

SEPARATION OF A MIXTURE BY COLUMN CHROMATOGRAPHY

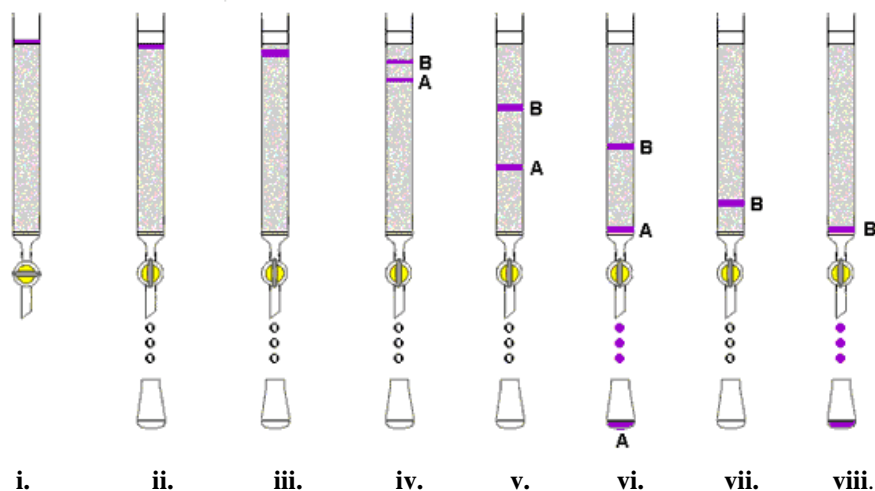
Review From General Chemistry review: *polarity, vapor pressure; London dispersion forces; dipole-dipole interactions; hydrogen bonding; intermolecular forces;*

Introduction

Chromatography is a technique used to analyze, identify, and/or separate mixtures of compounds. There are several types of chromatography techniques, for example, column, thin-layer, paper, gas, and liquid chromatography. All types of chromatography, however, have two common features: a mobile phase which is gaseous or liquid and a stationary phase which can be a solid or liquid adsorbent through which the mobile phase passes.

The way chromatographic separation works is well illustrated by column chromatography, a technique that uses a solid adsorbent as the stationary phase packed inside a glass or plastic column, and a liquid solvent mobile phase called the eluent.

In column chromatography a column is filled with small particles or beads of the stationary phase (insoluble in the eluent) and the column is equilibrated with a small amount of the eluent. Then a mixture of compounds, for instance, **A** and **B**, are dissolved in the solvent and applied to the top of the column as a narrow layer (part **i.** of the diagram below). If the stopcock of the column is carefully opened, a layer of the eluent solvent (mobile phase) moves down the packed column by gravity. As the eluent moves through the column, the compounds in the mixture partition between the moving (mobile) phase and adsorbent (stationary) phase due to the differences in the compounds' physical properties (e.g., polarity, molecular weight, vapor pressure, *etc.*) and therefore the different interactions the compounds have with the stationary phase and mobile phase (e.g., London dispersion forces, dipole-dipole interactions, hydrogen bonding, *etc.*). A compound that is attracted less strongly to the stationary phase will move through the column faster and elute, or come off, the column more rapidly in the eluent. In contrast, a compound that is more strongly attracted to the stationary phase will move through the column more slowly and elutes later. Let us assume, for the purpose of this illustration, that component **B** binds more strongly to the stationary phase than component **A** does (see diagram below). Then, as the mixture travels down the column, the molecules of **B** will be retarded with respect to the molecules of **A** and, in time, the two components will separate into two bands and they will be eluted at different times. That is, complete separation of the initial mixture into its components is achieved. The collected volumes of the eluent are called fractions.



In this example, the stationary phase is placed in a column, whence the term column chromatography. In related techniques, the stationary phase may be a sheet of suitable material, such as paper (paper chromatography) or a layer of solid on a glass, aluminum or polymer sheet (thin-layer chromatography).

Several types of adsorbents can be used for column chromatography. The most commonly used adsorbents are alumina (Al_2O_3) and silica gel (SiO_2). The major determinant of activity of the adsorbent is the degree of

hydration of the surface of the adsorbent. The surface provides a highly polar environment so that more polar compounds will be adsorbed more strongly (due to hydrogen bonding or dipole-dipole attractions) than less polar ones. Thus, less polar compounds of the mixture move through the column more rapidly while more polar compounds have a stronger attraction to the adsorbent stationary phase than to the moving eluent and may require a more polar solvent in order to dislodge them from the column. [More exactly, the eluting ability of the solvent depends upon dielectric constant but polarity is a "fair" measure of this ability.]

A mobile phase consisting of a single eluent may not be sufficient to separate and elute each compound of a given mixture, particularly if the mixture to be separated includes compounds with a wide range of polarities. Then two eluents may have to be used: a low polarity solvent first to elute the lower polarity compounds, followed by a more polar solvent to elute the more polar compounds.

As practical guide, the following solvents are used most frequently in column chromatography to dissolve organic compounds and to dislodge a polar substance from alumina or silica gel. The solvents are arranged in increasing polarity, with cyclohexane being the least polar. In practice, the least polar solvent is used first and then the polarity of the solvent or solvent mixture is gradually increased to elute the more polar component.

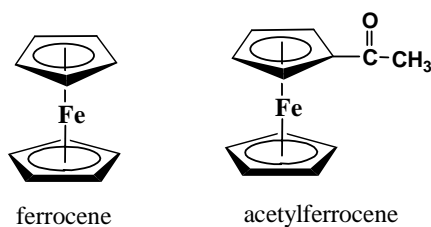
<i>Solvent</i>	<i>m.p.</i> (°C)	<i>b.p.</i> (°C)	<i>d</i> ^a	<i>μ</i> ^b (D)	<i>ε</i> ^c
cyclohexane	4-7	81	0.779	0.0	2.0
hexane	-95	68.8	0.659	0.0	1.9
carbon disulfide	-111	46	1.266	0	2.6
^t butyl methyl ether	-109	55	0.74	1.3	2.6
dichloromethane	-97	40	1.325	1.14	9.1
1,4-dioxane	11.8	101	1.03	0.45	2.2
ethyl acetate	-84	77	0.902	1.88	6.0
diethyl ether	-116	34.6	0.71	1.12	4.3
methanol	-97.8	65	0.791	1.7	32.6
water	0.0	100.0	1.000 ⁴	1.8	78.5

↑
polarity
decreases

^a Relative density in g·cm⁻³ at 25°C except as noted by a superscript to the density value

^b Dipole moment in Debyes ^c Dielectric constant at 25°C

Such is the case in today's experiment, where a mixture of two compounds of different polarities, ferrocene and acetylferrocene, will be separated. Both of these compounds are colored so it is easy to follow the progress of the chromatographic separation.



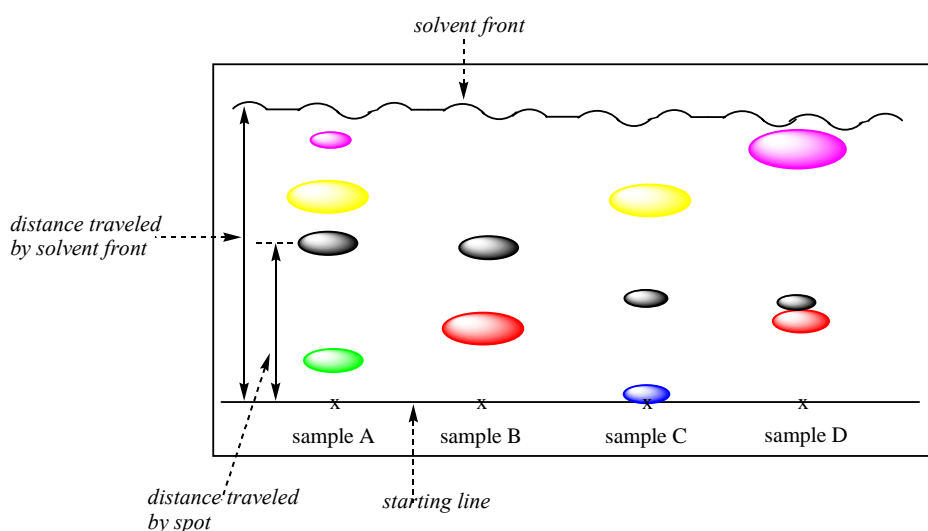
Thin-Layer Chromatography

Samples to be studied by thin-layer chromatography (TLC) are usually dissolved in suitable solvents and “spotted” along the starting line on the TLC plate using a capillary tube. *[It is very important to note that all markings on the TLC plate should be made by pencil. Never use pens or markers.]*

“Spotting” means briefly touching the capillary tube to the TLC plate, carefully blowing on the wet spot to evaporate the solvent, and repeating these steps a few times.

Glass vessels of various sizes are available for use as developing chambers. For example, wide-mouth jars (Mason jars) or beakers tightly covered with aluminum foil may be used. It is important that the chamber be airtight so that the solvent will not evaporate as it nears the top of the TLC plate.

After each sample has been spotted onto the plate, the plate is then carefully placed in the developing chamber and the chamber is then capped/closed tightly. The solvent moves from the bottom of the chamber along the plate by capillary action, and each component in the sample moves with the solvent at a particular rate which rate depends upon the component’s distribution between the mobile and stationary phases. Thus, some components move with the solvent front, other components move shorter distances, and some components may not move at all. The resulting chromatogram might appear as shown below.



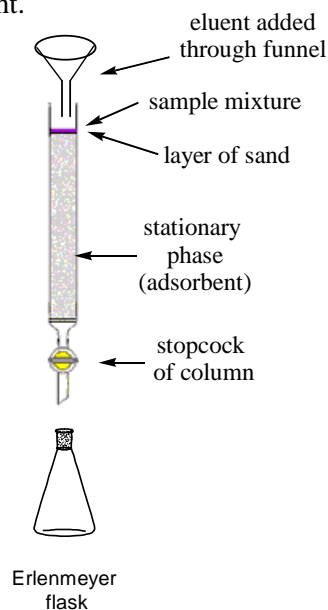
The ratio of the distance traveled by a component to the distance traveled by the solvent front is called the R_f value.

$$R_f = \frac{\text{distance traveled by component [cm or mm]}}{\text{distance traveled by solvent front [cm or mm]}}$$

The R_f value is a characteristic physical property of the component – just like melting point, boiling point, refractive index, *etc.* - and will not change unless the type of plate and/or type of solvent is changed. Ideally, the solvent front moves uniformly but in some cases it does not.

In our experiment we will use a TLC plate to identify which component of the mixture if which, and determine their relative polarity. The movements of the colored spots on TLC plate are easily observed. The simplicity by which these compounds may be separated by TLC, and the visibility and characteristic color of each compound make this mixture an ideal sample for a truly colorful laboratory experiment.

The set-up that is used in this column chromatography experiment is shown on the right.



Safety

- Observe the general safety precautions outlined in the section titled **SAFETY in the ORGANIC LAB**. In addition to the general safety measures the special safety precautions for this specific experiment are as follows:
- Acetylferrocene is toxic, therefore exercise caution when handling it.
- Hexane and diethyl ether are extremely flammable. There will be absolutely no flames allowed in the lab during the experiment.
- Avoid breathing in the vapor of the developing solution.

Procedure

1. Assemble the chromatography column, as shown above, and make sure it is clamped in a vertical position.
2. Close the stopcock (use both hands) and fill the column to about half full with hexane.
3. Add 4 g of alumina to about 6 mL of hexane in a 50-mL Erlenmeyer flask and swirl vigorously to form a slurry. Quickly pour the slurry into the column. It will be necessary to rinse the residual alumina with solvent several times to get most of it into the column. Open the stopcock and drain out excess solvent into a container labeled *WASTE*. As the alumina is added, gently tap the walls of the column with a rubber hose. This ensures even packing of the column and removal of any air bubbles which could cause nonuniform bed packing and interfere with uniform movement of compounds through the column (compounds may streak instead of moving as a band).
4. Never allow the column to run dry (i.e., there should always be a small column of liquid above adsorbent). A dry column can allow entrance of air bubbles. If you inadvertently allow the column to go dry, quickly add more solvent which will often prevent loss of your experiment.
5. Allow the solvent to drain just to the top of the alumina which should be flat. Add about 0.5-1 cm high column of sand to protect the surface of the alumina.
6. Label two dry 50-mL Erlenmeyer flasks as **#1** and **#2** and weigh each flask.
7. Weigh about 0.1 g of a 3:1 mixture of ferrocene : acetylferrocene in a 50-mL Erlenmeyer flask. Dissolve this sample in a minimal volume of dichloromethane (about 1 mL) by adding dichloromethane dropwise to the sample using a disposable Pasteur pipet. Swirl the solution well. When dissolved, pipet the sample onto the column (using the same disposable pipet) and rinse the sample flask with a minimal amount of dichloromethane (about 1 mL or less).
8. Carefully add hexane to fill the column up to the plastic funnel. Place the small, pre-weighed Erlenmeyer flask (flask **#1**) under the column. Open the stopcock and drain the hexane down to the sand. Repeat this by filling the column two or three times (or as necessary) with hexane to move the entire first band (first compound) off the column and into the pre-weighed Erlenmeyer flask (flask **#1**). Remember, never allow the column to run dry (i.e., there should always be a small column of liquid above the band of sand).

9. After the entire first band has been eluted, place the other small pre-weighed Erlenmeyer flask (flask #2) under the column. Obtain about 10 mL of hexane:diethyl ether = 1:1 mixture and pour a small portion of it onto the column to begin eluting the second band. Just like before, this step requires to fill the space above the alumina about three-four times (or as necessary) with the eluent mixture to be able to collect the entire amount of the second band.
10. Add a few crystals (a few milligrams) of the initial 3:1 mixture of ferrocene-acetylferrocene, and similar amounts of sample of “pure” (separated) ferrocene and of “pure” (separated) acetylferrocene, and similar amounts of authentic ferrocene and authentic acetylferrocene into 5 different vials. Label each vial. Dissolve each sample in about 10 drops of acetone.
11. Set up a TLC plate by drawing the starting line on the shorter edge. Mark the starting positions for the 5 samples. Make sure you handle TLC plates only at the edges and not touching the face of the plate.
12. Using an open-ended capillary tube, spot these five solutions on a TLC plate and label each spot under the starting line.
13. Place a small amount of 1:1 mixture of hexane:diethyl ether into a 250-mL jar so that the bottom of the jar is barely but completely covered with the mixture. Then gently lower the bottom of the TLC plate into the mixture and place the cap on the jar. When the solvent front reaches about 80% of the height of the TLC plate (approximately 4-5 minutes), take the plate out of the jar and quickly mark the solvent front across the plate. Determine the R_f values for each component. [*The solvent will evaporate from the plate quickly and may not leave a mark. Therefore you may wish to reach into the developing tank and make a small mark on the plate just before removing it from the jar.*]
14. Evaporate all the solvent from the two fractions (#1 and #2) you collected using a hot plate. Set the heating rate between 2-3 and stir the contents with a glass rod/boiling stick to avoid splashing. Take the flasks off the hot plate as soon as boiling stops. If needed, a slow stream of air blown over the product will help to remove the last traces of hexane and solidify the product.
15. Allow the flasks to cool to room temperature and weigh them to obtain the amount of samples collected and to determine the percent recoveries.
16. Allow the TLC plate to dry and staple it to your lab report.

Cleaning up and Disposal

- *Empty the contents of the chromatography column onto a piece of paper towel and let the solvent evaporate in the hood. After the solid alumina is dry, dispose of it in the trash can.*
- *Wash column with a few milliliters of hexane:diethyl ether = 1:1 mixture. Do not wash or rinse column with water.*
- *Clamp used column with stopcock pointing up. Turn stopcock to open position.*
- *Pour all organic liquids into the “ORGANIC WASTE” collection flask under the front hood.*

Questions

Before Lab

BL #1: What would happen if a ball-point or felt-tip pen were used to mark the starting line (baseline) and the labels on the TLC plate?

BL #2: Would the chromatographic process proceed properly if the TLC jar were not covered? Why or why not?

BL #3: Determine the R_f values for each component of sample A on the TLC plate above (page iii).

BL #4: Based on the chemical structures of the two compounds, which one, ferrocene or acetylferrocene would you expect to elute from the column first? Explain your answer.

After Lab

AL #1: Once a chromatographic column has been prepared, why is it important

a) to prevent air bubbles from forming in the column?

b) to allow the level of solvent in the column to drop to the top of the alumina before applying the solution of the sample?

AL #2: Describe the movement of acetylferrocene during the elution of the column with hexane.

AL #3: Explain what would happen if you had used only hexane as the eluent.

AL #4: Explain what would happen if you first used the mixed solvent, hexane-diethyl ether, instead of hexane to elute the column.

AL #5: Predict and explain the order of elution of 1-chlorobutane, 1-iodobutane, 1-fluorobutane, and 1-bromobutane from an alumina column using hexane as a solvent.

AL #6: How would you monitor the elution of a chromatography column if the compounds in the sample being separated are colorless?

Experiment Title: _____ **Name:** _____

Date: _____

Mass of dry flasks: **Flask #1 :** _____ **Flask #2 :** _____

Mass of flask + product: **Flask #1 :** _____ **Flask #2 :** _____

	<u>acetylferrocene</u>	<u>ferrocene</u>
Original mixture (0.1 g):	<u>0.033 g</u>	<u>0.067 g</u>

Amount recovered from column: _____ _____

% Recovered _____ _____

R_f of recovered compounds* _____ _____

R_f of original compounds* _____ _____

R_f of components in mixture* _____ _____

*Attach your TLC plate to your lab report.

Observations

Identify the compound that elutes first and second from the column. Justify your choices.

Conclusions