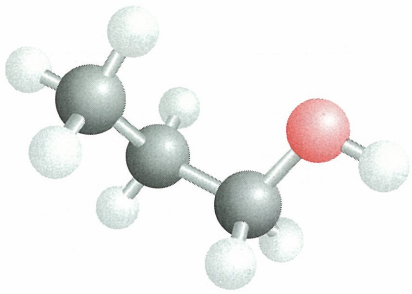
CHAPTER

8



Thin-Layer Chromatography: Analyzing Analgesics and Isolating Lycopene from Tomato Paste

Online Study Center

This icon will direct you to techniques, equipment setups, and online resources at http://college.hmco.com/PIC/williamsonMMOE5e.

PRELAB EXERCISE: Based on the number and polarity of the functional groups in aspirin, acetaminophen, ibuprofen, and caffeine, whose structures are shown on page 184, predict which of these four compounds has the highest R_f value and which has the lowest.

Chromatography is the separation of two or more compounds or ions caused by their molecular interactions with two phases—one moving and one stationary. These two phases can be a solid and a liquid, a liquid and a liquid, a gas and a solid, or a gas and a liquid. You very likely have seen chromatography carried out on paper towels or coffee filters to separate inks and food dyes. The cellulose paper is the stationary or solid phase, and a propanol-water mixture is the mobile or liquid phase. The samples are spotted near one edge of the paper, and this edge is dipped into the liquid phase. The solvent is drawn through the paper by capillary action, and the molecules are separated based on how they interact with the paper. Although there are several different forms of chromatography, the principles are essentially the same.

Thin-layer chromatography (*TLC*) is a sensitive, fast, simple, and inexpensive analytical technique that you will use repeatedly while carrying out organic experiments. It is a micro technique; as little as 10^{-9} g of material can be detected, although the usual sample size is from 1×10^{-6} g to 1×10^{-8} g. The stationary phase is normally a polar solid adsorbent, and the mobile phase can be a single solvent or a combination of solvents.

TLC requires micrograms of material.

Uses of Thin-Layer Chromatography

1. **To determine the number of components in a mixture.** TLC affords a quick and easy method for analyzing such things as a crude reaction mixture, an extract from a plant substance, or the ingredients in a pill. Knowing the

2. Why is it necessary to remove the stopper from a separatory funnel when liquid is being drained from it through the stopcock?
3. The pK_a of *p*-nitrophenol is 7.15. Would you expect this to dissolve in sodium bicarbonate solution? The pK_a of 2,5-dinitrophenol is 5.15. Will it dissolve in bicarbonate solution?
4. The distribution coefficient, k = conc. in ligroin ÷ conc. in water, between ligroin and water for solute A is 7.5. What weight of A would be removed from a solution of 10 g of A in 100 mL of water by a single extraction with 100 mL of ligroin? What weight of A would be removed by four successive extractions with 25-mL portions of ligroin? How much ligroin would be required to remove 98.5% of A in a single extraction?
5. In experiment 1, how many moles of benzoic acid are present? How many moles of sodium bicarbonate are contained in 1 mL of a 10% aqueous solution? (A 10% solution has 1 g of solute in 9 mL of solvent.) Is the amount of sodium bicarbonate sufficient to react with all of the benzoic acid?
6. To isolate benzoic acid from a bicarbonate solution, it is acidified with concentrated hydrochloric acid, as in experiment 1. What volume of acid is needed to neutralize the bicarbonate? The concentration of hydrochloric acid is expressed in various ways on the inside back cover of this laboratory manual.
7. How many moles of 4-*t*-butylphenol are in the mixture to be separated in experiment 1? How many moles of sodium hydroxide are contained in 1 mL of 5% sodium hydroxide solution? (Assume the density of the solution is 1.0.) What volume of concentrated hydrochloric acid is needed to neutralize this amount of sodium hydroxide solution?
8. Draw a flow sheet to show how you would separate the components of a mixture containing an acid substance, toluic acid, a basic substance, *p*-bromo-aniline, and anthracene, a neutral substance.
9. Write equations showing how caffeine could be extracted from an organic solvent and subsequently isolated.
10. Write equations showing how acetaminophen might be extracted from an organic solvent such as an ether, if it were soluble.
11. Write detailed equations showing the mechanism by which aspirin is hydrolyzed in boiling, slightly acidic water.



General Resources
Web Links

number and relative amounts of the components aids in planning further analytical and separation steps.

2. **To determine the identity of two substances.** If two substances spotted on the same TLC plate give spots in identical locations, they *may* be identical. If the spot positions are not the same, the substances cannot be the same. It is possible for two or more closely related but not identical compounds to have the same positions on a TLC plate. Changing the stationary or mobile phase will usually effect their separation.
3. **To monitor the progress of a reaction.** By sampling a reaction at regular intervals, it is possible to watch the reactants disappear and the products appear using TLC. Thus, the optimum time to halt the reaction can be determined, and the effect of changing such variables as temperature, concentrations, and solvents can be followed without having to isolate the product.
4. **To determine the effectiveness of a purification.** The effectiveness of distillation, crystallization, extraction, and other separation and purification methods can be monitored using TLC, with the caveat that a single spot does not guarantee a single substance.
5. **To determine the appropriate conditions for a column chromatographic separation.** In general, TLC is not satisfactory for purifying and isolating macroscopic quantities of material; however, the adsorbents most commonly used for TLC—silica gel and alumina—are also used for column chromatography, which is discussed in Chapter 9. Column chromatography is used to separate and purify up to 1 g of a solid mixture. The correct adsorbent and solvent to use for column chromatography can be rapidly determined by TLC.
6. **To monitor column chromatography.** As column chromatography is carried out, the solvent is collected in a number of small flasks. Unless the desired compound is colored, the various fractions must be analyzed in some way to determine which ones have the desired components of the mixture. TLC is a fast and effective method for doing this.

The Principles of Chromatography

To thoroughly understand the process of TLC (and other types of chromatography), we must examine the process at the molecular level. All forms of chromatography involve a dynamic and rapid equilibrium of molecules between the liquid and the stationary phases. For the chromatographic separation of molecules A and B shown in Figure 8.1, there are two states:

1. **Free**—dissolved in the liquid or gaseous mobile phase
2. **Adsorbed**—sticking to the surface of the solid stationary phase

Molecules A and B are continuously moving back and forth between the dissolved (free) and adsorbed states, with billions of molecules adsorbing and billions of other molecules desorbing from the solid stationary phase each second. The equilibrium between the free and adsorbed states depends on the relative strength of the

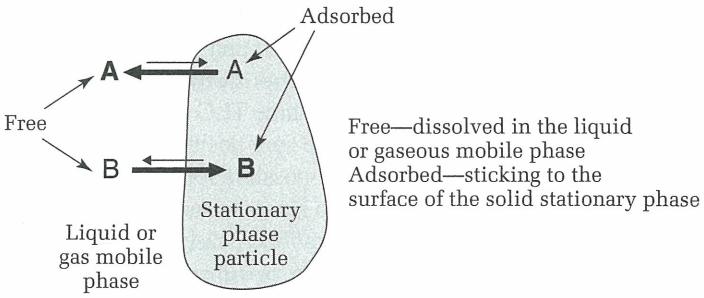


FIG. 8.1
The mixture of molecules A and B is in a dynamic equilibrium between the free and adsorbed states.

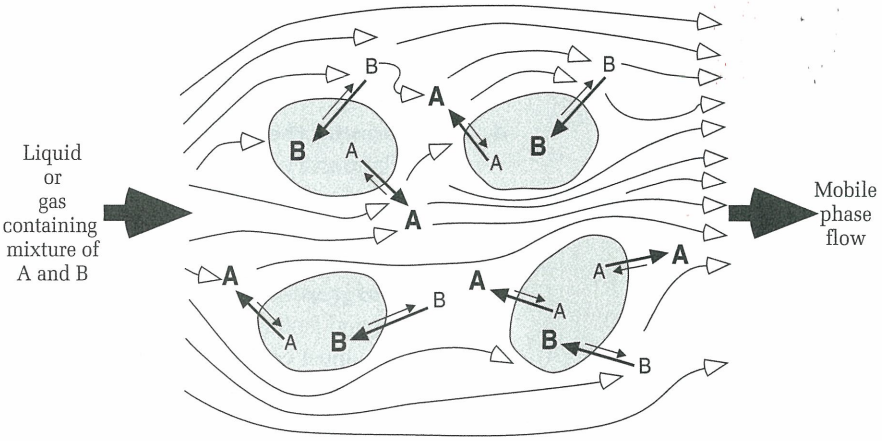


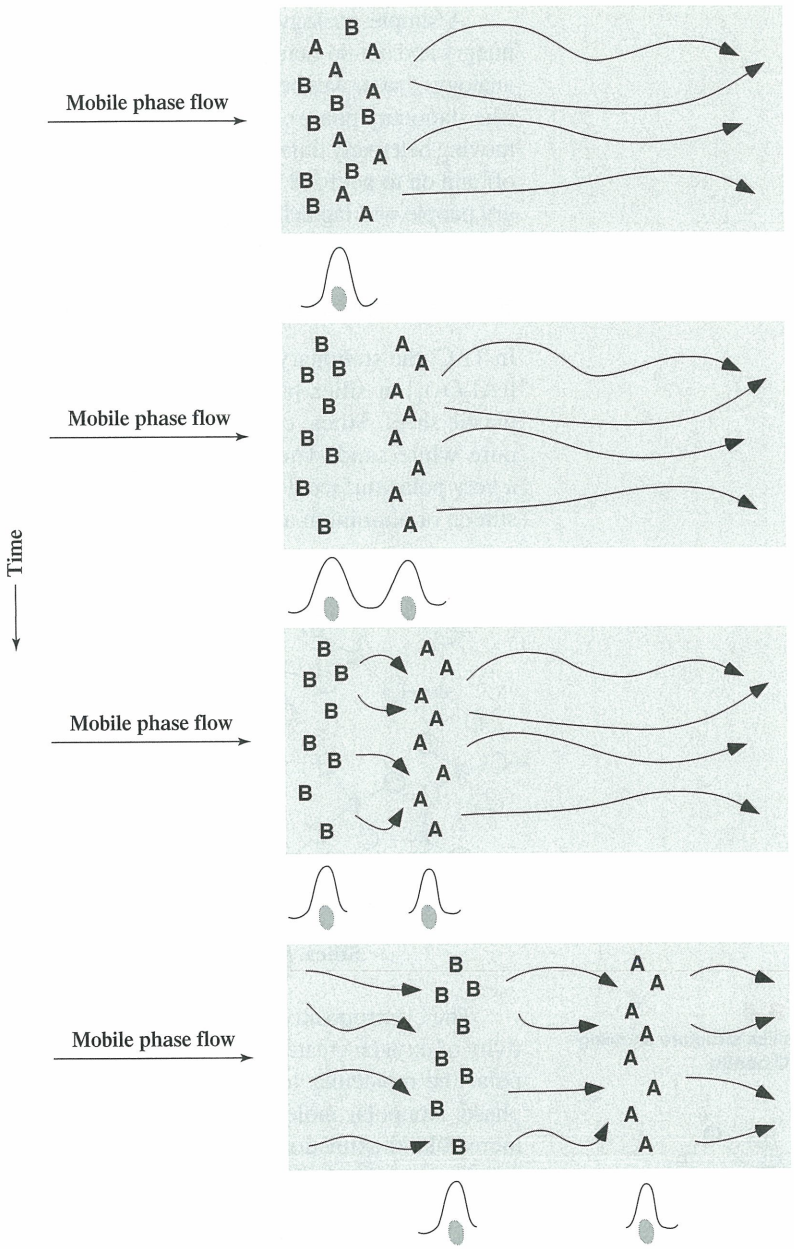
FIG. 8.2
The mixture of molecules A and B is in a dynamic equilibrium between the stationary adsorbent and a *flowing* mobile phase.

attraction of A and B to the liquid phase molecules *versus* the strength of attraction of A and B to the stationary phase structure. As discussed in the introduction to Chapter 3, the strength of these attractive forces depends on the following factors:

- Size, polarity, and hydrogen bonding ability of molecules A and B
- Polarity and hydrogen bonding ability of the stationary phase
- Polarity and hydrogen bonding ability of the mobile phase solvent

Molecules distribute themselves, or *partition*, between the mobile and stationary phases depending on these attractive forces. As implied by the equilibrium arrows in Figure 8.1, the A molecules are less polar and are thus weakly attracted to a polar stationary phase, spending most of their time in the mobile phase. In contrast, equilibrium for the more polar B molecules lies in the direction of being adsorbed onto the polar stationary phase. The equilibrium constant k (also called the *partition coefficient*) is a measure of the distribution of molecules between the mobile phase and the stationary phase and is similar to the distribution coefficient for liquid/liquid extraction. This constant changes with structure.

Simply adding a mixture to a combination of a liquid phase and a stationary phase will not separate it into its pure components. For separation to happen, the liquid phase must be mobile and be flowing past the stationary phase, as depicted in Figure 8.2. Because the A molecules spend more time in the mobile phase, they will be carried through the stationary phase and be eluted faster and move farther



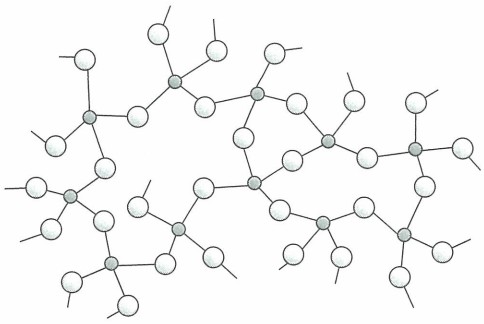
■ FIG. 8.3
A chromatographic separation. Over time, the mobile phase carries the less weakly adsorbed A molecules ahead of the more strongly adsorbed B molecules.

in a given amount of time. Because the B molecules are adsorbed on the stationary phase more than A molecules, the B molecules spend less time in the mobile phase and therefore migrate through the stationary phase more slowly and are eluted later. The B molecules do not migrate as far in the same amount of time. The consequence of this difference is that A is gradually separated from B by moving ahead in the flowing mobile phase as time passes, as shown in Figure 8.3.

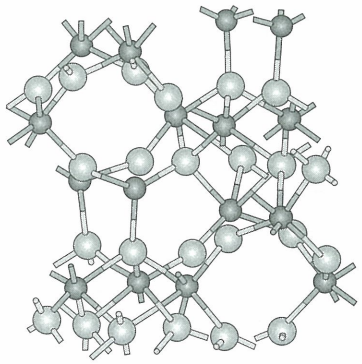
A simple analogy may help to illustrate these concepts. Imagine a group of hungry and not-so-hungry people riding a moving sidewalk (the mobile phase in this analogy) that moves beside a long buffet table covered with all sorts of delicious food (the stationary phase). Hungry people, attracted to the food, will step off and on the moving belt many times in order to fill their plates. The not-so-hungry people will step off and on to get food far less often. Consequently, the more strongly attracted hungry people will lag behind, while the not-so-hungry ones will move ahead. The two types of people are thus separated based on the strength of their attraction for the food.

Stationary Phase Adsorbents

In TLC, the stationary phase is a polar adsorbent, usually finely ground alumina $[(Al_2O_3)_x]$ or silica $[(SiO_2)_x]$ particles, coated as a thin layer on a glass slide or plastic sheet. Silica, commonly called *silica gel* in the laboratory, is simply very pure white sand. The extended covalent network of these adsorbents creates a very polar surface. Partial structures of silica and alumina are shown below. The silicon or aluminum atoms are the smaller, darker spheres:

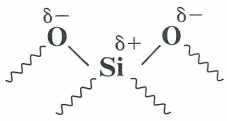


Silica, $(SiO_2)_x$



Alumina, $(Al_2O_3)_x$

FIG. 8.4
A partial silica structure showing polar Si–O bonds.



The electropositive character of the aluminum or silicon and the electronegativity of oxygen create a very polar stationary phase (Fig. 8.4). Therefore, the more polar the molecules to be separated, the stronger the attraction to the stationary phase. Nonpolar molecules will tend to stay in the mobile phase. In general, the more polar the functional group, the stronger the adsorption on the stationary phase and the more slowly the molecules will move. In an extreme situation, the molecules will not move at all. This problem can be overcome by increasing the polarity of the mobile phase so that the equilibrium between the free and adsorbed state is shifted toward the free state.

Although silica is the most common stationary phase used for TLC, many other types are used, ranging from paper to charcoal, nonpolar to polar, and reverse phase to normal phase. Several different types of stationary phases are listed according to polarity in Table 8.1.

Silica gel and alumina are commonly used in column chromatography for the purification of macroscopic quantities of material (*see* Chapter 9). Of the two,

TABLE 8.1 • Common Stationary Phases Listed by Increasing Polarity

Increasing polarity ↓

Polydimethyl siloxane*
Methyl- or Phenylsiloxane*
Cyanopropylsiloxane*
Carbowax [poly(ethyleneglycol)]*
Reverse phase (hydrocarbon-coated silica, e.g., C_{18})
Paper
Cellulose
Starch
Calcium sulfate
Silica (silica gel)
Florisil (magnesium silicate)
Magnesium oxide
Alumina (aluminum oxide; acidic, basic, or neutral)
Activated carbon (charcoal or Norit pellets)

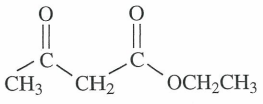
*Stationary phase for gas chromatography

alumina, when anhydrous, is the more active; that is, it will adsorb substances more strongly. It is thus the adsorbent of choice for the separation of relatively nonpolar substrates, such as hydrocarbons, alkyl halides, ethers, aldehydes, and ketones. To separate more polar substrates, such as alcohols, carboxylic acids, and amines, the less active adsorbent, silica gel, is often used.

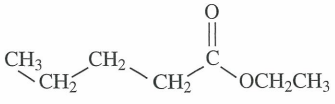
Molecular Polarity and Elution Sequence

Elution sequence is the order in which the components of a mixture move during chromatography.

Assuming we are using a polar adsorbent, how can we determine how rapidly the compounds in our particular mixture move, that is, their elution sequence? Because the more polar compounds will adsorb more strongly to the polar stationary phase, they will move the slowest and the shortest distance on a TLC plate. Nonpolar compounds will move rapidly; they will elute first or move the greatest distance on the TLC plate. Table 8.2 lists several common compound classes according to how they move or elute on silica or alumina.



Ethyl acetoacetate



Ethyl pentanoate

You should be able to look at a molecular structure, identify its functional group(s), and easily determine whether it is more or less polar than another structure with different functional groups. Note that the polarity of a molecule increases as the number of functional groups in that molecule increases. Thus, ethyl acetoacetate, with both ketone and ester groups, is more polar than ethyl pentanoate, which has only an ester group. However, it should be noted that chromatography is not an exact science. The rules discussed here can be used to help predict the order of elution; however, only performing an experiment will give definitive answers.

TABLE 8.2 • Elution Order for Some Common Functional Groups with a Silica or Alumina Stationary Phase

Increasing polarity of functional group ↓

Highest/fastest (elute with nonpolar mobile phase)
Alkane hydrocarbons
Alkyl halides (halocarbons)
Alkenes (olefins)
Dienes
Aromatic hydrocarbons
Aromatic halides
Ethers
Esters
Ketones
Aldehydes
Amines
Alcohols
Phenols
Carboxylic acids
Sulfonic acids
Lowest/slowest (need polar mobile phase to elute)

Mobile Phase Solvent Polarity

The key to a successful chromatographic separation is the mobile phase. You cannot change the polarities of the compounds in your mixture, and you normally use silica gel or alumina as the stationary phase. In extreme situations very polar substances chromatographed on alumina will not migrate very far from the starting point (i.e., give low R_f values), and nonpolar compounds chromatographed on silica gel will travel with the solvent front (i.e., give high R_f values). These extremes of behavior are markedly affected, however, by the solvents used to carry out the chromatography. A polar solvent will carry along with it polar substrates, and nonpolar solvents will do the same with nonpolar compounds—another example of the generalization "like dissolves like." By using different solvents, either alone or as mixtures, we can adjust the polarity of the mobile phase and affect the equilibria between the free and adsorbed states. Changing the polarity of the mobile phase can optimize the chromatographic separation of mixtures of compounds with a wide variety of polarities.

Table 8.3 lists, according to increasing polarity, some solvents that are commonly used for both TLC and column chromatography. Because the polarities of benzene, carbon tetrachloride, or chloroform can be matched by other, less toxic solvents, these three solvents are seldom used. In general, the solvents for TLC and column chromatography are characterized by having low boiling points that allow them to be easily evaporated and low viscosities that allow them to migrate

Avoid using benzene, carbon tetrachloride, and chloroform. Benzene is known to be a carcinogen when exposure is prolonged; the others are suspected carcinogens.

TABLE 8.3 • Common Mobile Phases Listed by Increasing Polarity

Increasing polarity ↓

Helium
Nitrogen
Pentanes (petroleum ether)
Hexanes (ligroin)
Cyclohexane
Carbon tetrachloride*
Toluene
Chloroform*
Dichloromethane (methylene chloride)
t-Butyl methyl ether
Diethyl ether
Ethyl acetate
Acetone
2-Propanol
Pyridine
Ethanol
Methanol
Water
Acetic acid

*Suspected carcinogens

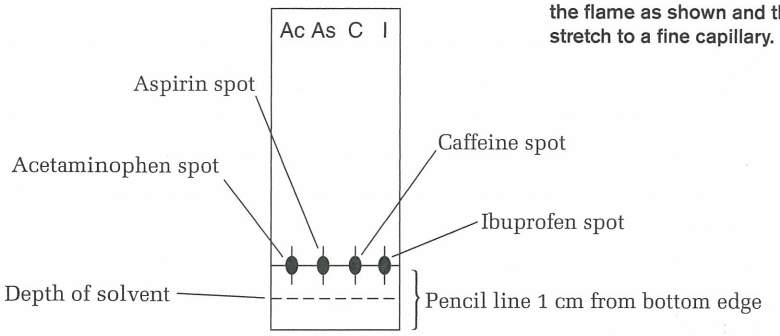
rapidly. A solvent more polar than methanol is seldom needed. Often, two solvents are used in a mixture of varying proportions; the polarity of the mixture is a weighted average of the two. Hexane and ether mixtures are often employed.

Finding a good solvent system is usually the most critical aspect of TLC. If the mobile phase has not been previously determined, start with a nonpolar solvent such as hexane or ligroin and observe the separation. If the mixture's components do not move very far, try adding a polar solvent such as ether or ethyl acetate to the hexane. Compare the separation to the previous plate. In most cases, a combination of two solvents is the best choice. If the spots stay at the bottom of the plate, add more of the polar solvent. If they run with the solvent front (move to the top), increase the proportion of the nonpolar solvent. Unfortunately, some trial and error is usually involved in determining which solvent system is the best. There is a large amount of literature on the solvents and adsorbents used in the separation of a wide variety of substances.

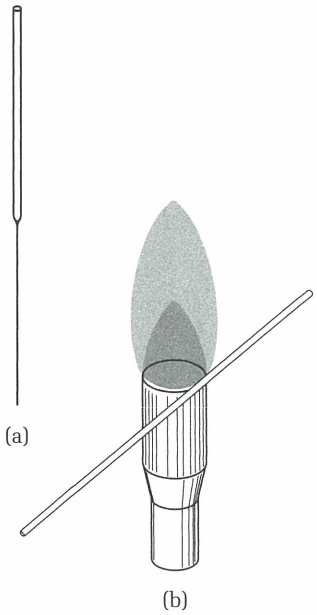
Spotting the TLC Plate

It is recommended that you use commercially available TLC plates, poly(ethylene terephthalate) (Mylar) sheets coated with silica gel using polyacrylic acid as a binder; these fluoresce under ultraviolet (UV) light.[1] The TLC plates must be

■ FIG. 8.5
A marked and spotted TLC plate.



■ FIG. 8.6
(a) A spotting capillary. (b) Soften the glass by heating at the base of the flame as shown and then stretch to a fine capillary.



handled gently, or the 100-mm-thick coating of silica gel can be easily scratched off. With a pencil, lightly draw a faint line 1 cm from the end and then three or four short hash marks to guide spotting. Lightly write identifying letters at the top of the plate to keep track of the placement of the compound spots (Fig. 8.5). Note that a pencil is always used to mark TLC plates because the graphite (carbon) is inert. If ink is used to mark the plate, it will chromatograph just as any other organic compound and give flawed results.

You need to dissolve only a few milligrams of material because one can detect a few micrograms of compound on a TLC plate. Choose a volatile solvent. Even if the material is only partially soluble, you will normally be able to observe the compound because only low concentrations are needed. It is extremely important that the spots be as small as possible and that they be applied using a 1% (not more than 2%) solution of the compounds being separated.

Too much sample is a frequent problem. Use a 1% solution of the mixture. Apply very small spots.

⚠ **CAUTION:** Bunsen burners should be used only in lab areas that are far from flammable organic solvents.

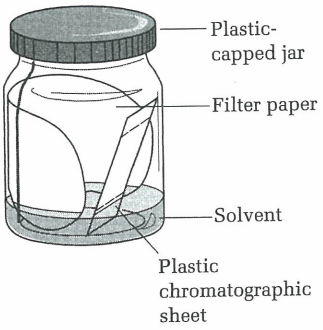
Once the sample is prepared, a spotting capillary must be used to add the sample to the plate. Spotting capillaries can be made by drawing out open-end melting point tubes or Pasteur pipette stems in a burner flame (Fig. 8.6).[2] The bore of these capillaries should be so small that once a liquid is drawn into them, it will not flow out to form a drop. Practice spotting just pure solvent onto an unmarked TLC plate. Dip the capillary into the solvent and let a 2–3 cm column of solvent flow into it by

1. Whatman flexible plates for TLC, cat. no. 4410 222 (Fisher cat. no. 05-713-162); cut with scissors to 1″ × 3″ or 2.5 × 7.5 cm Baker-flex, Silica Gel IB-F (J.T. Baker, Phillipsburg, N.J.). Unlike student-prepared plates, these coated sheets give very consistent results. A supply of these plates makes it a simple matter to examine most of the reactions in this book for completeness of reaction, purity of product, and side reactions.

2. Three-inch pieces of old and unusable gas chromatography capillary columns are also effective spotting capillaries.

capillary action, hold this vertically over the *coated* side of the plate, and lower the pipette until the tip just touches the adsorbent. Only then will liquid flow onto the plate; quickly withdraw the capillary when the spot is about 1 mm in diameter. The center of the letter *o* on this page is more than 1 mm in diameter. The distance between the sides of the letter *n* is 1 mm. The solvent should evaporate quickly, leaving your mixture behind on the plate. You may have to spot the plate a couple of times to ensure that sufficient material is present; do not spot too much sample because this will lead to a poor separation. Smearing, smudging, and overlapping of spots will make the identification of separated components difficult. Practice spotting a number of times until you develop good spotting technique. You are now ready to spot the mixture solutions as described in Experiments 1 and 2.

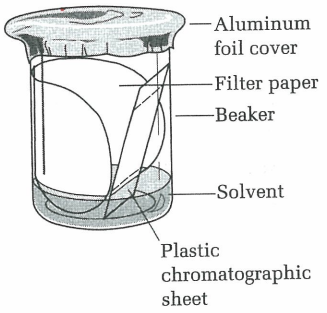
FIG. 8.7
Using a wide-mouth bottle to develop a TLC plate.



Development

Once the dilute solution of the mixture has been spotted on the plate, the next step is the actual chromatographic separation, called *plate development*. The marked and spotted TLC plate is inserted into a 4-oz wide-mouth bottle (Fig. 8.7) or beaker (Fig. 8.8) containing 4 mL of an organic solvent or solvent mixture. The bottle is lined with filter paper that is wet with solvent to saturate the atmosphere within the container. Use tweezers to place the plate in the development chamber; oils from your fingers can sometimes smear or ruin a TLC plate. Also make sure that the origin spots are not below the solvent level in the chamber. If the spots are submerged in the solvent, they are washed off the plate and lost. The top of the bottle is put in place and the time noted. (If a beaker is used, the beaker is to be covered with aluminium foil.) The solvent travels up the thin layer by capillary action, and if the substance is a pure colored compound, one soon sees a spot traveling either along with the solvent front or, more commonly, at some distance behind the solvent front. Once the solvent has run within a centimeter of the top of the plate, remove the plate with tweezers. Immediately, before the solvent evaporates, use a pencil to draw a line across the plate where the solvent front can be seen. The proper location of this solvent front line is important for R_f calculations.

FIG. 8.8
Using a foil-covered beaker to develop a TLC plate.



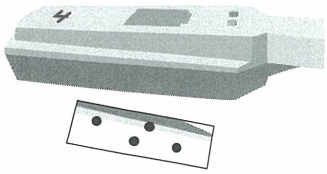
Visualization

If you are fortunate enough to be separating organic molecules that are colored, such as dyes, inks, or indicators, then visualizing the separated spots is easy. However, because most organic compounds are colorless, this is rarely the case.

For most compounds a UV light works well for observing the separated spots. TLC plates normally contain a fluorescent indicator that makes them glow green under UV light of wavelength 254 nm. Compounds that adsorb UV light at this wavelength will quench the green fluorescence, yielding dark purple or bluish spots on the plate. Simply hold the plate by its edges under a UV lamp as shown in Figure 8.9, and the compound spots become visible to the naked eye. Lightly circle the spots with a pencil so that you will have a permanent record of their location for later calculations.

Another useful visualizing technique is to use an iodine (I_2) chamber. Certain compounds, such as alkanes, alcohols, and ethers, do not absorb UV light sufficiently

FIG. 8.9
A UV lamp used to visualize spots.



⚠ Never look into a UV lamp.

to quench the fluorescence of the TLC plate and therefore will not show up under a UV lamp. However, they will adsorb iodine vapors and can be detected (after any residual solvent has evaporated) by placing the plate for a few minutes in a capped 4-oz bottle containing some crystals of iodine. Iodine vapor is adsorbed by the organic compound to form brown spots. These brown spots should be outlined with a pencil immediately after removing the plate from the iodine bottle because they will soon disappear as the iodine sublimes away; a brief return to the iodine chamber will regenerate the spots. Using both the UV lamp and iodine vapor visualization methods will ensure the location of all spots on the TLC plate.

Many specialized spray reagents have also been developed to give specific colors for certain types of compounds.

R_f Values

R_f is the ratio of the distance the spot travels from the origin to the distance the solvent travels.

In addition to qualitative results, TLC can also provide a chromatographic parameter known as an R_f value. The R_f value is the retardation factor or the ratio-to-front value expressed as a decimal fraction. The R_f value is the ratio of the distance the spot travels from the point of origin to the distance the solvent travels. The R_f value can be calculated as follows:

$$R_f = \frac{\text{distance spot travels}}{\text{distance solvent travels}}$$

This number should be calculated for each spot observed on a TLC plate. Figure 8.10 shows a diagram of a typical TLC plate and how the distances are measured to calculate the R_f value. The best separations are usually achieved when the R_f values fall between 0.3 and 0.7.

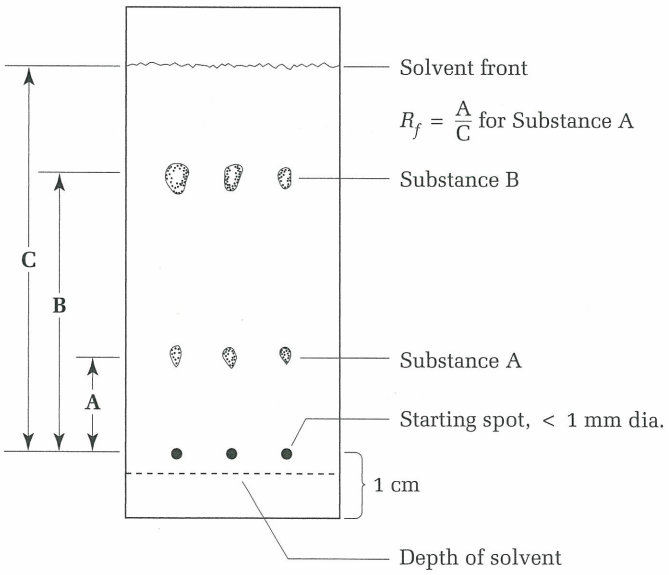


FIG. 8.10
A developed TLC plate with spots visualized and R_f values determined.

If two spots travel the same distance or have the same R_f value, then it might be concluded that the two components are the same molecule. Just as many organic molecules have the same melting point and color, many can have the same R_f value, so identical R_f values do not necessarily mean identical compounds. For comparisons of R_f values to be valid, TLC plates must be run under the exact same conditions for stationary phase, mobile phase, and temperature. Even then, additional information such as a mixed melting point or an IR spectrum should be obtained before concluding that two substances are identical.

Comparison of Different Types of Chromatography

Table 8.4 summarizes the terminology used in chromatography and how these apply to different types of chromatography. All of the chromatographic types involve the same principles but vary in the nature of the stationary phase and the mobile phase and the measure of separation.

EXPERIMENTS

1. Analgesics

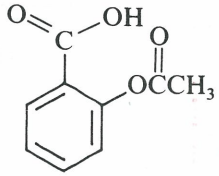
Analgesics are substances that relieve pain. The most common of these is aspirin, a component of more than 100 nonprescription drugs. In Chapter 41, the history of this most popular drug is discussed. In this experiment, analgesic tablets will be analyzed by TLC to determine which analgesics they contain and whether they contain caffeine, which is often added to counteract the sedative effects of the analgesic.

In addition to aspirin and caffeine, the most common components of the currently used analgesics are acetaminophen and ibuprofen. In addition to one or

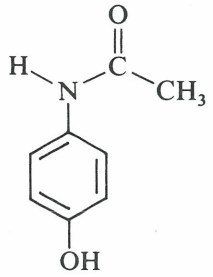
TABLE 8.4 • Chromatography Terms and Their Definitions with Examples

Chromatography Term	Definition	Examples
Mixture	A collection of different compounds	Aspirin, ibuprofen, caffeine, and fluorene/fluorenone
Stationary phase	A fixed material that can adsorb compounds	Alumina, silica gel, and silicone gum
Mobile phase	A moving liquid or gas that dissolves compounds and carries them along	Hexane, CH_2Cl_2, and ethyl acetate (TLC and column chromatography); helium gas (gas chromatography)
Adsorption	The strength of attraction between the compounds and the stationary phase	London forces, hydrogen bonds, and dipole-dipole attractive forces
Separation	A measure of the elution or migration rate of compounds	R_f (TLC); elution volume (column chromatography); retention time (gas chromatography)

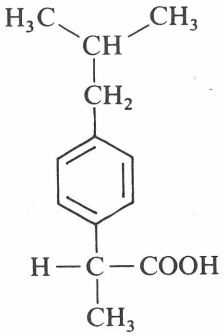
more of these substances, each tablet contains a binder—often starch, microcrystalline cellulose, or silica gel. And to counteract the acidic properties of aspirin, an inorganic buffering agent is added to some analgesics. An inspection of analgesic labels will reveal that most cold remedies and decongestants contain both aspirin and caffeine in addition to the primary ingredient.



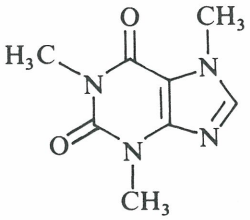
Aspirin
Acetylsalicylic acid



Acetaminophen
4-Acetamidophenol



Ibuprofen
2-(4-Isobutylphenyl)propionic acid



Caffeine

To identify an unknown by TLC, the usual strategy is to run chromatograms of known substances (the standards) and the unknown at the same time. If the unknown has one or more spots that correspond to spots with the same R_f values as the standards, then those substances are probably present.

Proprietary drugs that contain one or more of the common analgesics and sometimes caffeine are sold under some of the following brand names: Bayer Aspirin, Anacin, Datril, Advil, Excedrin, Extra Strength Excedrin, Tylenol, and Vanquish. Note that ibuprofen has a chiral carbon atom. The *S*-(+)-enantiomer is more effective than the other.

Procedure

Before proceeding, practice the TLC spotting technique described earlier. Following that procedure, draw a light pencil line about 1 cm from the end of a chromatographic plate. On this line spot aspirin, acetaminophen, ibuprofen, and

caffeine, which are available as reference standards. Use a separate capillary for each standard (or rinse the capillary carefully before reusing). Make each spot as small as possible, preferably less than 0.5 mm in diameter. Examine the plate under UV light to see that enough of each compound has been applied; if not, add more. On a separate plate, run the unknown and one or more of the standards.

Because of the insoluble binder, not all of the unknown will dissolve.

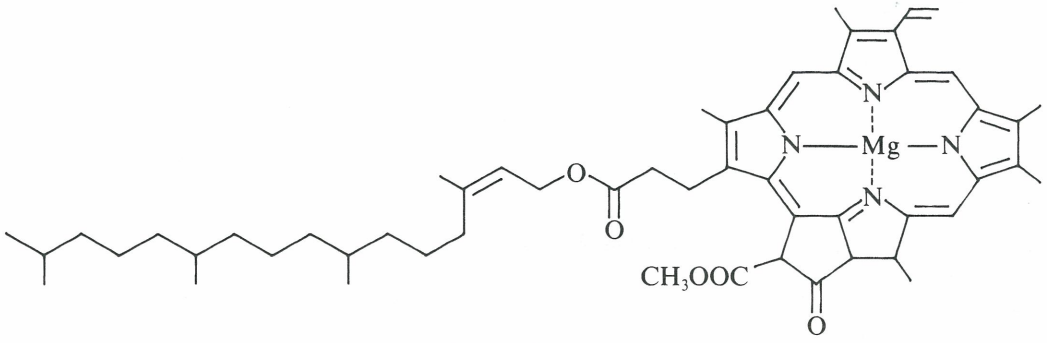
The unknown sample is prepared by crushing a part of a tablet, adding this powder to a test tube or small vial along with an appropriate amount of ethanol, and then mixing the suspension. Not all of the crushed tablet will dissolve, but enough will go into solution to spot the plate. The binder—starch or silica—will not dissolve. Weigh out only part of the tablet to try to prepare a 1% solution of the unknown. Typically, ibuprofen tablets contain 200 mg of the active ingredient, aspirin tablets contain 325 mg, and acetaminophen tablets contain 500 mg.

To the developing jar or beaker (*see* Fig. 8.7 or Fig. 8.8 on page 181), add 4 mL of the mobile phase, a mixture of 95% ethyl acetate and 5% acetic acid. Insert the spotted TLC plates with a tweezers. After the solvent has risen nearly to the top of the plate, remove the plate from the developing chamber, mark the solvent front with a pencil, and allow the solvent to dry. Examine the plate under UV light to see the components as dark spots against a bright green-blue background. Outline the spots with a pencil. The spots can also be visualized by putting the plate in an iodine chamber made by placing a few crystals of iodine in the bottom of a capped 4-oz jar. Calculate the R_f values for the spots and identify the components in the unknown.

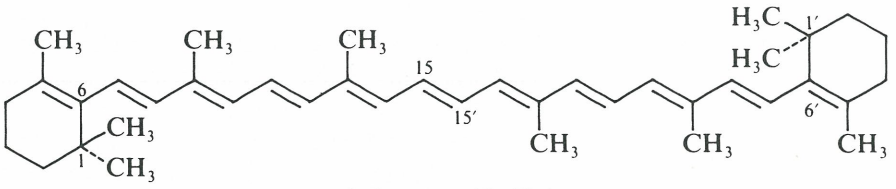
Cleaning Up. Solvents should be placed in the organic solvents container; dry, used chromatographic plates can be discarded in the nonhazardous solid waste container.

2. Plant Pigments

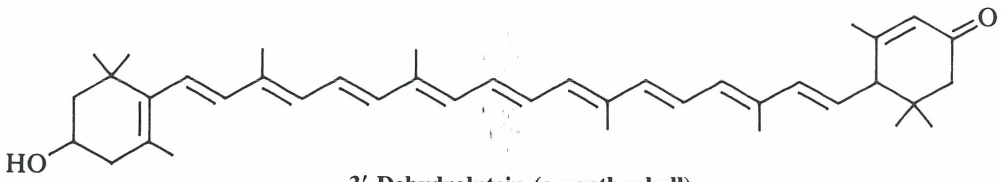
The botanist Michael Tswett discovered the technique of chromatography and applied it, as the name implies, to colored plant pigments. The leaves of plants contain, in addition to chlorophyll-a and chlorophyll-b, other pigments that are revealed in the fall when the leaves die and the chlorophyll rapidly decomposes. Among the most abundant of the other pigments are the carotenoids, which include the carotenes and their oxygenated homologs, the xanthophylls. The bright orange β-carotene is the most important of these because it is transformed in the liver to vitamin A, which is required for night vision.



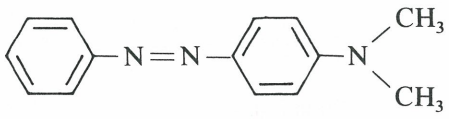
Chlorophyll-a



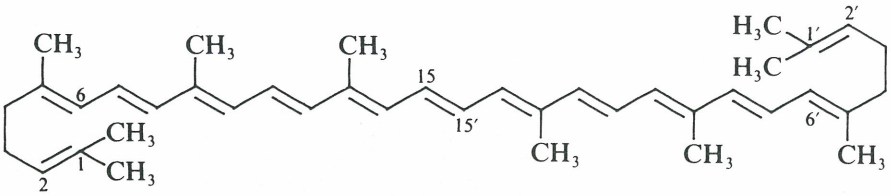
β-Carotene ($C_{40}H_{56}$)
mp 183°C, λ_{max}^{hexane} 451 nm



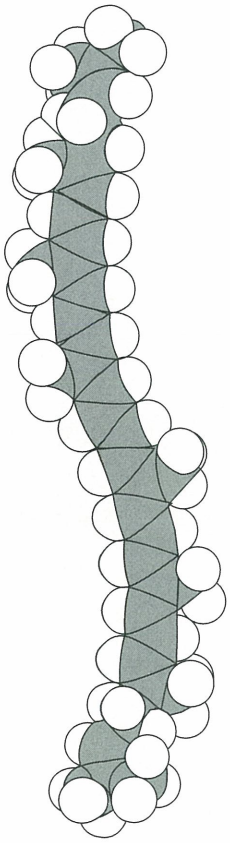
3′-Dehydrolutein (a xanthophyll)



Butter Yellow
(carcinogenic)

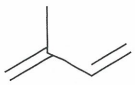


Lycopene ($C_{40}H_{56}$)
MW 536.85
mp 173°C, λ_{max}^{hexane} 475 nm



■ **FIG. 8.11**
An energy-minimized, space-filling model of lycopene. The molecule is flat, but steric hindrance of the methyl groups causes the molecule to bend into an S shape.

Cows eat fresh, green grass that contains carotene, but they do not metabolize the carotene entirely, so it ends up in their milk. Butter made from this milk is therefore yellow. In the winter the silage cows eat does not contain carotene because that compound readily undergoes air oxidation, and the butter made at that time is white. For some time an azo dye called Butter Yellow was added to winter butter to give it the accustomed color, but the dye was found to be a carcinogen. Now winter butter is colored with synthetic carotene, as is all margarine.



Isoprene

Lycopene (Fig. 8.11), the red pigment of the tomato, is a C_{40}-carotenoid made up of eight isoprene units. β-Carotene, the yellow pigment of the carrot, is an isomer of lycopene in which the double bonds at C_1—C_2 and C'_1—C'_2 are replaced by bonds extending from C_1 to C_6 and from C'_1 to C'_6 to form rings. The chromophore in each case is a system of 11 all-*trans* conjugated double bonds; the closing of the two rings causes β-carotene to absorb at shorter wavelengths than lycopene does, shifting its color from red to yellow.

Lycopene from tomato paste and β-carotene from strained carrots

In 1911, Richard Willstätter and Heinrich R. Escher isolated 20 mg of lycopene per kilogram of fresh tomatoes, which contain about 96% water.[3] They then found a more convenient source in commercial tomato paste: the seeds and skin were eliminated, and the water content was reduced by evaporation in vacuum to a content of 26% solids. From this they isolated 150 mg of lycopene per kilogram of paste. The expected yield in the following experiment is 0.075 mg, which is not enough to weigh on a balance.

As an interesting variation, try extracting lycopene from commercial ketchup.

A jar of strained carrots sold as baby food serves as a convenient source of β-carotene. The German investigators isolated 1 g of β-carotene per kilogram of dried, shredded carrots of unstated water content.

The following procedure calls for the dehydration of tomato or carrot paste with ethanol and extraction with dichloromethane, an efficient solvent for lipids.

Experimental Considerations

CAUTION: Do not breathe the vapors of dichloromethane. Carry out the extraction in the hood.

Carotenoids are very sensitive to light-catalyzed air oxidation. Perform this experiment as rapidly as possible; keep the solutions as cool and dark as possible. This extraction produces a mixture of products that can be analyzed by both TLC and column chromatography (*see* Chapter 9). If enough material for TLC only is desired, use one-tenth the quantities of starting material and solvents employed in the following procedure. This extraction can also be carried out with hexane if the ventilation is not adequate enough to use dichloromethane. However, hexane is more prone to form emulsions than the chlorinated solvent.

Procedure

IN THIS EXPERIMENT some tomato or carrot paste is treated with acetone, which will remove water and lipids but not the highly colored carotenoid hydrocarbons. The carotenoids are extracted by dichloromethane and analyzed by TLC.

A 5-g sample of fresh tomato or carrot paste (baby food) is transferred to the bottom of a 25 × 150 mm test tube, followed by 10 mL of acetone. The mixture is stirred and shaken before being filtered on a Hirsch funnel. Scrape as much of the material from the tube as possible and press it dry on the funnel. Let the tube drain thoroughly. Place the filtrate in a 125-mL Erlenmeyer flask.

3. Willstätter, R.; Escher, H. R. *Z. Physiol. Chem.* **1911**, *64*, 47–61.

Return the solid residue to the test tube, shake it with a 10-mL portion of dichloromethane, and again filter the material on a Hirsch funnel. Add the filtrate to the 125-mL flask. Repeat this process two more times and then pour the combined filtrates into a separatory funnel. Add water and sodium chloride solution (which aids in the breaking of emulsions) and shake the funnel gently. This aqueous extraction will remove the acetone and any water-soluble components from the mixture, leaving the hydrocarbon carotenoids in the dichloromethane. Dry the colored organic layer over anhydrous calcium chloride and filter the solution into a dry flask. Remove about 0.5 mL of this solution and store it under nitrogen in the dark until it can be analyzed by TLC. Evaporate the remainder of the dichloromethane solution to dryness under a stream of nitrogen or under vacuum on a rotary evaporator. This material can be used for column chromatography (*see* Chapter 9). If it is to be stored, fill the flask with nitrogen and store it in a dark place.

Air can be used for the evaporation, but nitrogen is better because these hydrocarbons air oxidize with great rapidity.

Thin-Layer Chromatography

Spot the mixture on a TLC plate about 1 cm from the bottom and 8 mm from the edge. Make one spot concentrated by repeatedly touching the plate, but ensure that the spot is as small as possible, certainly less than 1 mm in diameter. The other spot can be of lower concentration. Develop the plate with an 80:20 hexane-acetone mixture. With other plates you could try cyclohexane and toluene as eluents and also hexane-ethanol mixtures of various compositions.

Many spots may be seen. There are two common carotene and chlorophyll isomers and four xanthophyll isomers.

The container in which the chromatography is carried out should be lined with filter paper that is wet with the solvent so that the atmosphere in the container will be saturated with the solvent vapor. After elution is completed, remove the TLC plate and mark the solvent front with a pencil and outline the colored spots. Examine the plate under UV light. Also place the plate in an iodine chamber to visualize the spots.

Cleaning Up. The aqueous saline filtrate containing acetone can be flushed down the drain. Recovered and unused dichloromethane should be placed in the halogenated organic waste container; the solvents used for TLC should be placed in the organic solvents container. If local regulations allow, evaporate any residual solvent from the drying agents in the hood and place the dried solid in the nonhazardous waste container. Otherwise, place the wet drying agent in a waste container designated for this purpose. Used plant material and dry TLC plates can be discarded in the nonhazardous waste container.



Procedure

In a small mortar grind 2 g of green or brightly colored fall leaves (do not use ivy or waxy leaves) with 10 mL of ethanol, pour off the ethanol (which serves to break up and dehydrate the plant cells), and grind the leaves successively with three 1-mL portions of dichloromethane that are decanted or withdrawn with a Pasteur pipette and placed in a test tube. The pigments of interest are extracted by the dichloromethane. Alternatively, place 0.5 g of carrot paste (baby food) or tomato paste in a test tube, stir and shake the paste with 3 mL of ethanol until the paste has a somewhat dry or fluffy appearance, remove the ethanol, and extract the

dehydrated paste with three 1-mL portions of dichloromethane. Stir and shake the plant material with the solvent to extract as much of the pigments as possible.

Fill the tube containing the dichloromethane extract from leaves or vegetable paste with a saturated sodium chloride solution and shake the mixture. Remove the aqueous layer; add anhydrous calcium chloride pellets to the dichloromethane solution until the drying agent no longer clumps together. Shake the mixture with the drying agent for about 5 min and then withdraw the solvent with a Pasteur pipette and place it in a test tube. Add to the solvent a few pieces of Drierite to complete the drying process. Gently stir the mixture for about 5 min, transfer the solvent to a test tube, wash off the drying agent with more solvent, and then evaporate the combined dichloromethane solutions under a stream of nitrogen while warming the tube in your hand or in a beaker of warm water. Carry out this evaporation in the hood.

These hydrocarbons air oxidize with great rapidity.

Immediately cork the tube filled with nitrogen and then add 1 or 2 drops of dichloromethane to dissolve the pigments for TLC analysis. Carry out the analysis without delay by spotting the mixture on a TLC plate about 1 cm from the bottom and 8 mm from the edge. Make one spot concentrated by repeatedly touching the plate, but ensure that the spot is as small as possible—less than 1.0 mm in diameter. The other spot can be of lower concentration. Develop the plate with a 70:30 hexane-acetone mixture. With other plates try cyclohexane and toluene as eluents and also hexane-ethanol mixtures of various compositions. The container in which the chromatography is carried out should be lined with filter paper that is wet with the solvent so that the atmosphere in the container will be saturated with solvent vapor. After elution is completed, mark the solvent front with a pencil and outline the colored spots. Examine the plate under the UV light. Are any new spots seen? Report colors and R_f values for all of your spots and identify each as lycopene, carotene, chlorophyll, or xanthophyll.

Cleaning Up. The ethanol used for the dehydration of the plant material can be flushed down the drain along with the saturated sodium chloride solution. Recovered and unused dichloromethane should be placed in the halogenated organic waste container. The solvents used for TLC should be placed in the organic solvents container. If local regulations allow, evaporate any residual solvent from the drying agents in the hood and place the dried solid in the nonhazardous waste container. Otherwise, place the wet drying agent in a waste container designated for this purpose. Used plant material and dry TLC plates can be discarded in the nonhazardous waste container.

3. For Further Investigation

Many of the pigments in plants are made up of compounds called *anthocyanins*. Grind about 4 g of colored plant tissue (flower petals, blueberries, strawberries, cranberries, apple skins, red cabbage, red or purple grapes, etc.) and a small amount of alumina or fine sand with about 4 mL of a mixture of 99% methanol and 1% hydrochloric acid. Spot the extract on cellulose TLC plates and elute with a solvent mixture of 20% concentrated hydrochloric acid, 40% water, and 4% formic acid. Note the number and color of the spots. Look up the structures of the possible anthocyanins, of which many are glycosides of the aglycones delphinidin, peonidin, malvidin, and cyanidin, among others.

4. Colorless Compounds

You will now apply the thin-layer technique to a group of colorless compounds. The spots can be visualized under UV light if the plates have been coated with a fluorescent indicator; chromatograms can also be developed in a 4-oz bottle containing crystals of iodine. During development, spots will appear rapidly but remember that they also disappear rapidly. Therefore, outline each spot with a pencil immediately on withdrawal of the plate from the iodine chamber. Some suggested solvents are pure cyclohexane, pure toluene, toluene (3 mL) plus dichloromethane (1 mL), or toluene (4.5 mL) plus methanol (1/2 mL).

The compounds for trial are to be selected from the following list (all 1% solutions in toluene; those compounds with an asterisk are fluorescent under UV light):

1. Anthracene*
2. Cholesterol
3. 2,7-Dimethyl-3,5-octadiyne-2,7-diol
4. Diphenylacetylene
5. *trans,trans*-1,4-Diphenyl-1,3-butadiene*
6. *p*-Di-*t*-butylbenzene
7. 1,4-Di-*t*-butyl-2,5-dimethoxybenzene
8. *trans*-Stilbene
9. 1,2,3,4-Tetraphenylnaphthalene*
10. Tetraphenylthiophene
11. *p*-Terphenyl*
12. Triphenylmethanol
13. Triptycene

Except for tetraphenylthiophene, the structures for all of these molecules will be found in this book.

Make your own selections.

It is up to you to make selections and to plan your own experiments. Do as many as time permits. One plan would be to select a pair of compounds that are estimated to be separable and that have R_f values determinable with the same solvent. One can assume that a hydroxyl compound will travel less rapidly with a hydrocarbon solvent than a hydroxyl-free compound; you will therefore know what to expect if the solvent contains a hydroxylic component. An aliphatic solvent should carry along an aromatic compound with aliphatic substituents better than one without such groups. However, instead of relying on assumptions, you can do brief preliminary experiments on used plates on which previous spots are visible or outlined. If you spot a pair of compounds on such a plate and let the solvent rise about 3 cm from the starting line before development, you might be able to tell if a certain solvent is appropriate for a given sample. Alternatively, make some spots on a plate (new or used) and then touch each spot with a different solvent held in a capillary. In Figure 8.12a, the mixture did not move away from the point of origin; in Figure 8.12b, two concentric rings are seen between the origin and the solvent front. This is how a good solvent behaves. In Figure 8.12c, the mixture of compounds traveled with the solvent front.

Preliminary trials on used plates

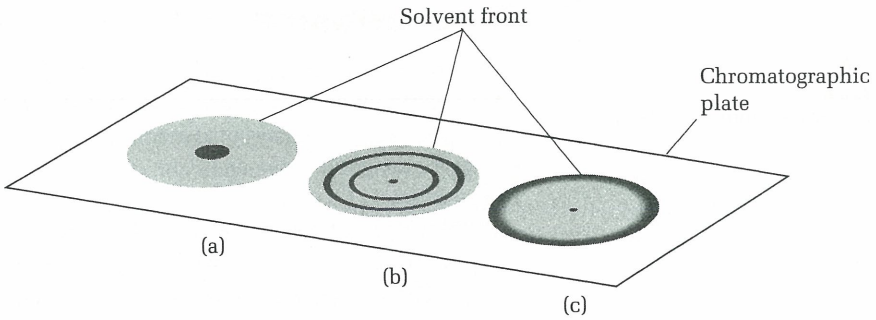


FIG. 8.12
A fast method for determining the correct solvent for TLC. See the text for the procedure.

Once a solvent is chosen, run a complete chromatogram on the two compounds on a fresh plate. If separation of the two compounds seems feasible, put two spots of one compound on a plate, let the solvent evaporate, and put spots of the second compound over the first ones. Run a chromatogram and see if you can detect two spots in either lane (with colorless compounds, it is advisable not to attempt a three-lane chromatogram until you have acquired considerable practice and skill).

Cleaning Up. Solvents should be placed in the organic solvents container, and dry, used chromatographic plates can be discarded in the nonhazardous solid waste container.

Discussion

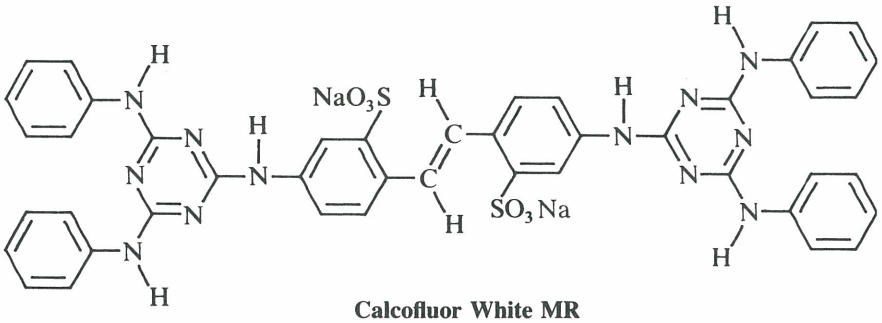
If you have investigated hydroxylated compounds, you doubtless have found that it is reasonably easy to separate a hydroxylated from a nonhydroxylated compound or a diol from a mono-ol. How, by a simple reaction followed by a thin-layer chromatogram, could you separate cholesterol from triphenylmethanol? Heating a sample of each with acetic anhydride and a trace of pyridine catalyst for 5 min on a steam bath, followed by chromatography, should do it. A first trial of a new reaction leaves questions about what has happened and how much, if any, starting material is present. A comparative chromatogram of the reaction mixture with starting material may tell the story. How crude is a crude reaction product? How many components are present? The thin-layer technique may give the answers to these questions and suggest how best to process the product. A preparative column chromatogram may afford a large number of fractions of eluent (say, 1 to 30). Some fractions probably contain nothing and should be discarded, while others should be combined for evaporation and workup. How can you identify the good and the useless fractions? Take a few used plates and put numbered circles on clean places of each; spot samples of each of the fractions; and, without any chromatography, develop the plates with iodine. Negative fractions for discard will be obvious, and the pattern alone of positive fractions may allow you to infer which fractions can be combined. Thin-layer chromatograms of the first and last fractions of each suspected group would then show whether or not your inferences are correct.

Fluorescence

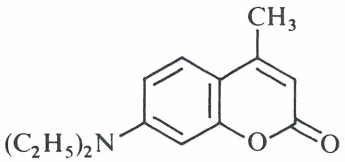
Never look into a UV lamp.

Four of the compounds listed in Experiment 4 are fluorescent under UV light. These compounds give colorless spots that can be picked up on a chromatogram by fluorescence (after removal from the UV-absorbing glass bottle). If a UV light source is available, spot the four compounds on a used plate and observe the fluorescence.

Take this opportunity to examine a white shirt or handkerchief under UV light to see if it contains a brightener, that is, a fluorescent white dye or optical bleach. These substances are added to counteract the yellow color that repeated washing gives to cloth. Brighteners of the type of Calcofluor White MR, a sulfonated *trans*-stilbene derivative, are commonly used in detergent formulations for cotton; the substituted coumarin derivative is typical of brighteners used for nylon, acetate, and wool. Detergents normally contain 0.1%–0.2% of optical bleach. The amount of dye on a freshly laundered shirt is approximately 0.01% of the weight of the fabric.



Calcofluor White MR



7-Diethylamino-4-methylcoumarin

Online Study Center
General Resources
Additional Experiments

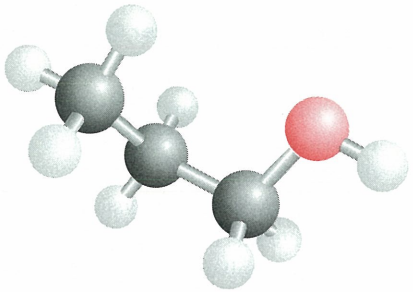
QUESTIONS

1. Why might it be very difficult to visualize the separation of *cis*- and *trans*-2-butene by TLC?
2. What error is introduced into the determination of an R_f value if the top is left off the developing chamber?
3. What problem will ensue if the level of the developing liquid is higher than the applied spot in a TLC analysis?

4. In what order (from top to bottom) would you expect to find naphthalene, butyric acid, and phenyl acetate on a silica gel TLC plate developed with dichloromethane?
5. In carrying out an analysis of a mixture, what do you expect to see when the TLC plate has been allowed to remain in the developing chamber too long, so that the solvent front has reached the top of the plate?
6. Arrange the following in order of increasing R_f with TLC: acetic acid, acetaldehyde, 2-octanone, decane, and 1-butanol.
7. What will be the result of applying too much compound to a TLC plate?
8. Why is it necessary to run TLC in a closed container and to have the interior vapor saturated with the solvent?
9. What will be the appearance of a TLC plate if a solvent of too low polarity is used for the development? a solvent of too high polarity?
10. A TLC plate showed two spots with R_f values of 0.25 and 0.26. The plate was removed from the developing chamber, the residual solvent was allowed to evaporate from the plate, and then the plate was returned to the developing chamber. What would you expect to see after the second development was complete?
11. One of the analgesics has a chiral center. Which compound is it? One of the two enantiomers is far more effective at reducing pain than the other.
12. Using a ruler to measure distances, calculate the R_f value for substance B in Figure 8.10.

CHAPTER

9



Column Chromatography: Fluorenone, Cholesteryl Acetate, Acetylferrocene, and Plant Pigments

Online Study Center

This icon will direct you to techniques, equipment setups, and online resources at http://college.hmco.com/PIC/williamsonMMOE5e.

PRELAB EXERCISE: Compare column chromatography and thin-layer chromatography (TLC) with regard to the (1) quantity of material that can be separated, (2) time needed for the analysis, (3) solvent systems used, and (4) ability to separate compounds.

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids when carrying out microscale experiments. It becomes expensive and time consuming, however, when more than about 10 g of material must be purified. Column chromatography involves the same chromatographic principles as detailed for TLC in Chapter 8, so be sure that you understand those before doing the experiments in this chapter.

As discussed in Chapter 1, organic chemists obtain new compounds by synthesizing or isolating natural products that have been biosynthesized by microbes, plants, or animals. In most cases, initial reaction products or cell extracts are complex mixtures containing many substances. As you have seen, recrystallization, distillation, liquid/liquid extraction, and sublimation can be used to separate and purify a desired compound from these mixtures. However, these techniques are frequently not adequate for removing impurities that are closely related in structure. In these cases, column chromatography is often used. The broad applicability of this technique becomes obvious if you visit any organic chemistry research lab, where chromatography columns are commonplace.

Three of the five experiments in this chapter involve synthesis and may be your first experience in running an organic reaction. Experiments 1 and 2 involve the synthesis of a ketone. In Experiment 3 an ester of cholesterol is prepared. Experiment 4 demonstrates the separation of colored compounds. Experiment 5 involves the isolation and separation of natural products (plant pigments), which is analogous to Experiment 2 in Chapter 8 but on a larger scale.