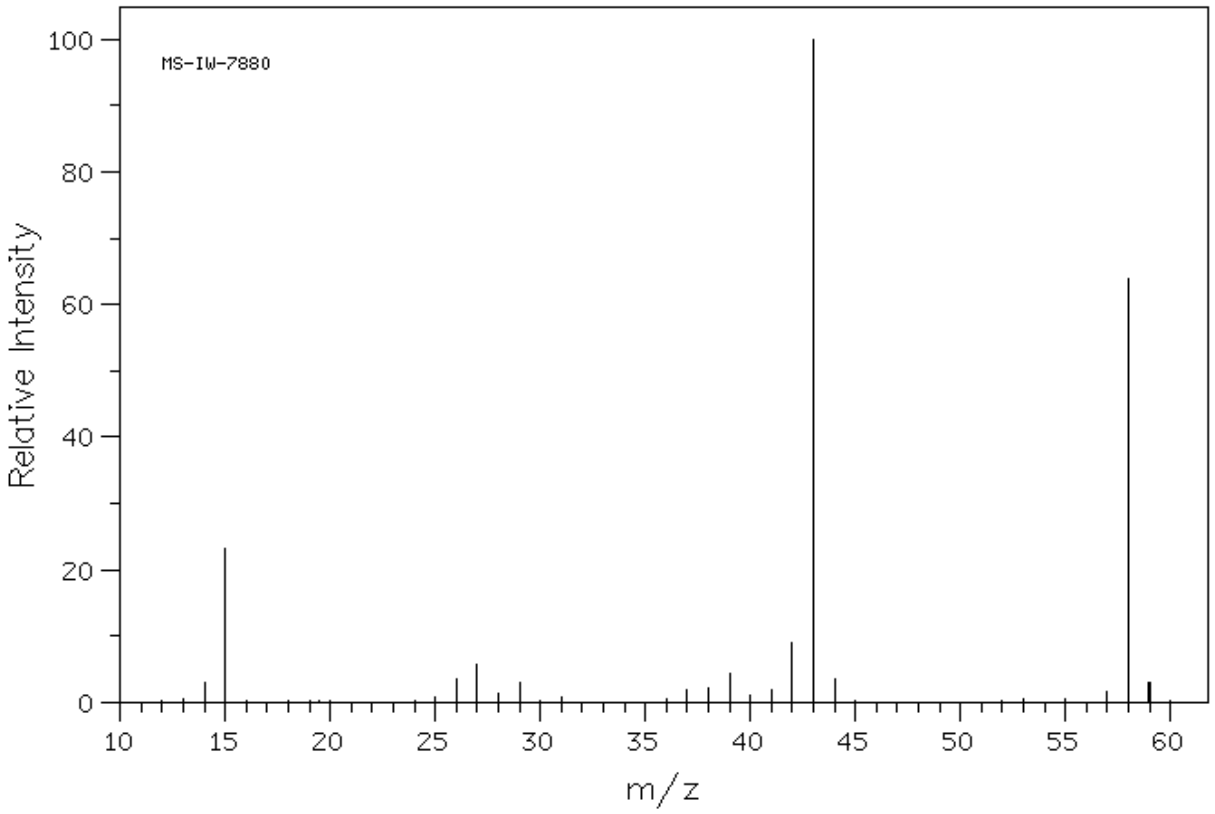
3. The tropylium ion occurs in a mass spectrum by the rearrangement of what chemical species?
Answer _____

4. Write an equation for the Diels-Alder reaction between 1,3-butadiene and ethane. Separate the reactants from products by reversible arrows. In the space beneath each chemical in the equation, indicate the mass of the chemical species.

1,3-butadiene ethane product

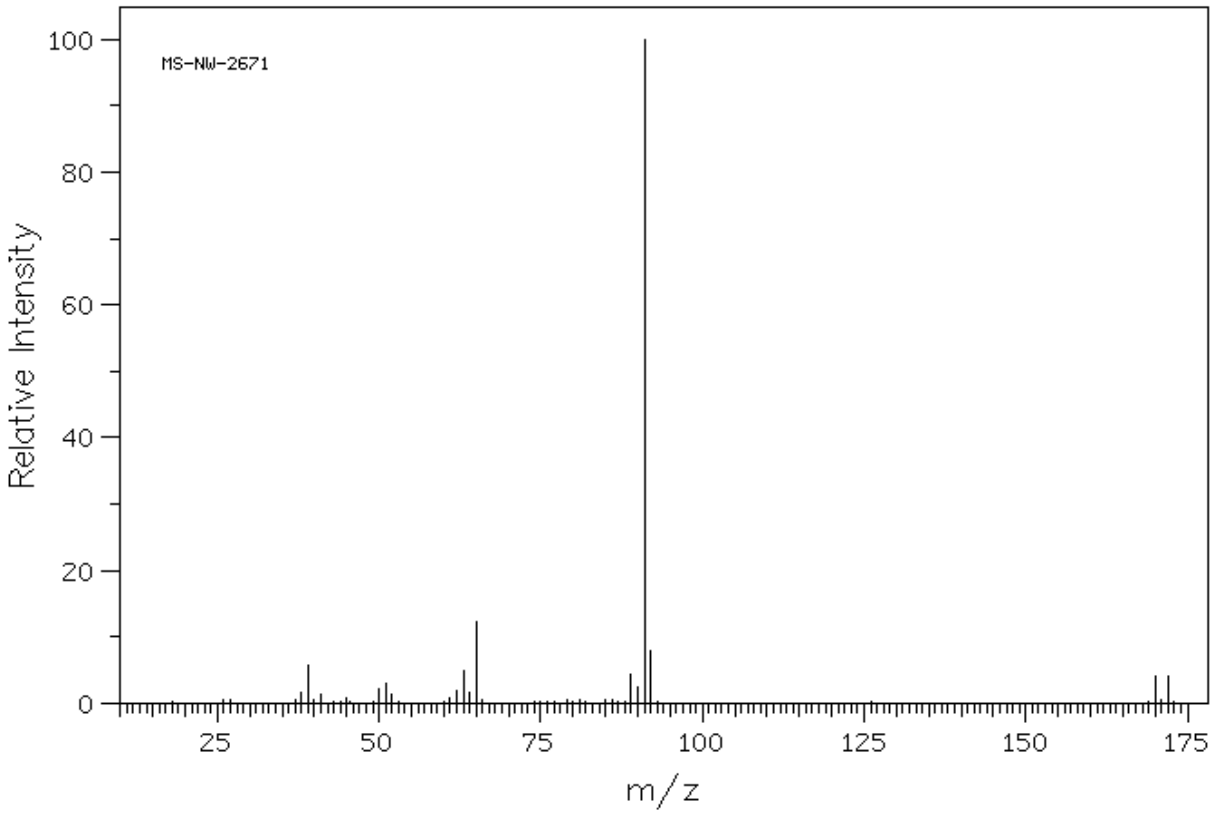
5. In the space below, show the mechanism for a McClafferty rearrangement of 2-pentanone. Indicate the mass of each chemical species in the equation.

6. The mass spectrum of acetone[19] is shown below. How do you account for the base peak at *m*/*z* = 43?



[19] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).

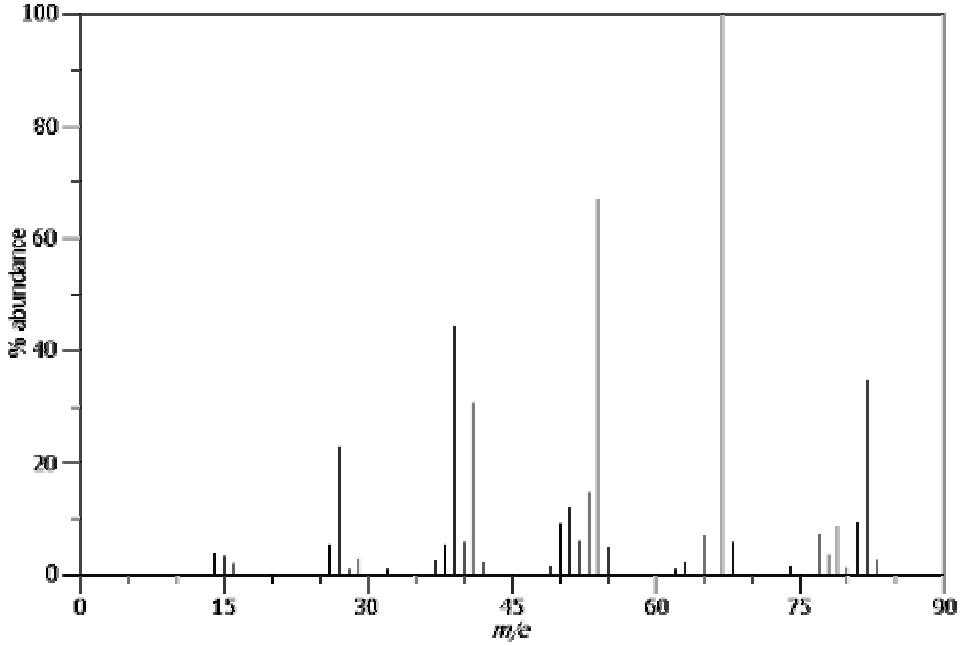
6. Draw the structure of the compound whose mass spectrum[20] is shown below.



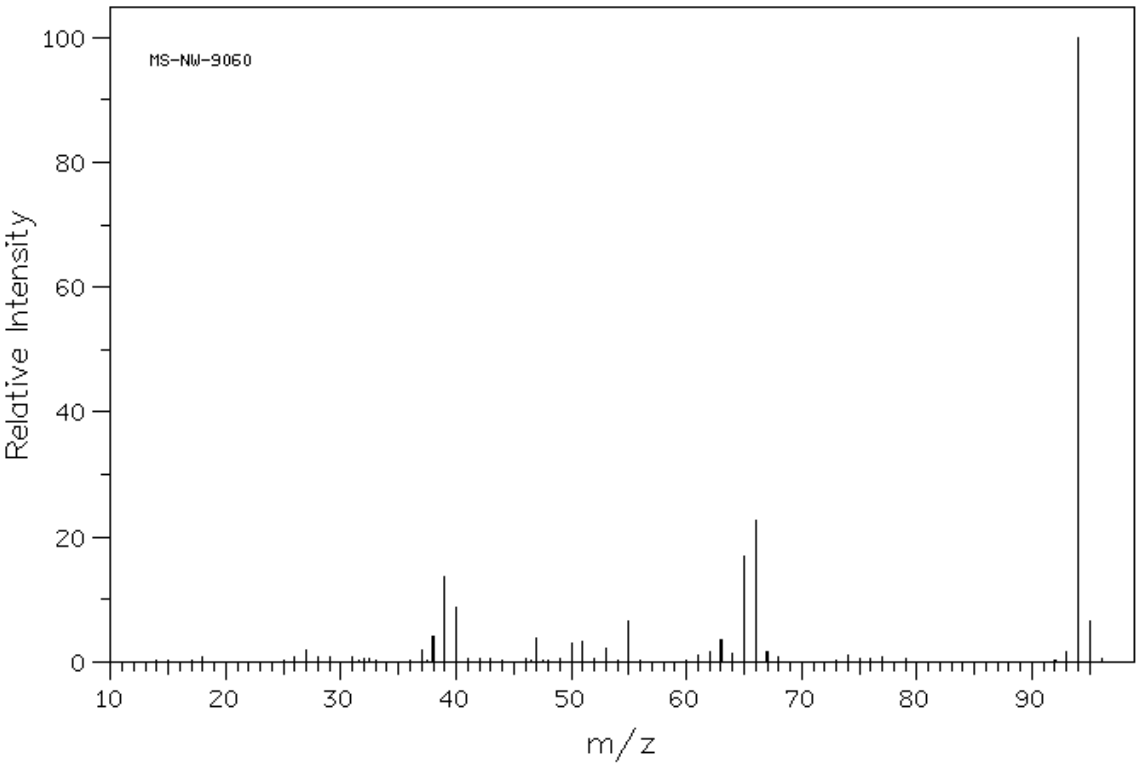
7. The molecular formula of the compound whose mass spectrum[21] is shown below is C_6H_{12}.

[20] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).

What is the structure of the compound?



8. Draw the structure of the compound whose mass spectrum[22] is shown below. Account for the significant mass peaks at m/z = 65 and 66.



[21] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).
[22] SDBSWeb: http://www.aist.go.jp/RIODB/SDBS/ (December 22, 2003).

9. List three *m*/*z* peaks you would expect to see in the mass spectrum of 2-hexanone.

10. List three *m*/*z* peaks you would expect to see in the mass spectrum of benzoic acid.