

1. Resolution of Mixtures 2: Chromatography

Objective

The field of separation science is one of the most important in chemistry today. The particular branch of chemistry called **analytical chemistry** is concerned with the separation of mixtures and the analysis of the amount of each component in the mixture. In this experiment, you will perform two chromatographic separations of mixtures. Choice I studies the resolution of felt-tip pen inks by paper chromatography. Choice II deals with the resolution of a mixture of colored indicator dyes by thin-layer chromatography.

General Introduction to Chromatography

The word **chromatography** means color-writing. The name was chosen at the beginning of this century when the method was first used to separate colored components from plant leaves. Chromatography in its various forms is perhaps the most important known method of chemical analysis of mixtures.

Paper and thin-layer chromatography are simple techniques that can be used to separate mixtures into the individual *components* of the mixture. The methods are very similar in operation and principle, differing primarily in the medium used for the analysis.

Paper chromatography uses ordinary filter paper, which consists primarily of the polymeric carbohydrate *cellulose*, as the medium on which the mixture to be separated is applied. **Thin-layer chromatography** (universally abbreviated as **TLC**) uses a thin coating of aluminum oxide (alumina) or silicagel on a glass microscope slide or plastic sheet to which the mixture to be resolved is applied.

A single drop or spot of the unknown mixture to be analyzed is applied about half an inch from the end of a strip of filter paper or a TLC slide. The filter paper or TLC slide is then placed in a shallow layer of solvent or solvent mixture in a jar or beaker. Since filter paper or the coating of the TLC slide is permeable to liquids, the solvent begins rising by capillary action.

As the solvent rises to the level at which the spot of mixture was applied, various effects can occur, depending on the constituents of the spot. Those components of the spot that are completely soluble in the solvent will be swept along with the solvent front as it continues to rise. Those components that are not at all soluble in the solvent will be left behind at the original location of the spot. Most components of the unknown spot mixture will take an intermediate approach as the solvent front passes. Components in the spot that are *somewhat* soluble in the solvent will be swept along by the solvent front, but to *different extents*, reflecting their specific solubilities. By this means, the original spot of mixture is spread out into a series of spots or bands, with each spot or band representing one single component of the original mixture.

The separation of a mixture by chromatography is not solely a function of the solubility of the components in the solvent used, however. The filter paper or

TLC slide coating used in chromatography is not inert, but consists of molecules that may *interact* with the molecules of the components of the mixture being separated. Each component of the mixture is likely to have a different extent of interaction with the filter paper or slide coating. This differing extent of interaction between the components of a mixture and the molecules of the support forms an equally important basis for the separation. Filter paper or the TLC slide coating adsorbs molecules on its surface to differing extents, depending on the structure and properties of the molecules involved.

To place a paper chromatography or TLC separation on a quantitative basis, a mathematical function called the **retention factor**, R_f , is defined:

$$R_f = \text{distance traveled by spot} / \text{distance traveled by solvent front}$$

The retention factor depends on what solvent is used for the separation and on the specific composition of the filter paper or slide coating used for a particular analysis. Because the retention factors for particular components of a mixture may vary if an analysis is repeated under different conditions, a *known* sample is generally analyzed at the *same time* as an *unknown* mixture on the same sheet of filter paper or slide. If the unknown mixture produces spots having the same R_f values as spots from the known sample, then an identification of the unknown components has been achieved.

Paper chromatography and TLC are only two examples of many different chromatographic methods. Mixtures of volatile liquids are commonly separated by a method called **gas chromatography**. In this method, a mixture of liquids is vaporized and passed through a long tube (column) of solid adsorbent material coated with an appropriate liquid, by the action of a carrier gas (usually helium). As with paper chromatography, the components of the mixture will have different solubilities in the liquid coating and different attractions for the solid adsorbent material. Separation of the components of the mixture thus occurs as the mixture progresses through the tube. The individual components of the mixture exit the tube one by one and are usually detected by electronic means. A final very important chromatographic technique is called high performance liquid chromatography (HPLC). In HPLC, liquid mixtures to be analyzed are blown through a column of adsorbent material under high pressure from a pump, resulting in a very quick passage through the column. HPLC is routinely used in medical and forensic laboratories to analyze biological samples. For example, blood samples can be analyzed for the presence of alcohol or illicit drugs in just a few minutes using HPLC.

Choice I. Paper Chromatography of Inks

Introduction

Although paper chromatography is a very simple technique, it is still used frequently for analyses of mixtures of colored substances (or for substances which can be made colored by treatment with an appropriate reagent). For example, biologists often use paper chromatography for quick analysis of plant pigments. Paper chromatography can also be used for simple analyses of protein extracts (amino acids).

In this Choice, you will do some very simple paper chromatographic analyses of some felt-tip pen inks. As you know, inks for such pens come in many different, bright colors—particularly in pen sets used by small children for working on their coloring books. Such brightly colored inks, however, are often *mixtures* of primary color inks. For example, a felt-tip pen having what appears to be bright purple ink may actually contain a mixture of blue and red inks. Similarly, what appears to be orange ink may be a mixture of red and yellow inks. Although this Choice is very simple, you will clearly see the basis for chromatographic analyses, and will perhaps gain some insight into the great importance of the various chromatographic methods.

SAFETY PRECAUTIONS

- **Wear safety glasses at all times while in the laboratory.**
- **Acetone is highly flammable. No flames are permitted in the laboratory. Acetone may be toxic if inhaled or absorbed through the skin.**

Apparatus/Reagents Required

filter paper for chromatography (5 × 10 cm), latex surgical gloves, ruler, pencil, heat gun, acetone-water mixture (50% v/v), felt-tip pens (water-soluble and permanent)

Procedure

Record all observations directly in your notebook in ink.

Because the skin contains oils which can interfere with the chromatogram, latex surgical gloves should be worn from this point onward in the procedure to prevent contamination of the chromatogram. A pencil is used in the following procedure for marking the chromatogram, since ink from a ball-point pen would also undergo chromatography.

Obtain a sheet of filter paper prepared for the chromatographic analysis. Draw a light pencil line across the paper about a half inch from each end. On the lower pencil line, lightly mark three or four small circles. These circles will be where the felt-tip pen inks are to be applied to the paper.

From your instructor, obtain several felt-tip pens containing *water-soluble* inks. Apply a single small spot of a different ink to each of the pencil circles you drew on the filter paper. Allow the spots to dry completely. Record in your notebook the original color of each ink applied to the filter paper, as well as any code numbers marked on the barrel of the pen.

When the spots are completely dry, apply a second spot of each ink to its respective place on the filter paper. Applying a second spot of the same dye builds up a larger sample of the dye on the filter paper.

Clean a 400-mL beaker (or special chromatography jar) for use in developing the chromatogram. Cut a square of plastic wrap for use as a cover for the beaker.

Add distilled water to the beaker to a depth of approximately one-quarter of an inch.

Make certain that the ink spots on the filter paper are completely dry. If the spots are dry, fold the filter paper in half lengthwise.

Carefully lower the filter paper (with the spots at the bottom) into the water in the beaker. Make certain not to wet the spots as you lower the filter paper, and do not move the beaker to avoid sloshing water onto the spots. Cover the beaker with the plastic wrap.

Allow the water to rise in the filter paper until it reaches the upper pencil line. Then carefully remove the filter paper and set it on a clean paper towel. Quickly dry the filter paper using a heat-gun or hair dryer.

Determine R_f for each of the ink spots and record.

Obtain from your instructor several *permanent* ink markers (which are *not* water soluble). Repeat the chromatographic procedure using a new strip of filter paper and a 50% acetone-water mixture as the solvent (*Caution*). Determine and record the R_f values for the permanent inks.

Save your two chromatograms for submitting with your lab report.

Choice II. Thin-Layer Chromatography of Indicator Dyes

Introduction

Indicators are organic compounds that are typically used to signal a change in pH in acid/base titration analyses. Such indicators are dyes that exist in different colored forms at different pHs, and the change in color of the indicator is the signal that the titration analysis is complete. In most cases, indicator dyes are very *intensely* colored, and only a very tiny quantity of the indicator is needed.

In this experiment, you will perform a thin-layer chromatographic analysis of a mixture of the dyes bromcresol green, methyl red, and xlenol orange. These dyes have been chosen because they have significantly different retention factors, and a nearly complete separation should be possible in the appropriate solvent system. You will also investigate the effect of the solvent on TLC analyses, by attempting the separation in several different solvent systems.

In real practice, thin-layer chromatography has several uses. When a new compound is synthesized, for example, a TLC of the new compound is routinely done to make certain that the new compound is pure (a completely pure compound should only give a single TLC spot; impurities would result in additional spots). TLC is also used to separate the components of natural mixtures isolated from biological systems: for example, the various pigments in plants can be separated by TLC of an extract made by boiling the plant leaves in a solvent. Once the components of a mixture have been separated by TLC, it is

even possible to isolate small quantities of each component by scraping its spot from the TLC slide and redissolving the spot in some suitable solvent.

SAFETY PRECAUTIONS

- **Wear safety glasses at all times while in the laboratory.**
- **The organic indicator dyes used in this experiment will stain skin if spilled; many such dyes are toxic or mutagenic.**
- **The solvents used for the chromatographic separation are highly flammable and their vapors are toxic. No flames should be burning in the room while these solvents are in use. Use the solvents only in the exhaust hood.**

Apparatus/Reagents Required

Bakerflex plastic TLC slides (1 × 4 inch), latex surgical gloves, ruler, pencil, plastic wrap or Parafilm, micropipets, hot plate, heat gun, ethanolic solutions of the indicator dyes (methyl red, xylene orange, bromocresol green), acetone, ethyl acetate, hexane, ethanol

Procedure

Clean and dry six 400-mL beakers to be used as the chambers for the chromatography. Obtain several squares of plastic wrap or Parafilm to be used as covers for the beakers.

The chromatographic separation will be attempted in several solvent mixtures to investigate which gives the most complete resolution of the three dyes. All of these solvents are highly flammable; no flames should be open in the lab. A total of only 10–15 mL of each solvent mixture is necessary. Prepare mixtures of the solvents below, in the proportions indicated by volume, and transfer each to a separate 400-mL flask. Cover the flasks after adding the solvent mixture, and label the flask with the identity of the mixture it contains.

Acetone 60% / hexane 40%

Ethyl acetate 60% / hexane 40%

Acetone 50% / ethyl acetate 50%

Acetone 50% / ethanol 50%

Ethyl acetate 50% / ethanol 50%

Hexane 50% / ethanol 50%

Wearing plastic surgical gloves to avoid oils from the fingers, prepare six plastic TLC slides by marking *lightly* with pencil (not ink) a line across both the top and bottom of the slide. Do not mark the line too deeply or you will remove the coating of the slide. See Figure 1-1.

On one of the lines you have drawn on each slide, mark four small pencil dots (to represent where the spots are to be applied). *Above* the other line on

each slide, mark the following letters: *R* (methyl red), *X* (xylenol orange), *G* (bromocresol green), and *M* (mixture). See Figure 1-1.

FIGURE 1-1
Plastic TLC
slide with
spots of the
three dyes
and the
mixture
applied. Keep
the spots
applied small.



Obtain small samples of the ethanol solutions of the three dyes (methyl red, xylenol orange, bromocresol green). Also obtain several micropipets: use a separate micropipet for each dye, and be careful not to mix up the pipets during the subsequent application of the dyes.

Apply a single small droplet of the appropriate dye to its pencil spot on each of the TLC slides you have prepared (wipe the outside of the micropipet if necessary before applying the drop to remove any excess dye solution). Keep the spots of dye as small as possible.

Apply one droplet of each dye to the spot labeled *M* (mixture) on each slide, being sure to allow each previous spot to dry before applying the next dye. Allow the spots on the TLC slides to dry before proceeding.

Gently lower one of the TLC slides, spots downward, into one of the solvent systems. Be careful not to wet the spots, or to slosh the solvent in the beaker; do not move or otherwise disturb the beaker after adding the TLC slide. Carefully cover the beaker with plastic wrap.

Allow the solvent to rise on the TLC slide until it reaches the upper pencil line (this will not take very long).

When the solvent has risen to the upper pencil mark, remove the TLC slide and quickly mark the exact solvent front before it evaporates. Mark the TLC slide with the identity of the solvent system used for development. Set the TLC slide aside to dry completely.

Repeat the process using the additional TLC slides and solvent systems. Be certain to mark each slide with the solvent system used.

Determine R_f for each dye in each solvent system and record. Which solvent system led to the most complete resolution of the dye mixture? If no mixture gave a complete resolution, your instructor may suggest other solvents for you to try, or other proportions of the solvents already used.

Save your TLC slides and staple to the lab report page for this experiment.

Choice II. Thin-Layer Chromatography of Indicator Dyes

1. In preparing a TLC slide or filter paper for chromatography, a baseline is drawn for positioning the spots in pencil. Why is ink never used for drawing the baseline?

2. The indicator dyes used in this experiment are also used in acid/base titration analyses because they change color at particular values of pH. Use a handbook of chemical data to find the colors of each of these dyes under low and high pH ranges.

Methyl red

Xylenol orange

Bromocresol green

3. TLC slides are most commonly coated with alumina or, less commonly, silicagel. Use an encyclopedia of chemistry or a handbook to find out the composition of each of these materials.

Resolution of Mixtures 2: Chromatography

Date: Student name:
Course: Team members:
Section:
Instructor:

Results/Observations

Choice I. Paper Chromatography of Inks

Water-soluble inks

| Spot | Pen color | Identification number |
|------|-----------|-----------------------|
| a. | | |
| b. | | |
| c. | | |
| d. | | |

Distance traveled by solvent front

| Spot | Distance traveled by spot | R_f |
|------|---------------------------|-------|
| a. | | |
| b. | | |
| c. | | |
| d. | | |

Acetone-soluble inks

| Spot | Pen color | Identification number |
|------|-----------|-----------------------|
| a. | | |
| b. | | |
| c. | | |
| d. | | |

Resolution of Mixtures 2: Chromatography

Date: Student name:
Course: Team members:
Section:
Instructor:

Results/Observations

Choice II. Thin-Layer Chromatography of Indicator Dyes

For each of the solvent mixtures studied, calculate R_f for each of the spots:

| | | |
|-----------------------|--|------------------|
| Acetone/hexane | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |
| Ethyl acetate/hexane | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |
| Acetone/ethyl acetate | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |

| | | |
|-----------------------|--|------------------|
| Acetone/ethanol | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |
| Ethyl acetate/ethanol | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |
| Hexane/ethanol | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |
| Other mixture | Distance traveled by solvent front | |
| | Distance traveled by spot | Calculated R_f |
| Xylenol orange | | |
| Bromcresol green | | |
| Methyl red | | |

Which solvent mixture gave the most complete resolution of the three dyes? Which solvent mixture gave the poorest resolution?

