What's Mass Got To Do With It?
A Self-Guided Introduction to Mass Spectrometry

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Scenario:
You hurry into your chemistry lab only to find your teacher slumped unconscious over the lab table. The only sign of any chemical is one drop of a colorless liquid on top of the lab counter. What is the chemical? Is it responsible for the current state of your teacher? How can the small volume be analyzed? What good is one drop? Will your teacher live to give another assignment?

► 1. *Suppose the unknown liquid was diethyl ether, with a density of 0.70 g/cm^3 and assume that the approximation of 20 drops to 1 milliliter is reasonable. How many molecules would be in the one drop of the liquid found on the lab table?*

Objectives:

1. To experience how the structure of a substance can be determined by various analytical instruments commonly found in college labs.
2. To learn how a mass spectrometer works.
3. To learn how to analyze isotopic abundance from the mass spectrum of an element.
4. To learn how to identify a compound from a mass spectra.
5. To learn how fragmentation patterns of organic compounds contribute necessary information concerning the structure and hence the identity of the molecule.

Why Should You Care About The Mass Spectrometer?

Mass spectrometry is a powerful analytical technique that is used to identify unknown compounds. Even with masses as small as 10^{-12} g, chemicals can be identified using this instrument. Did you know that the Mass Spectrometer is used to.....

- ✓ determine the chemical composition of substances retrieved from outer space?
- ✓ detect and identify the use of steroids by athletes?
- ✓ monitor the breath of patients under anesthesia?

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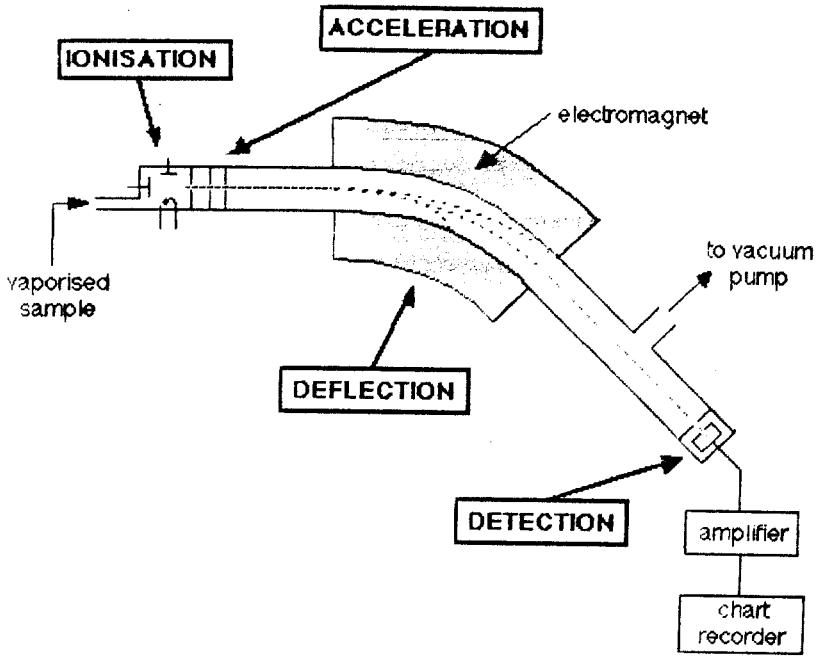
How Does a Mass Spectrometer Work?

Imagine trying to deflect the path of a ball being rolled in front of you by applying a sideways blast of water from a garden hose. The path of the ball will be deflected in a curved path depending on three variables.

► 2. *Name two of the three variables on which the degree of curvature depends.*

► 3. *Explain how material in a comet flying past planet Earth can also be an analogy for this kind of deflection. What is different about this analogy?*

Diagram of a Mass Spectrometer



Note; the sample to be analyzed in the mass spectrometer must be in the gaseous phase (vaporized) to be turned to an ion. Ionization is accomplished by using a stream of electrons to knock out one (or more) electron from the molecule. This species is now called the "molecular ion". With sufficient energies, the molecules can be broken into fragments that are also ionized. The molecular ions and fragments are accelerated to the same kinetic energy, and then deflected by an electromagnetic field. The ions passing through the spectrometer are detected electrically, and that signal is amplified.

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► 4. *If an electron were knocked out of a water molecule, what species would be formed? Include formula, mass, and charge.*

► 5. *If an electron were knocked out of a butane molecule, what species would be formed? Include formula, mass, and charge.*

► 6. *Which so you think is more likely, and why; to knock one electron out of a molecule, or to knock two electrons out of a molecule?*

► 7. *Charged particles travel from one side of the MS to the other. Can you imagine why the interior of the MS chamber must be kept at a high vacuum?*

Activity; A Mechanical Analog to the Mass Spectrometer

As the instructor demonstrates this instrument, draw and label the instrument in the space below.

► 8. *On your diagram above, draw the deflected pathways of 3 hypothetical balls, A, B, and C of increasing mass.*

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► 9. *Complete the chart of comparisons between the demonstration and a Mass Spectrometer.*

Factors That Influence Deflection of Particle	In Demonstration Analogy	In Mass Spectrometer
Mass		
Charge		
Force		

If we assume the electromagnetic force of the mass spectrometer remains constant during a particular run, then the 2 variables that influence deflection are mass and charge. These are combined into the mass/charge ratio and given the symbol m/z. Since it is so much more difficult to remove 2 electrons as opposed to one electron from the same molecule, we can safely assume during this introduction that the molecular ions will all have a +1 charge.

► 10. *In your labeled diagram with the trajectory of 3 balls indicated, label the trajectories with the highest and the lowest m/z ratio.*

What is Elemental Mass Spectroscopy?

In elemental mass spectroscopy, the elements in a compound are fractured, isolated and analyzed. This technique is usually done on inorganic compounds. Below is a sample of carbon dioxide in the MS. Note the molecular ion (M^+) is CO_2^+.

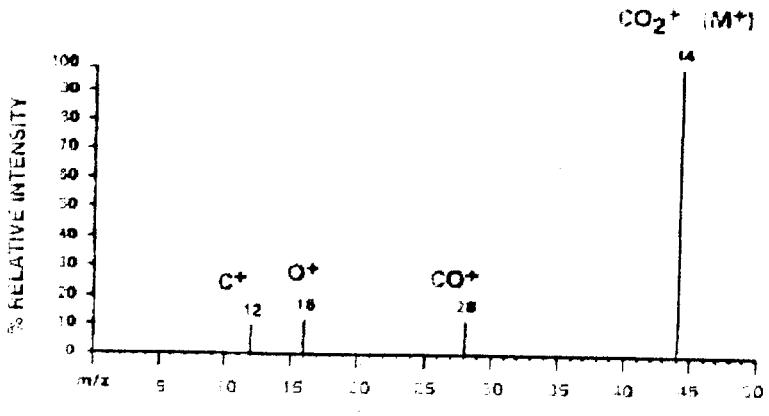

Figure 2 Mass spectrum of carbon dioxide, CO_2. Molecular ion is seen at m/z 44.

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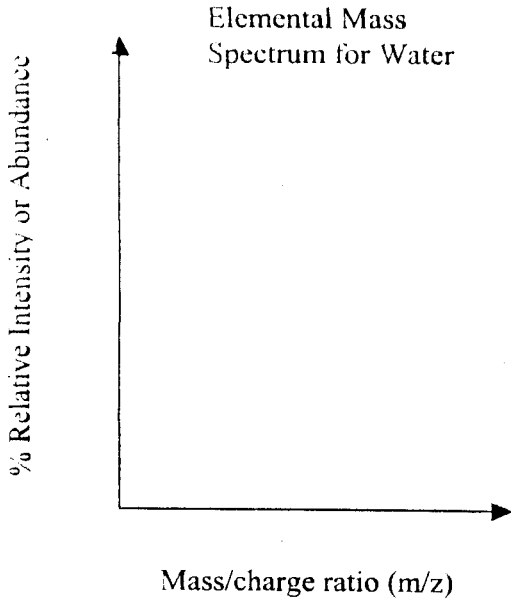
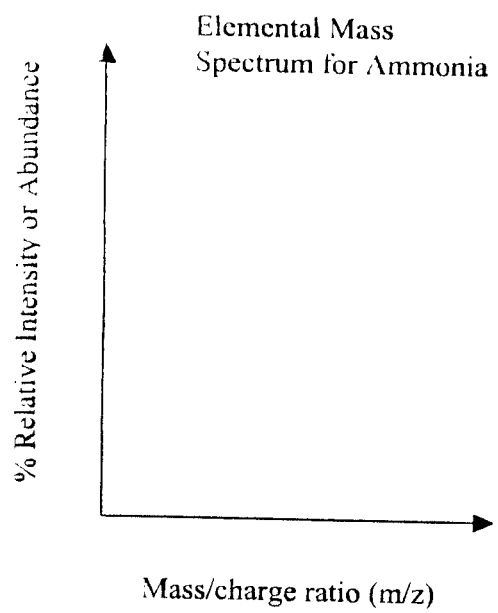
➤ 11. Now *you draw the elemental mass spectra of water and ammonia. Number the m/z axes. You will not know the relative intensities of the ions, but you can predict the m/z ratio of the possible charged particles for each of these compounds.*





A trained mass spectrometrist, and eventually you (!) can interpret the masses and relative abundances of the ions in a mass spectrum and determine the structure and elemental composition of the molecule. It has been said that "*a mass spectrometrist is someone who figures out what something is by smashing it with a hammer and looking at the pieces*".

➤ 12. *Notice that water and ammonia have very similar masses. Explain how a mass spectrometer can distinguish between these two chemical substances.*

What is Isotope Ratio Mass Spectroscopy?

➤ 13. *What is meant by an isotope and the isotopic abundance of an element? Why does boron have an atomic mass of 10.81, yet no atom of boron has that mass?*

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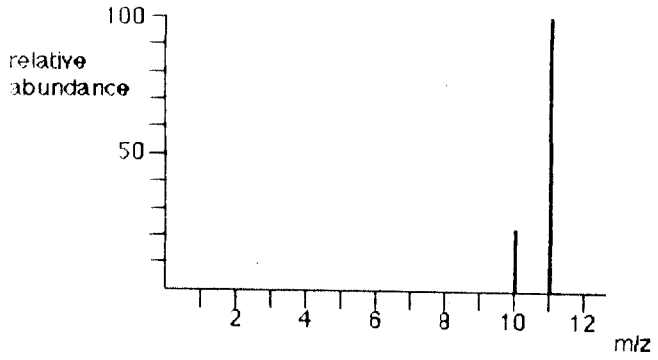
Isotopic Mass Spectrum of the Element Boron



Note that on this graph the most abundant isotope is assigned the value of 100, and the other isotope is assigned a relative value. Do not confuse this with the real % abundance.

Element Name	Element Symbol	Exact Atomic Mass	Abundance
Boron	B -10	10.012938	19.80%
Boron	B -11	11.009305	80.20 %

► 14. *Use the mass spectrum of the isotopes of zirconium found on the next page to first predict the atomic mass number of the element, and then actually calculate the atomic mass number from the masses of the isotopes.*

Element Name	Element Symbol	Exact Atomic Mass	Abundance
Zirconium	Zr -90	89.9047026	51.54 %
	Zr -91	90.9056439	11.22 %
	Zr -92	91.9050386	17.15 %
	Zr -94	93.9063148	17.38 %
	Zr -96	95.908275	2.80 %

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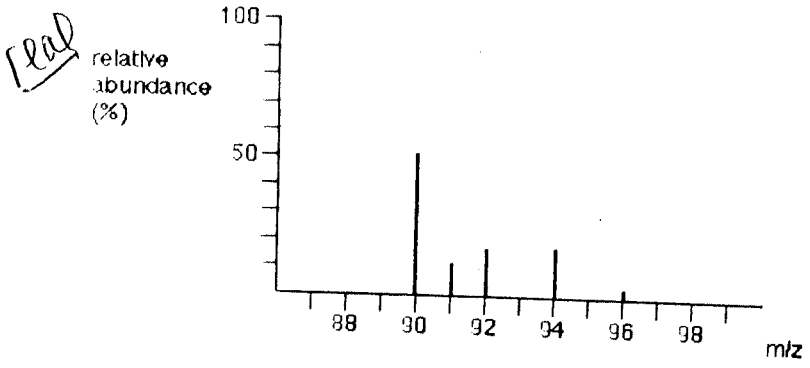
Isotopic Mass Spectrum of the Element Zirconium



What Are Some Common Elements Found in Organic Compounds?
and
What Are The Naturally Occurring Isotopes of These Elements?

(Note, numbers in parentheses are estimated and uncertain)

Element Name	Element Symbol	Exact Atomic Mass	Abundance
Carbon	C -12	12.000 000 0	98.93(8)
	C -13	13.003 354 8378(10)	1.07(8)
Hydrogen	H -1	1.007 825 032 1(4)	99.9885(70)
	H -2	2.014 101 778 0(4)	0.0115(70)
Oxygen	O -16	15.994 914 6221(15)	99.757(16)
	O -17	16.999 131 50(22)	0.038(1)
	O -18	17.999 160 4(9)	0.205(14)
Nitrogen	N -14	14.003 074 005 2(9)	99.632(7)
	N -15	15.000 108 898 4(9)	0.368(7)
Chlorine	Cl - 35	34.968 852 71(4)	75.78(4)
	Cl - 37	36.965 902 60(5)	24.22(4)
Bromine	Br - 79	78.918 3376(20)	50.69(7)
	Br - 81	80.916 291(3)	49.31(7)

Does the Presence of Isotopes Affect the Mass Spectra of Molecules?

You bet! But the good news is we will just quickly look at the effect of isotopes on mass spectra and you will not be held responsible for this level of analysis. But it is still good for you to see what is complicating the mass spectra.

So, take the simplest organic compound methane. If you had 10 000 molecules, (remember, one drop of anything would have many more molecules than this!) a mass spectrometer would record the individual masses of each of these molecules.

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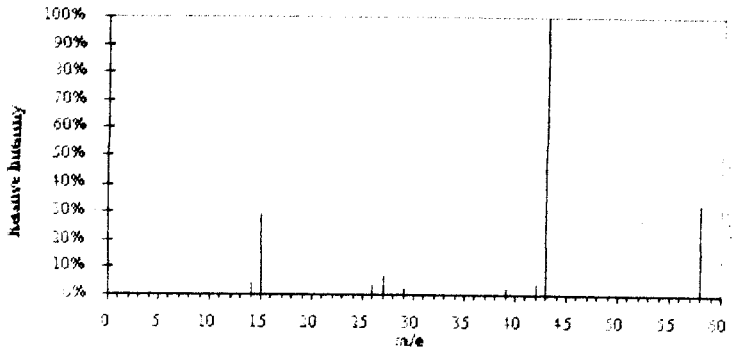
Now, would all the molecules of methane have the same mass? Unfortunately, no. Because of the existence of isotopes, the occasional methane molecule with C-13 (about 1% of all the molecules, or about 100 of the 10 000 molecules) would have larger mass, and the occasional methane molecule that had a deuterium atom (H-2) (about 0.01% or 1 molecule out of 10 000 methane molecules) would have a larger mass. Now you can imagine that with very large numbers of methane molecules, some molecules would have both a C-13 atom and an H-2 atom in the same molecule, some molecules could have two deuterium atoms, and a very small number could have a C-13 and two H-2 atoms making up the methane. All of this goes to say that when we look at the fragments of an organic compound in a mass spectrometer, we will see major peaks representing the commonly occurring isotopes, and minor peaks representing the other isotopic variations of masses.

► 15. *What masses of molecular ions could be identified in the mass spectrometer for the organic compound dichloromethane? Remember, the mass spectrometer records the actual mass of each molecule, not the average of the isotopic abundance. Using the provided table of isotopic abundances, list some possible masses for $C_2H_2Cl_2$ in the order of greatest to least frequency.*

How Do We Analyze Mass Spectra of Organic Compounds?

We have already looked at the mass spectra of simple molecules like carbon dioxide, water and ammonia. Now look at the mass spectrum of acetone, or 2-propanone or CH_3COCH_3.

Mass Spectrum of Acetone



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As you look at the above spectrum of acetone, note the following;

- ✓ The molecular ion at m/z = 58 represents the whole acetone molecule after being made into a +1 ion but without being fragmented.
- ✓ The mass spectrum shows many fragmented ions in addition to the molecular ion.
- ✓ The masses of the fragments have smaller masses around them due to isotopic abundances of the elements C, H, and O.

► 16. *Let's think about how an organic compound would most likely fracture. Not all the bonds in an organic molecule are of the same strength. Use reference materials to find the bond enthalpies of the following bonds commonly found in organic molecules. Then draw a structural formula for acetone (2-propanone) and identify where this molecule is likely to be fractured into smaller pieces.*

Bond Enthalpies of Bonds Commonly Found in Organic Molecules						
Bond type	C—C	C—H	C—O	O—H	C = O	C = C
Bond enthalpies (kJ/mol)						

What Does the Fragmentation Pattern Tell Us About an Organic Compound?

A lot! From the molecular ion we can determine the molar mass of the molecule. That is well and good, but hardly enough information to identity an organic compound. But if we include an analysis of the fragmentation pattern from the mass spectrometer, we can determine the structure of the molecule. And since structure identifies the organic compound, we can name that molecule!

► 17. *If you know an organic compound has a molecular formula of $C_5H_{12}O$ and a molar mass of 88.17 g/mol, what possible substances could this be? (I can think of two different isomeric functional groups and several different substances within each functional group family.)*

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► 18. *Draw the structures for functional group isomers ethanol and dimethyl ether. Identify one fragment each molecule would have common to each molecule, and one fragment that would characterize only one of the molecules.*

What Are Some Common Simple Fragments Found in Organic Molecules?

► 19. *The following list of fragments would be helpful to know. (Complete the mass expected to be found (considering only the most abundant isotopic species) for each of these common fragments.*

Common Fragments	$-CH_3$ Methyl group	$-C_2H_5$ Ethyl group	—CHO	$-CH_2-$	—COOH Carboxylic acid group	—OH Alcohol group
Mass of fragment						

► 20. *Imagine what a mass spectrum of ethanol looks like. What would be the m/z ratio for the molecular ion? The methyl group would break off the easiest. Then the alcohol group would break off. What would be some of the m/z ratios identified on the mass spectrum for this compound? (Do not take into consideration the isotopes of these elements)*

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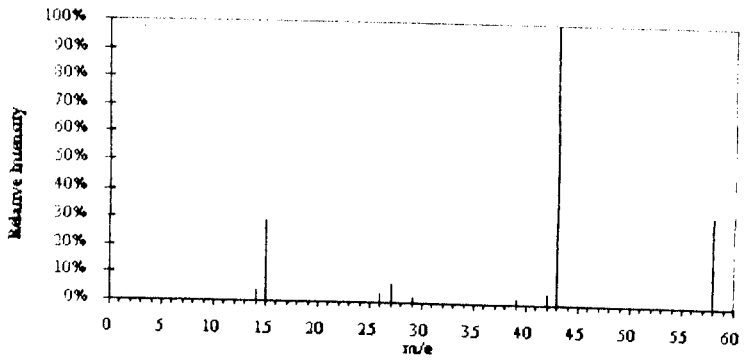
Here once again is the mass spectrum of acetone, otherwise known as 2-propanone.



► 21. *If the molecular ion represents acetone, what fragment was lost between the masses of 58 and 43? Identify what fragment broke off for the mass of 43 to be identified.*

► 22. *In the acetone MS, what fragment is represented by the mass lost between the fragment mass of 43 and the fragment mass of 27.*

How Do We Make Sense of the Fragmentation Pattern in Mass Spectra of Unknown Compounds ?

You can see how we can make sense of the fragmentation patterns in the mass spectra of organic compounds to confer structure and hence identify the substance. But with larger molecules the possible combinations of fragment losses becomes overwhelming. Consider a strand (molecule) of DNA with different fragments of amino acids being chopped off. We can actually sequence the amino acids in the protein by means of the mass spectrometer. This accomplishment, by the way, was the Nobel Prize in Chemistry awarded in 2002.

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Activity
An Analogy for Protein Amino Acid Sequencing Using the Mass Spectrometer

The Problem

A protein is one big molecule. It consists of huge numbers of amino acids bonded end-to-end in a long chain. Using the mass spectrometer, we can determine the molar mass of the protein, but the possible combinations of fragments is overwhelming. How is the sequencing of amino acids accomplished to determine the structure of a protein? Consider a small fragment of protein with a sequence of 9 amino acids. Because each one of the amino acids in the sequence could be one of 20 amino acids, the possible number of sequences is 20^9. That is 512 000 000 000 possible combinations, and you do not want to spend your evening figuring them all out. But with the aid of computers and statistical analysis, this problem can be solved. Let's look at an analogy.

Your Turn

You will need a stack of puzzle cards from the teacher for puzzles 1 and 2.

Puzzle 1

Your puzzle is a word made up of letters that have been fragmented as if they were passed through a mass spectrometer. Each word has been broken into one, two, or three-letter fragments. These have been written on cards in your stack. The idea is that the letters next to each other in the word are like atoms bonded to each other in a molecule. You should shuffle the cards in the stack, and turn one card over at a time until you can break through the code and solve the puzzle. For example, if the word is helium, you would see represented the following possible one, two, and three letter fragments; h, he, hel, e, el, eli, l, li, liu, i, iu, and ium. (This would be harder if these cards were shuffled.) Now you try puzzle #1.

Puzzle 2

This is the same idea as puzzle #1, but let's kick it up a notch.

Puzzles 1 and 2 are easier than the clues given by a mass spectrometer, because you were looking at the letters in the words, and not some property of the letters that make up the word. So now let's bring the analogy closer to a real mass spectrometer and identify a numerical property of the letter instead of the letter itself. This would be like the mass-to-charge ratio provided by the MS rather than the specific atom fragments. So we will assign each letter of the alphabet a corresponding "mass" using the first 26 prime numbers.

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	2	3	5	7	11	13	17	19	23	29	31	37	41	43	47	53	59	61	67	71	73	79	83	89	97

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A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	2	3	5	7	11	13	17	19	23	29	31	37	41	43	47	53	59	61	67	71	73	79	83	89	97

Now the next puzzles can be challenging. As an example consider the word CAT. For CAT the letter fragments are C, CA, CAT, A, AT, and T. These fragments correspond to the "masses" of 3, 4 (3 + 1), 71 (3 + 1 + 67), 1, 68 (1 + 67), and 67. Notice that the mass of 71 is complicated by the fact that "U" also has a mass of 71. When the puzzles are presented, the order of the "masses" will be in increasing mass, as in 1, 3, 4, 67, 68, and 71. In puzzles 3, and 4, you are given all the possible 1, 2, and 3 letter fragments for a word, including the entire word ("molar mass"). Puzzle 5 gives you ALL possible combinations of letter fragments. Use the table to solve each of the following puzzles.

Puzzle 3 11, 41, 71, 82, 112, 123

Puzzle 4 1, 7, 8, 37, 38, 45, 67, 74, 75, 112

Puzzle 5 3, 7, 10, 31, 37, 38, 41, 43, 71, 74, 80, 81, 84, 102, 105, 109, 111, 112, 118, 119, 121, 143, 150, 155, 186, 192, 193, 223, 230

➤ 23. *What are the answers to Puzzles 1, 2, 3, 4, and 5? Record answers by puzzles.*

➤ 24. *The letters that make up the answer to puzzle #4 came from 4 different words. What are the words? Are the fragment masses the same for each of these words? Explain.*

➤ 25. *The words TON and NOT have very different meanings in the English language. Do these two words have different fragment masses? Would this make a difference in a mass spec of a molecule contained three elements that were all different from one another?*

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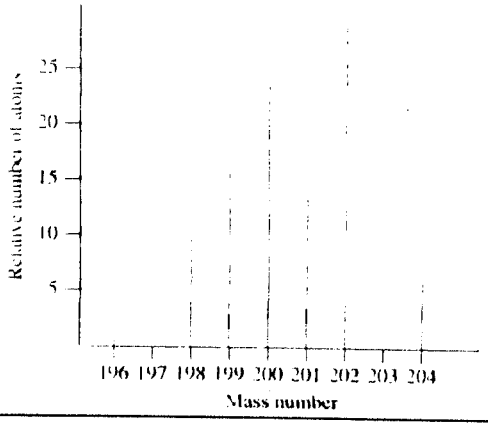
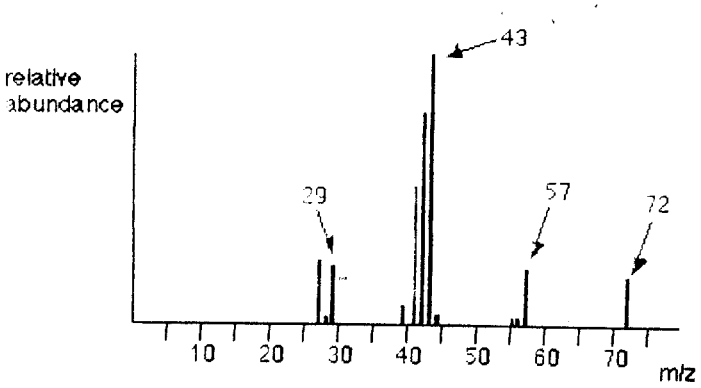
► 26. *When mercury is placed in a mass spectrometer, multiple masses are observed in the mass spectrum. How do you explain this?*

Mass Spectrum for Mercury



What Do Mass Spectra of Simple Organic Compounds Look Like?

simplified mass spectrum of pentane - $CH_3CH_2CH_2CH_2CH_3$



► 27. *What is the mass of the molecular ion in the spectrum of pentane? (We will always assume a charge of +1, so the mass/charge ratio becomes the same number as the mass.)*

It is important to realize that the pattern of lines in the mass spectrum of an organic compound tells you something quite different from the pattern of lines in the mass spectrum of an element. With an element, each line represents a different *isotope* of that element. With a compound, each line represents a different *fragment* produced when the molecular ion breaks up.

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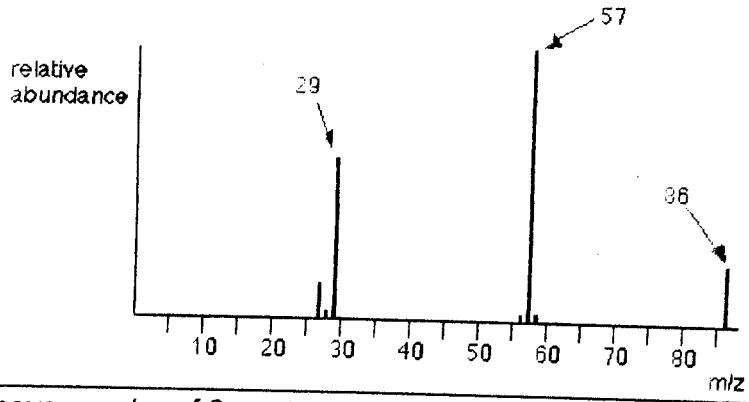
► 28. *What fragments are represented by the breaking of the molecular ion as it forms the fragments of mass 57, mass 43, and mass 29? Look at the Δ mass to determine fragments.*

► 29. *Pentane is isomeric with 2,2-dimethylpropane. Would these two compounds have the same mass of the molecular ion? Predict the fragmentation pattern you would see with 2,2-dimethylpropane.*

Here is a MS for 3-pentanone; otherwise known as pentan-3-one.

simplified mass spectrum of pentan-3-one - $CH_3CH_2COCH_2CH_3$



► 30. *In the above spectra of 3-pentanone the molecular ion has the mass of 86. Identify the loss fragments that resulted in the recorded masses of 57 and 29. Also identify and draw the fragments that are actually detected and producing the masses of 57 and 29.*

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In Summary;

Mass Spectrometry at its simplest is a technique for measuring the mass and therefore the molecular weight of a molecule. In addition, it is often possible to gain structural information about a molecule by measuring the masses of the fragments produced when molecules are fragmented in the mass spectrometer. This instrument has minimized the need for time consuming chemical and physical tests to identify functional groups and determine molar mass. When mass spectrometry in used in conjunction with other instruments such as Infrared Spectrometer and Hydrogen Nuclear Magnetic Resonance (H-NMR), chemical characterization becomes even faster and more confident. Complex molecules, such as proteins, can be readily sequenced by using the Mass Spectrometer to fragment and analyze the fragment masses. Nobel Prizes honor such recent accomplishments.

Final Scenario:
The rush of students to the care of the teacher caused the teacher to arouse from her seemingly unconscious state. It was only the grading of mountains of lab reports the night before that had diminished her quantity of required sleep. She nodded off while trying to finish her grading. On the third bob of her head before dropping off to a deep sleep, one drop of drool had dribbled from her open mouth, and sat unidentified by the side of her head.

Which goes to prove the old saying....

Old teachers never die, they just pass everyone.

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Name: _____ Block: ___ Date: _____

PAPER CHROMATOGRAPHY OF LEAF OR PETAL

Background Information:

Paper chromatography is a process that uses special filter paper to separate and identify the different substances in a mixture. Chromatography means "to write with color." The substances in the mixture dissolve in the alcohol and move up the paper. The heavier substances move up the paper more slowly. The lighter substances move up the paper more quickly. So heavy and light substances get separated from one another on the paper.

Plants contain chlorophyll, a green pigment, as well as carotenoids, pigments that range in color from red to orange to yellow.

Question: Do green leaves also contain other pigments?

Hypothesis: Record on the Analysis Sheet.

Materials:

Isopropyl alcohol
spinach leaf
chromatography paper
paperclip
beaker
ruler
scissors
pencil
tape
colored pencils

Procedure:

1. Obtain a strip of chromatography paper.

2. Use a ruler to measure and draw a light pencil line 2-cm above the bottom of the paper strip.

3. Here is the tricky part! Place the edge of the spinach leaf over the pencil line and using the edge of a coin gently press on the spinach leaf to create a single green line over the pencil line. You want this line to be thin and *concentrated* with the pigment from the spinach leaf. Therefore, repeat this edging process carefully about 3-4 times. Be sure not to press too hard or you will poke a hole through the paper.

4. Tape the top of the paper strip to a pencil so that the end of the strip with the green line hangs down. The pencil should be able to sit across the top of the beaker with the bottom of the paper strip just touching the bottom of the beaker. Cut off any excess paper from the TOP of the strip if it is too long.

5. Remove the pencil/paper strip contraption from the beaker for the moment. Record observations in data table.

6. Carefully add isopropyl alcohol to the beaker until it reaches a depth of 1-cm in the beaker.

7. Lay the pencil across the top of the beaker with the paper strip extending into the alcohol. MAKE SURE THAT THE LEVEL OF THE ALCOHOL IS BELOW THE GREEN LINE ON YOUR PAPER STRIP! IF THE ALCOHOL IS GOING TO COVER THE GREEN LINE, POUR OUT SOME ALCOHOL BEFORE YOU GET THE GREEN LINE WET!

8. Observe as the alcohol gets absorbed and travels up the paper by capillary action. This may take up to 20 minutes. Do not touch your experiment during this time.

9. When the alcohol has absorbed to approximately 1-cm below the pencil, you may remove the pencil/paper strip from the beaker to dry on your counter. With a pencil, mark the distance the alcohol has traveled on the paper, as well as the distance each pigment has traveled.

10. Using colored pencils, draw your results in the data table.

11. Using a ruler and the following formula, measure the R_f values of each pigment.

    Since the fastest molecules will travel the greatest distance, or to the highest point along the strip, the relative distances can be measured, and the flow rate (migration) of the molecules (R_f) can be calculated by using the following formula:

    $$R_f = \frac{\text{Distance pigment traveled}}{\text{Distance solvent traveled}}$$

Data:

Filter Paper	Use colored pencils to draw your observations
Before Paper Chromatography	
After Paper Chromatography	

Distance Alcohol Traveled: _____ mm

Color of Pigment	Distance Traveled (mm)	R_f value

Name: _____ Block: ____ Date: _____

PAPER CHROMATOGRAPHY OF LEAF OR PETAL

Analysis:

1. What type of plant material did you test? _____

2. What pigments did it contain? (list all)

3. For this process what is considered the solute(s)? _____

   What is considered the solvent? _____

4. Explain how paper chromatography was able to separate the different pigments.

5. How could you have expanded this lab to include concentration of pigment?

Name: _____ Block: ___ Date: _____

A Mother's Day Investigation

Paper Chromatography

Background

Parent's spend their time cleaning up behind our investigations, our trials and errors. Today we are going to prepare a Mother's Day card via our investigation into chromatography. Simply put chromatography is a separation technique that relies on the solute's particle size and its attraction to the solvent.

Although they appear as one pure color, most inks and food colorings are comprised of a mixture of pigments -- sometimes as many as eight or nine. A filter paper wedge acts as a wick, drawing water up to a full circular filter paper spotted with ink or food coloring. As the water is then absorbed outward and flows past the black spots, each of the pigments gets subjected to two opposing forces: its attraction to the filter paper, which is acting to hold the pigment in place and is known as the "stationary phase", and its attraction to the water, which is acting to pull the pigment outward along with it and is known as the "mobile phase". Due to the nature of the molecules that make them up, some of these pigments are more attracted to the filter paper than the water and thus stay more or less where they are spotted, while others are more attracted to the water than the paper and thus move out quite far. Thus, chromatography can be thought of as a race, separating out the fast pigments from the slower ones. The longer the chromatography is allowed to run, the more separated out the pigments become. If, however, the filter paper is not removed before the water reaches the edge, then the pigments, which cannot evaporate the way the water can, are deposited on the edge, one on top of the other. If given enough time, the pigments would eventually end up all together again in a circle along the entire edge of the filter paper. The color of this circle would, of course, be black. To prevent this from happening, the filter paper should be lifted up out of the water when the separation is greatest, effectively stopping the race in the middle and freezing the pigments in place.

Note that the slower pigments may have a strong attraction to both the water and the filter paper. However, the key is that the slower pigment is attracted to the paper more strongly than to the water; the slower pigment spends more time attached to the paper than dissolved in the moving water.

Procedure

1. Make sure your work space has a clean dry surface. Use pencil for any labels. (The pencil does not run.)
Obtain a piece of filter paper and poke a hole (2-3 mm diameter) through its center. Using a pencil, write your first and last names lightly along the edge of it.

2. Use a black pen to make five or six dots equally spaced out in a small circle around the center hole. The dots should be about 6-8 mm from the center.

3. Now select a second pen, a different color than the first, and use it to make dots in between the first dots. You should end up with 10-12 black dots in a circle around the center of the paper.

4. Wrap 1/3 of a paper towel around the base of a wooden skewer. Insert the skewer through the center hole. Fill a cup with water to within about a centimeter of the top, and dry the rim with a paper towel. Then, carefully place the filter paper on top of the cup so that the base of the paper towel extends down into the water and lightly touches the filter paper.

5. Go on to other parts of the separation lab while the water soaks into the paper.....

6. Once the water has spread to within 1-2 cm of the edge, carefully lift up the filter paper and set it on an empty cup to dry.

7. Drying maybe quickened by the use of the hair dryer. Once your paper is dry you may take a photo with your device to help you evaluate the changes at a later time.

8. Colored paper is available, along with coloring utensils so that you may make a Thank You card to your parents/ guardians, or a Mother's Day card for Mom, Grandmother or the other important lady in your life.

Data:

Using colored pencils illustrate the changes to the black ink spot.

Questions: (The questions below must be answered based on the black visa-vi pen.)

1. What is the solute(s) for this lab? _____
2. What is the solvent for this lab? _____
3. Which color had the strongest attraction to the solvent? How do you know?
4. Which color had the weakest attraction to the solvent?
5. Why might black ink be composed of many colors?

Isolation of Chlorophyll and Carotenoid Pigments from Spinach

Adapted from: Pavia, D.L.; Lampman, G.M.; Kriz, G.S.; Engel, R.G. *Introduction to Organic Laboratory Techniques: A Microscale Approach 3rd Edition* Saunders College Publishing: New York, NY, 1999 and also Quach, H.T.; Steeper, R.L.; Griffin, G.W., *J. Chem. Educ.*, **2004**, *81*, 385-387; http://www.uwlax.edu/faculty/koster/spinach.htm

BACKGROUND:

The leaves of plants contain a number of colored pigments generally falling into two categories, chlorophylls and carotenoids. Chlorophylls *a* and *b* are the pigments that make plants look green. These highly conjugated compounds capture the non-green light energy used in photosynthesis.

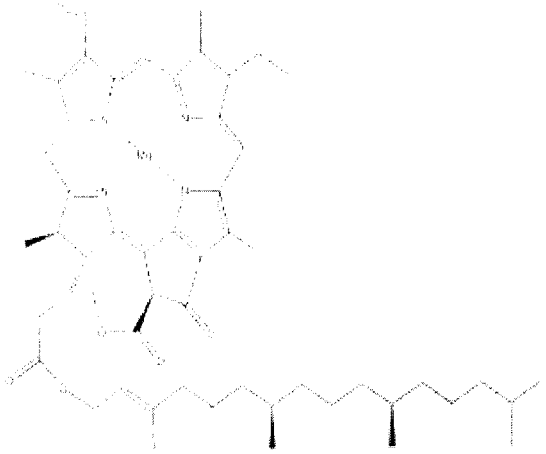
Carotenoids are part of a larger collection of plant derived compounds called terpenes. These naturally occurring compounds contain 10, 15, 20, 25, 30 and 40 carbon atoms which suggests that there is a compound with 5 carbons that serves as their building block. Their structures are consistent with the assumption that they were made by joining together isoprene units, usually in a "head" to "tail" fashion. Isoprene is the common name for 2-methyl-1,3-butadiene. The branched end is the "head" and the unbranched is the "tail". That isoprene units are linked in such a way to form terpenes – isoprene rule. Carotenoids are terpenes (eight isoprene units). Lycopene, the compound responsible for the red coloring of tomatoes and watermelon, and β-carotene the causes carrots and apricots to be orange, are examples of carotenoids.

B-carotene is also the coloring agent used in margarine. When ingested β-carotene is cleaved to form two molecules of vitamin A and is the major dietary source of the vitamin. Vitamin A, also called retinol, plays an important role in vision.

Spinach leaves contain chlorophyll *a* and *b* and β-carotene as major pigments as well as smaller amounts of other pigments called xanthophyll which are oxidized versions of carotenes and pheophytins which look like chlorophyll except that the Mg^{2+} has been replaced by two H^{+}. In this experiment we will isolate and separate the spinach pigments using differences in polarity to effect the separation. Since the pigments have different colors we can watch this process visually. The structures of the major components are pictured below. Notice that since β-carotene is a hydrocarbon it is very non-polar. Both chlorophylls contain C-O and C-N bonds which are polar and also contain magnesium bonded to nitrogen which is a polar bond (almost ionic!). Both chlorophylls are much more polar than β-carotene. If you look carefully at the two chlorophylls you can see that they only differ in one spot. Chlorophyll *a* has a methyl group where chlorophyll *b* has an aldehyde. This makes chlorophyll *b* slightly more polar than chlorophyll *a*. After we isolate the pigment mixture from the leaves in a hexane solution we will use the difference in polarity to separate the various pigments using column chromatography. We will analyze the fractions using spectroscopy.

Column Chromatography

Column chromatography involves the separation of compounds by the differences in portioning between the mobile and stationary phases. It is like other methods in the stationary phase in that a stationary phase is place in a support through which the mobile phase is passed. The stationary phase acts as an adsorbent. Many compounds with varying functional groups may be used as the stationary phase and several types of interactions can aid in developing the desired separation (*ie*. Hydrogen bonding, electrostatic interactions, Van der Waals forces, etc.) The major advantages of the column chromatography are its ability to handle large quantities of material and the ability to change the eluting solvent throughout the course of the elution. This allows one to remove one component while a desired product remains essentially unmoved. Then a solvent change moves the desired product through the column. Solvent changes may include such things as changes in polarity, changes in pH, or changes in ionic strength. The last two are largely used in biological separations. Thus, by varying the stationary and by changing the solvent or solvent systems, an efficient separation maybe achieved. In this experiment the alumina separates components primarily on the basis of polarity; the more polar components are held to the alumina more tightly and therefore move through the system more slowly. Increasing the polarity of the solvent moves all the components through more quickly but has the largest effect on polar compounds.

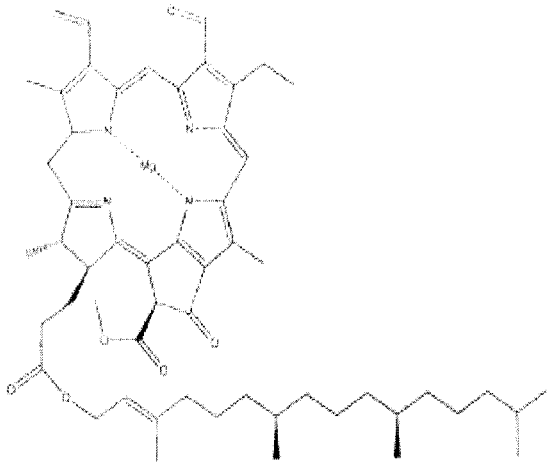


Chlorophyll a

Blue-green, polar

$C_{55}H_{72}MgN_4O_5$

M. W. 893.5026

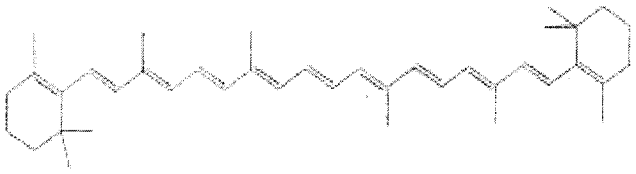


Chlorophyll b

Green, polar

$C_{55}H_{70}MgN_4O_6$

M. W. 907.4862

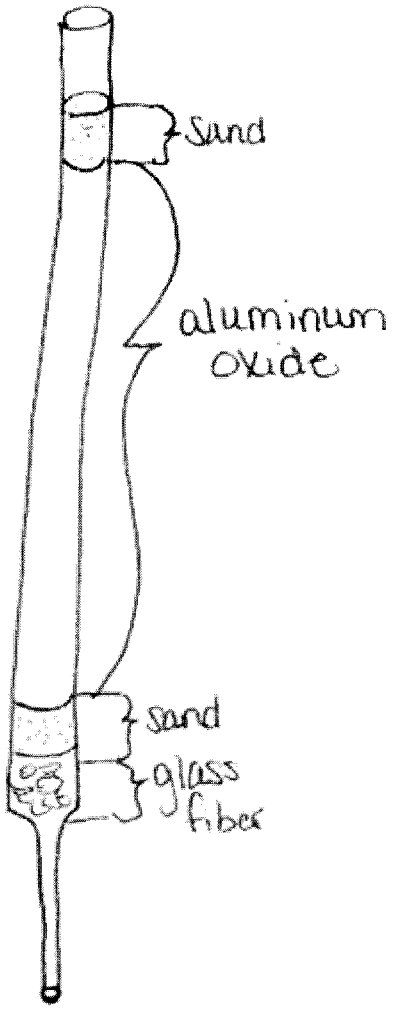


β-Carotene

yellow, nonpolar

$C_{40}H_{56}$

M.W. 536.8824



MATERIALS:

Acetone	Spinach Leaves (fresh)
Pentane	Mortar and Pestle
Sand	Glass Wool
Anhydrous magnesium sulfate	Aluminum oxide (can be substituted with $CaCl_2$ or florisil)
Glass vials	Centrifuge tubes (with caps)
Pasteur pipettes	Spectro-Vis / Logger Pro
Vernier Quest	

PROCEDURE:

Pigment Extraction

1. Obtain approximately 1.0 g of fresh spinach (do not use the stems).
2. Place in a mortar along with 1.0 g of anhydrous magnesium sulfate and 2.0 g sand. Grind with the pestle until a light green powder is obtained. (5-10 minutes)
3. Transfer the mixture to a centrifuge tube and add 2.0 ml of acetone.
4. Rinse the mortar and pestle with another 2.0 ml of acetone and add to the centrifuge tube.
5. Cap and shake the mixture, release pressure by opening cap over sink and facing away from everyone, re-cap and allow to stand for 10 minutes.
6. Shake again and centrifuge tubes for 5 minutes.
7. Using a Pasteur pipette, carefully transfer the liquid portion into a glass vial.
8. Rinse the solid in the centrifuge tube with 1.o ml of acetone and transfer this liquid to the glass vial.
9. Label the glass vial with your name and "EXTRACT".
10. Place your open glass vial in the rack in the fume hood where the liquids will be evaporated.
11. Once your vial is dry add 1.0 ml of hexane, swirl to re-suspend the extract. Observe for any water that may be left (it will be more dense and gather at the bottom of the vial). If any water appears to be present pipette just the hexane layer into a new vial. (Labelled!)

Preparation of the Column Chromatography Chamber

12. Obtain a clean dry Pasteur pipette, a very small plug of glass wool and a thin copper wire.
13. Use the copper wire to push the glass wool into the tip area of the pipette. (DO NOT push too hard!)
14. Add a 0.5 cm thin layer of sand on top of the glass wool.
15. Add Aluminum oxide until the pipette is 2/3 full, gently tapping along the way to pack and level the material.
    *The tighter the aluminum oxide is packed the better the separation will be, but the longer it will take.
16. Add 0.5 cm layer on next, carefully ensuring that it does not mix with the aluminum oxide. Gently tap to achieve a flat surface.

Elution

****Once this process has started it cannot be stopped, ensure that you have sufficient time to continue.****

17. Obtain 5 ml of the stock pentane solution, 5 ml of the 90% pentane – 10% acetone stock solution, 10 ml of the 70% pentane – 30% acetone stock solution and 5 ml of acetone. Be sure they are labelled and ready.
18. Label vials 1, 2, and 3, and a waste beaker.
19. Attach your column to a ring stand and place the waste beaker under it.
20. Slowly begin adding 2.0 ml of hexane, do not disturb the surface layer of sand.
    *once the process has begun the column must remain "wet" with a solvent at all times.
21. When the last of the 2.0 ml of hexane has reached the surface of the sand layer you may add 0.5ml of your extract.
22. When the last of the extract has reached the surface of the sand add 0.5 ml of pentane and drain to the sand.
23. Add the 90/10 mixture, continue adding until the yellow band is ready to be collected. (allow first yellow drop into waste beaker before switching)
24. Switch to vial number 1 and continue adding 90/10 mixture until all of the yellow band has been collected, switch back to waste beaker. *WATCH CAREFULLY*
25. When the 90/10 has reached the surface of the sand you may now begin adding 70/30 mixture, this should begin moving the green band down the column.
26. When the green band is ready to collect switch to vial two. (If you are running low on 70/30 mixture you may switch to pure acetone.)
27. If you need to use vial 3 for this band you may. Once the majority of this band has been collected switch back to the waste beaker and allow the remaining solvent to drain out.
28. If you do not have time to complete the spectroscopy cap your vials and wrap with parafilm.

Spectroscopy

29. Attach the Vernier Quest attach the Spectro-Vis and calibrate the Spectro-Vis.
30. Be sure to use the quartz cuvette, not the plastic ones, add water to a cuvette and rub the blank on the Spectro-Vis.
31. Add the contents of vial one, the β-carotene, to a cuvette. Insert into Spectro-Vis and click "run".
32. Click "Stop" and record the wavelength of the maximum peak.
33. Illustrate the curve on the graphs below.
34. Rinse the cuvette thoroughly with distilled water, add the contents of vial two, chlorophyll, to the cuvette. Insert into the cuvette and click "run".
35. Click "Stop" and record the wavelength of the maximum peak.
36. Illustrate the curve on the graph below.
37. If you have a 3rd vial: Rinse the cuvette thoroughly with distilled water, add the contents of vial two, chlorophyll, to the cuvette. Insert into the cuvette and click "run".
38. Click "Stop" and record the wavelength of the maximum peak.
39. Clean up all materials and return to proper storage area.

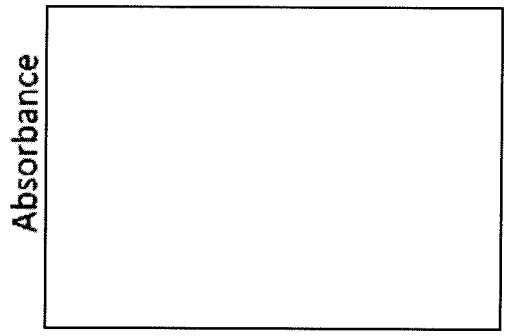
DATA TABLE:

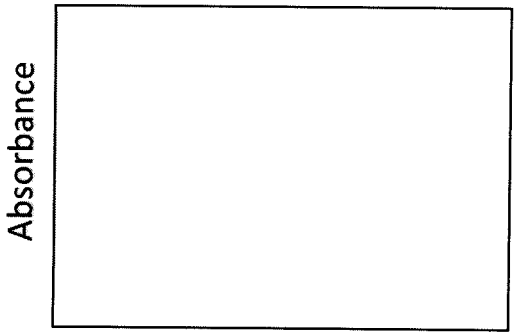
Volume of β-carotene collected: _____ml

Volume of chlorophyll collected: _____ml

Wavelength for maximum peak of β-carotene: _____nm

Wavelength of maximum peak(s) of chlorophyll _____nm and _____nm





DISCUSSION:

Answer the following discussion questions on a separate sheet of paper.

1. Explain the meaning and purpose of an adsorbent material.
2. If we wanted to separate chlorophyll *a* from chlorophyll *b*, what would you propose as the next elution? You may change either the stationary or mobile phases. Explain your choice.
3. B-carotene is yellow, and it absorbs in the blue region, λ_{max} = 450nm. How does this compare the maximum wavelength for your sample? Explain what may have made your results differ.
4. Both chlorophylls are green, and absorb in the red and blue regions. Chlorophyll *a* is more likely to have a greater peak at λ_{max} = 665nm and chlorophyll *b* will have a greater peak at λ_{max} = 480nm. Which of the two chlorophylls do you think your sample contained more of? Why?
5. Using the information from question number 4, how do the known wavelengths compare the maximum wavelength(s) for your sample? Explain what may have made your results differ.

Name: _____ **Block:** ____ **Date:** _____

Lab: An Investigation into "Quantitative Chromatography"

Purpose: The purpose of this lab is to investigate how concentration variations of the same chemical affect the results of chromatography.

Materials:

4 strips of chromatography paper	Containers of various metallic ions	300 ml beaker
9.0 ml Acetone	Capillary tubes	Plastic Wrap
1.0 ml 6M HCl	Concentrated Ammonia	Ruler & Pencil

Procedure:

1. Obtain 4 strips of chromatography paper, each 6-7 inches in length.
2. Mark the base of the strip with a line at 1.0 cm from the bottom and place a "X" in the middle of the line.
3. Label the strips 1 to 4 and the names of your group at the opposite end of the strip. (do not write further than 2 cm down the strip)
4. Choose one of the metallic ions from last lab to inoculate the strips.
5. On strip #1 use the capillary tube to "spot" the chromatography paper at the "X" until the spot is 3-4mm in diameter, allow this drop to dry. Repeat this step 3 more times. (for a total of four drops)
6. On strip #2 use the capillary tube to "spot" the chromatography paper at the "X" until the spot is 3-4mm in diameter, allow this drop to dry. Repeat this step 5 more times. (for a total of six drops)
7. On strip #3 use the capillary tube to "spot" the chromatography paper at the "X" until the spot is 3-4mm in diameter, allow this drop to dry. Repeat this step 7 more times. (for a total of eight drops)
8. On strip #4 use the capillary tube to "spot" the chromatography paper at the "X" until the spot is 3-4mm in diameter, allow this drop to dry. Repeat this step 9 more times. (for a total of ten drops)
9. While the strips are drying prepare the developing chamber by adding 1.0 ml of 6 M HCl to 9.0 ml Acetone in a 300 ml beaker. Swirl gently to mix.
10. Obtain a piece of plastic wrap large enough to cover beaker.
11. Place the four strips into the beaker of eluent so that only the bottom edge touches the liquid, fold the upper edge of the strip over the edge of the beaker. Cover with plastic wrap.
12. Allow the eluent to travel up the chromatography paper until it is approximately 2.0 cm from the upper edge (where the fold is).
13. Remove the strips and mark the eluent line.
14. Dry the strips and mark any coloration that you may see.
15. Enhance the coloration by placing in the enhancement chamber (already prepared by your teacher) for two minutes. Remove and make any additional notations necessary.
16. Staple or tape all four strips to a half sheet of notebook or computer paper.

Data:

Metallic ion used: _____mm____

Strip #	# of drops applied	Color	Depth of color	Color distance	Eluent distance	R_f Value	Color Band Width

Data Analysis:

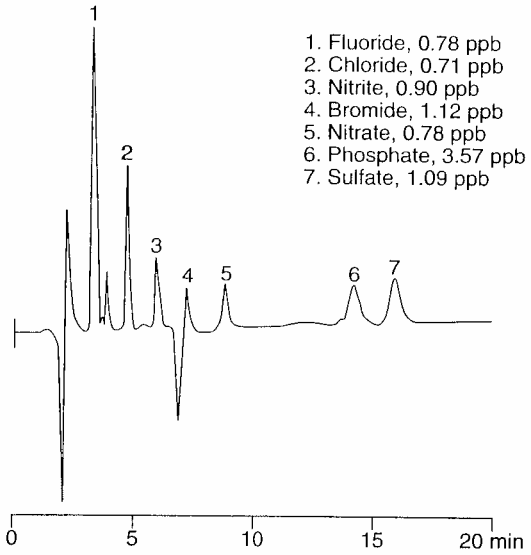
1. What did you notice that was the same for all four strips?

2. What did you notice that was different for the strips?

3. Was there a pattern? What do you think could be interpreted from this pattern?

4. How could you use the information collected to determine concentration of the samples?

5. Are there any changes or improvements that you would make to this lab that would help clarify the concept for you?



Trace levels of anions in high purity water can be determined by chromatography.

Experiment 4

Paper Chromatography

OBJECTIVES

- To become familiar with chromatography, a technique for separating the components of a mixture
- To separate a mixture of transition metal cations by paper chromatography

TECHNIQUES

The following techniques are used in the Experimental Procedure

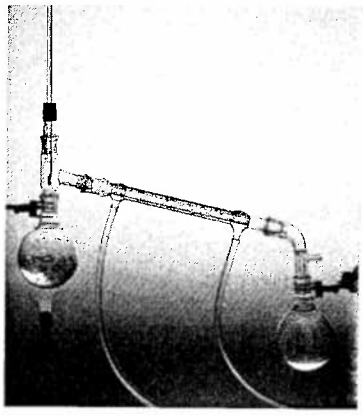
INTRODUCTION

Most substances found in nature, and many prepared in the laboratory, are impure; that is, they are a part of a mixture. One goal of chemical research is to devise methods to remove impurities from the chemical of interest.

A **mixture** is a physical combination of two or more pure substances wherein each substance retains its own chemical identity. For example, each component in a sodium chloride/water mixture possesses the same chemical properties as in the pure state: water consists of H_2O molecules, and sodium chloride is sodium ions, Na^+, and chloride ions, Cl^-.

The method chosen for separating a mixture is based on the differences in the chemical and/or physical properties of the components of the mixture. Some common *physical* methods for separating the components of a mixture include:

- **Filtration:** removing a solid substance from a liquid by passing the suspension through a filter (See Techniques 11B–E and Experiment 3 for details.).
- **Distillation:** vaporizing a liquid from a solid (or another liquid) and condensing the vapor (See margin photo.).
- **Crystallization:** forming a crystalline solid by decreasing its solubility by cooling the solution, evaporating the solvent, or adding a solvent in which the substance is less soluble (see Experiment 15.)
- **Extraction:** removing a substance from a solid or liquid mixture by adding a solvent in which the substance is more soluble (see Experiment 11.)



A distillation apparatus.

- **Centrifugation:** removing a substance from a mixture using a centrifuge (See Technique 11F for details.)
- **Sublimation:** vaporizing a solid and recondensing its vapor (not all solids sublime, however)
- **Chromatography:** separating the components of a mixture on the basis of their differing adsorptive tendencies on a stationary phase

Mobile phase: the phase (generally liquid) in which the components of the mixture exist

Eluent: the solvent in which the components of the mixture are moved along the stationary phase

Stationary phase: the phase (generally solid) to which the components of the mobile phase are characteristically adsorbed

In the chromatography[1] technique, two phases are required for the separation of the components (or compounds) of a mixture, the mobile phase and the stationary phase. The **mobile phase** consists of the components of the mixture and the solvent—the solvent being called the **eluent** or **eluting solution** (generally a mixture of solvents of differing polarities). The **stationary phase** is an adsorbent that has an intermolecular affinity not only for the solvent,[2] but also for the individual components of the mixture.

As the mobile phase passes over the stationary phase, the chromatogram develops. The components of the mobile phase have varying affinities for the stationary phase. The components of the mixture having a stronger affinity for the stationary phase move shorter distances while those with a lesser affinity move greater distances along the stationary phase during the time the chromatogram is being developed. Separation, and subsequent identification, of the components of the mixture is thus achieved. The leading edge of the mobile phase in the chromatogram is called the **eluent front.** When the eluent front reaches the edge of the chromatographic paper, the developing of the chromatogram is stopped.

Of the different types of chromatography including, for example, gas–solid chromatography, liquid–solid chromatography, column chromatography, thin-layer chromatography, and ion chromatography, this experiment uses paper chromatography as a separation technique.

In this experiment, chromatographic paper (similar to filter paper), a paper that consists of polar cellulose molecules, is the stationary phase. The mobile phase consists of one or more of the transition metal cations, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, dissolved in an acetone–hydrochloric acid eluent.[3] In this experiment, the chromatographic paper is first "spotted" (and marked with a pencil *only*) with individual solutions for *each* of the cations and then for a solution with a mixture of cations. The paper is then dried. The spotted chromatographic paper is then placed in contact with the eluent to form the mobile phase. By **capillary action** the transition metal ions are transported along the paper. Each transition metal has its own (unique) adsorptive affinity for the polar, cellulose chromatographic paper; some are more strongly adsorbed than others. Also, each ion has its own solubility in the eluting solution. As a result of these two factors, some transition metal ions move further along the chromatographic paper than others to form **bands** at some distance from the origin, therefore indicating that the ions are separating.[4]

Capillary action: action by which the ions have an adhesive attraction (ion-dipole attractive forces) to the fibers of the stationary phase (paper)

Band: the identifying position of the component on the chromatography paper

For a given eluting solution, stationary phase, temperature, and so on, each ion is characterized by its own R_f (ratio of fronts) factor:

$$R_{f,\,\text{ion}} = \frac{\text{distance from origin to final position of ion}}{\text{distance from origin to eluent front}} = \frac{D_{\text{ion}}}{D_{\text{solvent}}} \qquad (4.1)$$

[1] *Chromatography* means "the graphing of colors" where historically this technique was used to separate the various colored compounds from naturally occurring colored substances (e.g., dyes, plant pigments).

[2] This attraction is due to the similar type of intermolecular forces between the adsorbent (or the stationary phase) and the components of the mobile phase (e.g., dipole–dipole, ion–dipole, dipole–hydrogen bonding).

[3] The eluting solution (acetone–hydrochloric acid) actually converts the transition metal ions to their chloro ions, and it is in this form that they are separated along the paper.

[4] Consider, for example, a group of students moving through a cafeteria food line: the stationary phase is the food line; the mobile phase consists of students. You are well aware that some students go through the food line quickly because they are less attracted to the various food items. Others take forever to pass through the food line because they must stop to consider each item; they thus have a longer residence time in the food line.

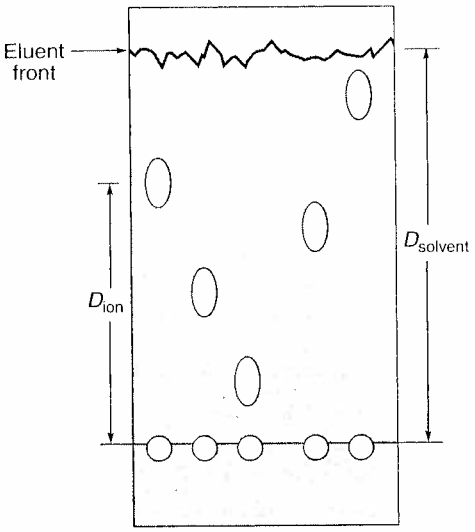


Figure 4.1 Determination of the R_f value for a transition metal ion.

The origin of the **chromatogram** is defined as the point where the ion is "spotted" on the paper. The eluent front is defined as the most advanced point of movement of the mobile phase along the paper from the origin (Figure 4.1).

Chromatogram: the "picture" of the separated components on the chromatography paper

The next step is to identify the exact position of these bands. Some transition metal ions are already colored; for others, a characteristic reagent for each metal ion is used to enhance the metal ion's appearance and location on the stationary phase.

In this experiment you will prepare a chromatogram for each transition metal ion, locate its characteristic band, and determine its R_f value. A chromatogram of a test solution(s) will also be analyzed to identify the transition metal ions present in a solution mixture.

EXPERIMENTAL PROCEDURE

Procedure Overview: Chromatographic paper is used to separate an aqueous mixture of transition metal cations. An eluent is used to move the ions along the paper; the relative solubility of the cations in the solution versus the relative adsorptivity of the cations for the paper results in their separation along the paper. An enhancement reagent is used to intensify the appearance of the metal cation band on the paper.

Obtain about 2 mL of three unknown cation mixtures from your instructor in carefully marked 75-mm test tubes. Record the number of each unknown on the second page of Report Sheet.

A. Preparation of Chromatography Apparatus

1. **Developing Chamber.** Obtain a 600-mL beaker and enough plastic wrap (e.g., Saran Wrap®) for a cover (Figure 4.2). In the fume hood, prepare 10 mL of an eluting solution that consists of 9 mL of acetone and 1 mL of 6 *M* HCl. (**Caution:** *Acetone is flammable; extinguish all flames; HCl is corrosive.*) Pour this eluent into the middle of the beaker using a stirring rod; be careful not to wet the sides of the beaker. The depth of the eluent in the beaker should be 0.75–1.25 cm but *less than* 1.5 cm. Cover the beaker with the plastic wrap for about 10 minutes.[5] Hereafter, this apparatus is called the **developing chamber.**

   Figure 4.3 shows a commercial developing chamber.

2. **Ammonia Chamber.** The ammonia chamber may already be assembled in the hood. *Ask your instructor.* Obtain a dry, 1000-mL beaker and place it in the *fume hood.* Pour 5 mL of conc NH_3 into a 30-mL beaker and position it at the center of the 1000-mL beaker (Figure 4.4). (**Caution:** *Do not inhale the ammonia fumes.*)

Figure 4.2 Use plastic wrap to cover the developing chamber.

[5]The "atmosphere" in the beaker should become saturated with the vapor of the acetone/HCl eluent.

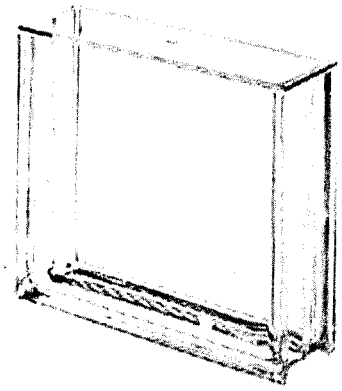


Figure 4.3 A commercial developing chamber.

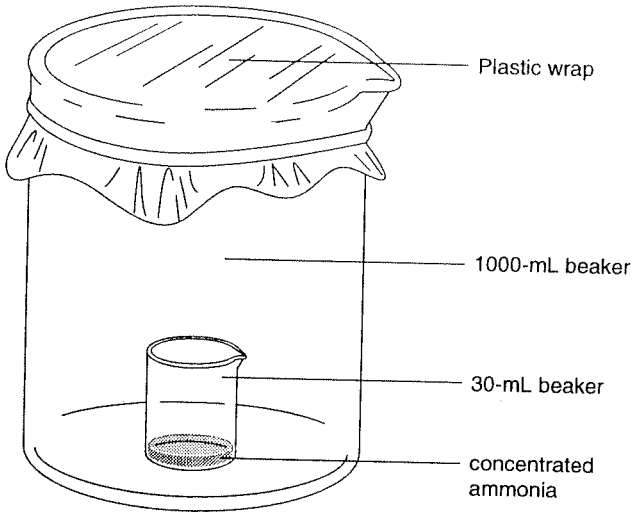


Figure 4.4 Apparatus for the ammonia chamber.

Cover the top of the 1000-mL beaker with plastic wrap. This apparatus will be used in Part C.3.

3. **Capillary Tube.** Obtain eight capillary tubes from the stockroom. When a glass capillary tube touches a solution, the solution should be drawn into the tip. When the tip is then touched to a piece of filter paper it should deliver a microdrop of solution. Try "spotting" a piece of filter paper with a known solution or water until the diameter of the drop is only 2–4 mm.
4. **Stationary Phase.** Obtain one piece of chromatographic paper (approximately 10 × 20 cm). Handle the paper only along its top 20-cm edge (by your designation) and lay it flat on a clean piece of paper, *not* directly on the lab bench. Draw a *pencil* line 1.5 cm from the *bottom* 20-cm edge of the paper (Figure 4.5). Starting 2 cm from the 10-cm edge and along the 1.5-cm line, make eight X's with a 2-cm separation. Use a pencil to label each X *below* the 1.5-cm line with the five cations being investigated and unknowns U1, U2, and U3.

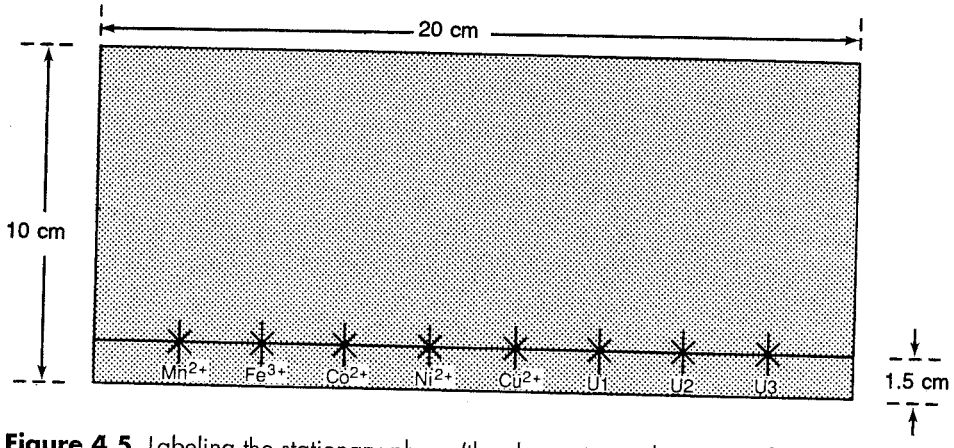


Figure 4.5 Labeling the stationary phase (the chromatographic paper) for the chromatogram.

B. Preparation of the Chromatogram

1. **Spot the Stationary Phase with the Knowns and Unknown(s).** Using the capillary tubes (remember you'll need *eight* of them, one for each solution) "spot" the chromatographic paper at the marked X's with the five known solutions containing the cations and the three unknown solutions. The microdrop should be 2–4 mm in diameter. Allow the "spots" to dry; a heat lamp or hair dryer may be used to hasten the drying—do *not* touch the paper along this bottom edge.

   Repeat the spotting/drying procedure two more times in order to increase the amount of metal ion at the "spot" on the chromatographic paper. Be sure to dry the sample between applications. Dry the paper with **caution:** *The heat lamp or hair dryer is hot!*

2. **Prepare the Stationary Phase for Elution.** Form the chromatographic paper into a cylinder and, near the top, attach the ends with tape, a staple, or small paper clip; do *not* allow the two ends of the paper to touch (Figure 4.6). Be sure the spots are dry and will *not* come into direct contact with the eluting solution!

3. **Develop the Chromatogram.** Place the paper cylinder into the developing chamber (Part A.1). The entire "bottom" of the cylindrical chromatographic paper must sit on the bottom of the developing chamber. Do *not* allow the paper to touch the wall. Make certain that the eluent is *below* the 1.5-cm line. Replace the plastic wrap. Do not disturb the developing chamber once the paper has been placed inside.

   When the eluent front has moved to within 1.5 cm of the top of the chromatographic paper remove the plastic wrap.

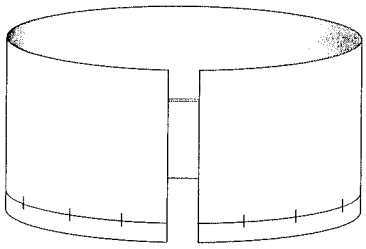


Figure 4.6 Formation of the stationary phase for placement in the developing chamber.

C. Analysis of the Chromatogram

1. **Detection of Bands.** Remove the paper from the developing chamber and *quickly* mark (with a pencil) the position of the *eluent front.*[6] Allow the chromatogram to dry. While the chromatogram is drying, cover the developing chamber with the plastic wrap. Analyze the paper and circle (with a pencil) any colored bands, those from the solutions containing the known cations and those from the unknown solutions.[7]

2. **Enhancement of the Chromatogram.** To enhance the appearance and locations of the bands, move the chromatogram to the fume hood. Position the paper in the ammonia chamber (Part A.2) and cover the 1000-mL beaker with the plastic wrap.

   After the deep blue color of Cu^{2+} is evident, remove the chromatogram and circle any new transition metal ion bands that appear. Table 4.1 identifies the colors of other cations in the presence of ammonia. Mark the *center* of each band with a pencil. Allow the chromatogram to dry.

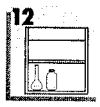


Table 4.1 Spot Solutions That Enhance the Band Positions of the Various Cations

	Cation				
	Mn^{2+}	Fe^{3+}	Co^{2+}	Ni^{2+}	Cu^{2+}
NH_3 test	Tan	Red-brown	Pink (brown)	Light blue	Blue
Spot solution	0.1 *M* $NaBiO_3$ (acidic) (purple)	0.2 *M* KSCN (blood red)	Satd KSCN in acetone (blue-green)	0.1 *M* NaHDMG (brick-red)	0.2 *M* $K_4[Fe(CN)_6]$ (red)

3. **Band Enhancement** (Optional, seek advice from your instructor). The "exact" band positions for the known cations and those of the mixture may be better defined using a second "spot solution." As necessary, use a capillary tube to spot the center of each band (from the known and unknown test solutions) with the corresponding spot solution identified in Table 4.1. This technique more vividly

[6]Do this quickly because the eluent evaporates.

[7]For the unknown solutions, there will most likely be more than one band.

locates the position of the band. Remember, the unknown test solution(s) may have more than one cation band.

4. **Analysis of Your Chromatogram.** Where is the center of each band? Mark the center of each band with a pencil. What is the color of each transition metal ion on the chromatogram?

   Calculate and record the R_f values for each transition metal ion.

5. **Composition of the Unknown(s).** Look closely at the bands for your unknown(s). What is the R_f value for each band in each unknown? Which ion(s) is(are) present in your unknown(s)? Record your conclusions on the Report Sheet. Submit your chromatogram to your instructor for approval.

Disposal: Dispose of the eluting solution in the "Waste Organics" container. Allow the conc NH_3 in the ammonia chamber to evaporate in the hood.

The Next Step

Ink is a mixture of dyes. (1) Research the use of a chromatographic technique for the separation of the dyes in ink. (2) Design a project to separate the components of leaves from different trees, shrubs, and/or grasses. (3) Design a project for the separation of amino acids in fruit juices.

ATTACH OR SKETCH THE CHROMATOGRAM THAT WAS DEVELOPED IN THE EXPERIMENT

Experiment 4 *Prelaboratory Assignment*

Paper Chromatography

Date _____ Lab Sec. _____ Name _____ Desk No. _____

1. a. Define the mobile phase in chromatography.

   b. What is the chemical composition of the mobile phase in this experiment?

2. a. Define the stationary phase in chromatography.

   b. What is the stationary phase in this experiment?

3. a. Define the eluent in chromatography.

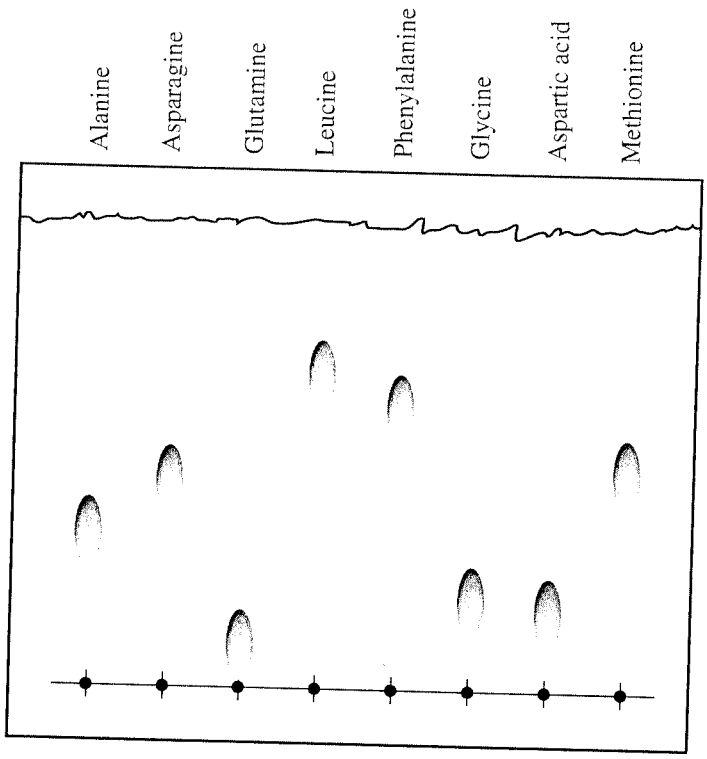
   b. What is the eluent (eluting solution) in this experiment?

4. Experimental Procedure, Part A. Distinguish between the functions of the developing chamber and the ammonia chamber.

5. Experimental Procedure, Parts A.4 and B.1. Specifically, what must be done to prepare the stationary phase for the developing chamber in this experiment?

6. The chromatogram for the separation of amino acids using a butanoic acid/acetic eluent is sketched at right.

   Use a ruler to determine and calculate the R_f for each amino acid.



7. A student developed a chromatogram and found that the eluent front traveled 69 mm and the Zn^{2+} cation traveled 24 mm. In the development of a chromatogram of a mixture of cations, the eluent front traveled 52 mm. If Zn^{2+} is a cation of the unknown mixture, where will its band appear in the chromatogram?

Experiment 4 *Report Sheet*

Paper Chromatography

Date _____ Lab Sec. _____ Name _____ Desk No. _____

C. Analysis of the Chromatogram

Distance of eluent front from the origin: _____ mm

	Color (original)	Color (with NH_3)	Color (with spot solution)	Distance (mm) Traveled	R_f
Mn^{2+}					
Fe^{3+}					
Co^{2+}					
Ni^{2+}					
Cu^{2+}					

Instructor's approval of chromatogram _____

Show your calculations for R_f.

Unknown number(s) U1=_____ U2=_____ U3=_____

Unknown	Band 1 Distance (mm) Traveled	R_f	Band 2 Distance (mm) Traveled	R_f	Band 3 Distance (mm) Traveled	R_f	Band 4 Distance (mm) Traveled	R_f
U1								
U2								
U3								

Show your calculations for R_f.

Cations present in U1				
Cations present in U2				
Cations present in U3				

Laboratory Questions

Circle the questions that have been assigned.

1. Part A.1 and Part B.3.
   a. Why is it important to keep the developing chamber covered with plastic wrap during the development of the chromatogram?
   b. The developing chamber is *not* covered with plastic wrap during the development of the chromatogram. How does this technique error affect the D_{ion} for a cation? Explain.
2. Part A.4. Why was a pencil used to mark the chromatogram and not a ballpoint or ink pen?
3. Part B.1. Explain why the cation samples are repeatedly "spotted" and dried on the chromatographic paper.
4. Part B.3. The eluent is to be below the 1.5-cm line on the chromatographic paper. Describe the expected observation if the eluent were above the 1.5-cm line.
5. Part C.2. Explain why the *center* of the band is used to calculate the R_f value for a cation rather than the leading edge of the band.
6. Part C.2. The ammonia chamber is to be covered during the enhancement of the chromatogram. What is the consequence of the ammonia chamber being left uncovered?
7. Suppose two cations have the same R_f value. How might you resolve their presence in a mixture using paper chromatography?