CHAPTER 10

Column Chromatography: Acetylferrocene, Cholesteryl Acetate, and Fluorenone

Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids when carrying out small-scale experiments. It becomes expensive and time consuming, however, when more than about 10 g of material must be purified.

The application in the present experiment is typical: A reaction is carried out, it does not go to completion, and so column chromatography is used to separate the product from starting material, reagents, and byproducts.

The theory of column chromatography is analogous to that of thin-layer chromatography. The most common adsorbents—silica gel and alumina—are the same ones used in TLC. The sample is dissolved in a small quantity of solvent (the eluent) and applied to the top of the column. The eluent, instead of rising by capillary action up a thin layer, flows down through the column filled with the adsorbent. Just as in TLC, there is an equilibrium established between the solute adsorbed on the silica gel or alumina and the eluting solvent flowing down through the column. Under some conditions the solute may be partitioning between an adsorbed solvent and the elution solvent; the partition coefficient, just as in the extraction process, determines the efficiency of separation in chromatography. The partition coefficient is determined by the solubility of the solute in the two phases, as was discussed in the extraction experiment (Chapter 8).

Three mutual interactions must be considered in column chromatography: the polarity of the sample, the polarity of the eluting solvent, and the activity of the adsorbent.

Adsorbent

A large number of adsorbents have been used for column chromatography—cellulose, sugar, starch, inorganic carbonates—but most separations employ alumina (Al_2O_3) and silica gel (SiO_2). Alumina comes in three forms: acidic, neutral, and basic. The neutral form of Brockmann activity II or III, 150 mesh, is most commonly employed. The surface area of this alumina is about 150 m^2/g. Alumina as purchased will usually be activity I, meaning it will strongly adsorb solutes. It must be deactivated by adding water, shaking, and allowing the mixture to reach equilibrium over an hour or so. The amount of water needed to achieve certain activities is given in Table 10.1. The activity of the alumina on TLC plates is usually about III. Silica gel for column chromatography, 70–230 mesh, has a surface area of about 500 m^2/g and comes in only one activity.

TABLE 10.1 Alumina Activity

Brockmann activity	I	II	III	IV	V
Percent by weight of water	0	3	6	10	15

Solvents

The elutropic series for a number of solvents is given in Table 10.2. The solvents are arranged in increasing polarity, with *n*-pentane being the least polar. This is the order of ability of these solvents to dissolve polar organic compounds and to dislodge a polar substance adsorbed onto either silica gel or alumina, with *n*-pentane having the lowest solvent power.

As a practical matter, the following sequence of solvents is recommended in an investigation of unknown mixtures: elute first with petroleum ether; then ligroin, followed by ligroin containing 1%, 2%, 5%, 10%, 25%, and 50% *t*-butyl methyl ether; pure *t*-butyl methyl ether; *t*-butyl methyl ether and dichloromethane mixtures, followed by dichloromethane and methanol mixtures. A sudden change in solvent polarity will cause heat evolution as the alumina or silica gel adsorbs the new solvent. This will cause undesirable vapor pockets and cracks in the column.

Petroleum ether: mostly isometric pentanes; ligroin: mostly isomeric hexanes

TABLE 10.2 Elutropic Series for Solvents

n-Pentane (first)
Petroleum ether
Cyclohexane
Ligroin
Carbon disulfide
t-Butyl methyl ether
Dichloromethane
Tetrahydrofuran
Dioxane
Ethyl acetate
2-Propanol
Ethanol
Methanol
Acetic acid (last)

Solutes

The ease with which different classes of compounds elute from a column is indicated in Table 10.3. The order is similar to that of the eluting solvents—another application of "like dissolves like."

TABLE 10.3 Elution Order for Solutes

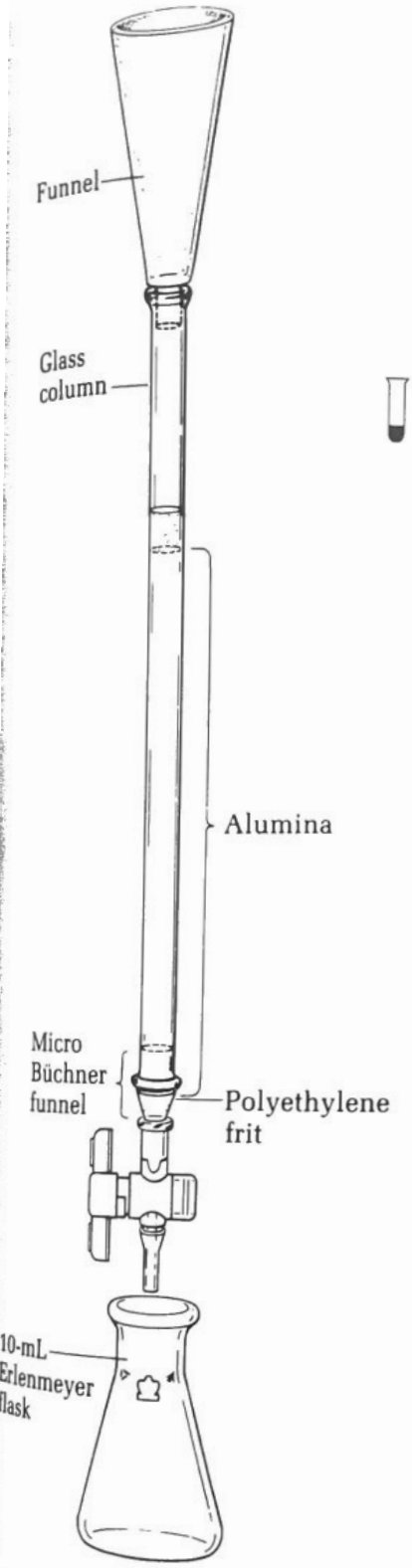
Alkanes (first)
Alkenes
Dienes
Aromatic hydrocarbons
Ethers
Esters
Ketones
Aldehydes
Amines
Alcohols
Phenols
Acids (last)

Sample and Column Size

In general the amount of alumina or silica gel used should weigh at least 30 times as much as the sample, and the column, when packed, should have a height at least 10 times the diameter. The density of silica gel is 0.4 g/mL, and the density of alumina is 0.9 g/mL, so the optimum size for any column can be calculated. A microscale column for the chromatography of about 50 mg of material is shown in Fig. 10.1.

Packing the Chromatography Column

Uniform packing of the chromatography column is critical to the success of this technique. The sample is applied as a pure liquid or, if it is a solid, as a very concentrated solution in the solvent that will dissolve it best, regardless of polarity. As elution takes place, this narrow band of sample will separate into several bands corresponding to the number of components in the mixture and their relative polarities and molecular weights. It is essential that the components move through the column as a narrow horizontal band in order to come off the column



IG. 10.1 Microscale hromatographic column.

in the least volume of solvent and not overlap with other components of the mixture. Therefore, the column should be vertical, and the packing should be perfectly uniform, without voids caused by air bubbles.

The preferred method for packing silica gel and alumina columns is the slurry method, whereby a slurry of the adsorbent and the first eluting solvent is made and poured into the column. When nothing is known about the mixture being separated, the column is prepared in petroleum ether, the least polar of the eluting solvents.

Microscale Procedure

Packing the Column. Assemble the column as depicted in Figure 10.1. To measure the adsorbent, fill the column one-half to two-thirds full, and then pour the powder into a 10-mL Erlenmeyer flask. Clamp the column in a vertical position, and close the valve. Always grasp the valve with one hand while turning it with the other. Fill the column with ligroin or hexane to the top of the glass column. Add about 8 mL hexane to the adsorbent in the flask, stir the mixture to eliminate air bubbles, and then (this is the hard part) swirl the mixture to get the adsorbent suspended in the solvent and immediately pour the entire slurry into the funnel. Open the valve, drain some solvent into the flask that had the adsorbent in it, and finish transferring the slurry to the column. Place an empty flask under the column, and allow the solvent to drain to about 5 mm above the top surface of the adsorbent. *Never allow the column to dry out;* this creates channels that will result in uneven bands and poor separation.

Adding the Sample and Eluting the Column. Dissolve the sample completely in the very minimum volume of dichloromethane (just a few drops) in an Erlenmeyer flask. Add to this solution 300 mg of the adsorbent, stir, and evaporate the solvent completely. Heat the flask *very gently* with *constant* stirring to avoid having the material bump. Remember that dichloromethane boils at 55°C. Pour this dry powder into the funnel of the chromatography column, wash it down onto the column with a few drops of hexane, and then tap the column to remove air bubbles from this layer of adsorbent-solute mixture just added. Open the valve, and carefully add new solvent in such a manner that the top surface of the column is not disturbed. Run the solvent down near to the surface several times to apply the sample as a narrow band at the top of the column. Then fill the column with the solvent, and elute the sample from the column.

Macroscale Procedure

xtinguish all flames; work in boratory hood.

The column can be prepared using a 50-mL burette such as the one shown in Fig. 10.2 or using the less expensive and equally satisfactory chromatographic tube shown in Fig. 10.3, in which the flow of solvent is controlled by a screw pinchclamp. Weigh out the required amount of silica gel (12.5 g in the first experiment), close the pinchclamp on the tube, and fill about half full with 90 : 10

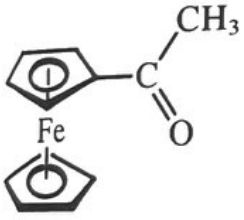
Cleaning Up After use, the tube is conveniently emptied by pointing the open end into a beaker, opening the pinchclamp, and applying gentle air pressure to the tip. If the plug of glass wool remains in the tube after the alumina leaves, wet it with acetone and reapply air pressure. Allow the adsorbent to dry in the hood, and then dispose of it in the nonhazardous waste container.

Experiments

1. Chromatography of a Mixture of Ferrocene and Acetylferrocene

MICROSCALE

Ferrocene
MW 186.04
mp 172–174°C



Acetylferrocene
MW 228.08, mp 85–86°C

TION: Toxic.

Both these compounds are colored (see Chapter 44 for their preparation), so it is easy to follow the progress of the chromatographic separation.

Prepare the microscale alumina column exactly as described above. Then add a dry slurry of 90 mg of a 50 : 50 mixture of acetylferrocene (*Caution!* toxic) and ferrocene that has been adsorbed onto 300 mg of alumina following the above procedure for preparing and adding the sample.

Carefully add hexane to the column, open the valve (use both hands), and elute the two compounds. The first to be eluted, ferrocene, will be seen as a yellow band. Collect this in a 10-mL flask. Any crystalline material seen at the tip of the valve should be washed into the flask with a drop or two of ether. Without allowing the column to run dry, add a 50 : 50 mixture of hexane and *t*-butyl methyl ether, and elute the acetylferrocene, which will be seen as an orange band. Collect it in a 10-mL flask. Spot a thin-layer silica gel chromatography plate with these two solutions. Evaporate the solvents from the two flasks, and determine the weights of the residues. An easy way to evaporate the solvent is to place it in a tared 25-mL filter flask and heat the flask in the hand under vacuum while swirling the contents (Fig. 10.5).

Recrystallize the products from the minimum quantities of hot hexane or ligroin. Isolate the crystals, dry them, and determine their weights and melting points. Calculate the percent recovery of the crude and recrystallized products based on the 45 mg of each in the original mixture.

The thin-layer chromatography plate is eluted with 30 : 1 toluene : absolute ethanol. Do you detect any contamination of one compound by the other?

Cleaning Up Empty the chromatography column onto a piece of aluminum foil in the hood. After the solvent has evaporated, place the alumina and sand in the nonhazardous waste container. Evaporate the crystallization mother liquor to dryness, and place the residue in the hazardous waste container.

MICROSCALE