Chapter 8: Column Chromatography

In column chromatography, the stationary phase is a solid adsorbent and the mobile phase is a liquid, just like in TLC. The big difference between the two techniques is that while in TLC the solvent moves up and through the adsorbent by capillary action, in column chromatography the solvent moves down and through the adsorbent by gravity or by external pressure. While TLC employs a very small amount of adsorbent in a thin layer on a plate, column chromatography uses a relatively large quantity contained in a cylindrical column. Column chromatography is generally applied as a purification technique: it isolates a desired compound from a mixture.

In column chromatography, the adsorbent is contained in an inert column constructed of metal, glass, or plastic. The mixture to be analyzed is dissolved in a small quantity of solvent and applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column.

Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called gravity column chromatography. If the solvent is forced down the column by positive air pressure, it is called flash chromatography, the method most often used in organic chemistry research laboratories. The term "flash chromatography" was coined by Professor W. Clark Still because it can be done in a "flash."

8.1 The Adsorbent

Silica gel (SiO_2) and alumina (Al_2O_3) are two adsorbents commonly used by the organic chemist for column chromatography. These adsorbents are sold in different mesh sizes, as indicated by a number on the bottle label: "silica gel 60" or "silica gel 230–400" are a couple examples. This number refers to the mesh of the sieve used to size the silica, specifically, the number of holes in the mesh or sieve through which the crude silica particle mixture is passed in the manufacturing process. If there are more holes per unit area, those holes are smaller, thus allowing only smaller silica particles to go through the sieve. The relationship is: the larger the mesh size, the smaller the adsorbent particles.

Adsorbent particle size affects how the solvent flows through the column. Smaller particles (higher mesh values) are used for flash chromatography, larger particles (lower mesh values) are used for gravity chromatography. For example, 70–230 silica gel is used for gravity columns and 230–400 mesh for flash columns.

Alumina is used more frequently in column chromatography than it is in TLC. Alumina is quite sensitive to the amount of water that is bound to it: the higher its water content, the less polar sites it has to bind organic compounds, and thus the less "sticky" it is. This stickiness or *activity* is designated as I, II, or III, with I being the most active. Alumina is usually purchased as activity I and deactivated with water before use according to specific procedures. Alumina comes in three forms: acidic, neutral, and basic. The neutral form of activity II or III, 150 mesh, is most commonly employed.

Cellulose, magnesium silicate, and activated charcoal (Norite) are also used by the organic chemist for column chromatography. Polymeric cross-linked solids are used in a variation of column chromatography called "gel-permeation" or "size-exclusion" chromatography. In this method, large molecules, such as

polymer chains of different sizes, are separated according to their size by their tendency to become entrained in the sieve-like structure of the solid support.

8.2 Choice of Solvent

The polarity of the solvent that is passed through the column affects the relative rates at which various species move through the column. As for TLC, polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is too nonpolar, the compounds will not move down the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique.

TLC is used to determine the solvent(s) that will be used to elute a column. In order to separate the compounds by column chromatography, you must first determine a solvent system that will separate the compounds. Since both TLC and column chromatography use the same adsorbents (silica or alumina), the solvents effective in achieving separation on a TLC plate for a particular mixture will also be effective in achieving separation on a column of the same adsorbent. The R_f values should also follow the same order on the column as they do on the TLC plate.

Sometimes more than one solvent will be used to elute a mixture from a column, beginning with a nonpolar solvent to elute the nonpolar compounds and then changing to a polar solvent to elute the polar compounds. The reason for use of a sequential solvent process in chromatography is that it increases the efficiency of the separation. You keep the slow compounds moving slowly until the less polar compounds are off the column, then elute the slow moving compounds by increasing the solvent polarity. The two solvent systems of different polarities are determined beforehand by TLC. Search for two different solvent systems, one that gives an R_f of 0.25 for one compound, and another that gives an R_f of 0.25 for the other compound. These two solvents systems, used sequentially, will separate the mixture on a flash chromatography column. If a mixture is known to contain only two compounds, after the first compound is off the column, a very polar solvent can then be used to speed up the elution of the second column.

In some cases it is necessary to use a *solvent gradient*, consisting of two solvents mixed together in different proportions so that the solvent polarity slowly increases. For instance, you might start eluting a column with pure hexanes, then switch to 90:10 hexanes-ethyl acetate, then 80:20, then 70:30, and so on. This is the most effective method for separating compounds with similar R_f values, though a certain amount of trial and error is involved in finding the right quantity and type of each solvent mixture to use. Automated chromatography machines are available that can supply a smoothly varying gradient of solvents throughout the column elution.

In column chromatography, another factor in solvent choice is volatility. Since you are attempting to isolate a pure sample of your compound, you will need to remove the solvent from the compound once it has come off the column. Volatile solvents are advantageous because they are easy to evaporate off from the desired compound after a column chromatography procedure.

8.3 Analysis of Fractions by TLC

If the compounds to be separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. However, more commonly the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labeled tubes and analyzed by TLC. Several fractions can be spotted on the same plate, but the original sample is usually spotted alongside them for comparison purposes. An example is shown in Figure 8-1. (The original mixture is labelled as "Unk" on these plates, for "Unknown".) In this case, fractions 1-4 contain only the faster-moving compound; fractions 5-6 contain both compounds; and fractions 7-10 contain only the slower-moving compound. To isolate the pure compounds, you should combine fractions 1-4 and remove the solvent, and then in a separate flask combine fractions 7-10 and remove the solvent. Unfortunately the material in fractions 5-6 is not useful unless you decide to run it through a second column.

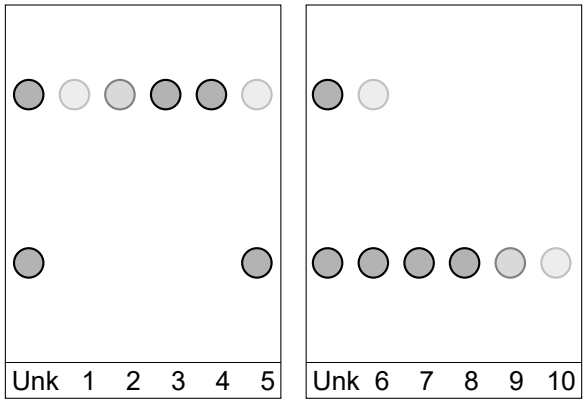


Figure 8-1: TLC results for a series of column fractions.

8.4 Procedure for Microscale Flash Column Chromatography

If you are separating compounds on a microscale (usually under 100 mg of sample), a disposable Pasteur pipet can be used to hold the packing material in flash chromatography (Figure 8-2). Pressure from a pipet bulb is sufficient to force the eluting solvents through the packing material. This is the method that is most commonly used in the teaching labs.

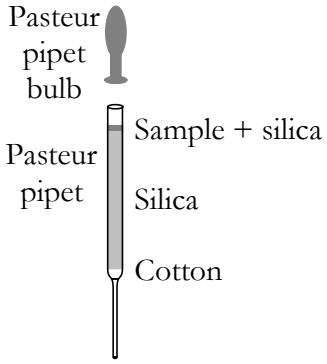


Figure 8-2: A microscale flash column can be performed with a Pasteur pipet.

The steps you will follow to elute this column are shown in Figure 8-3.

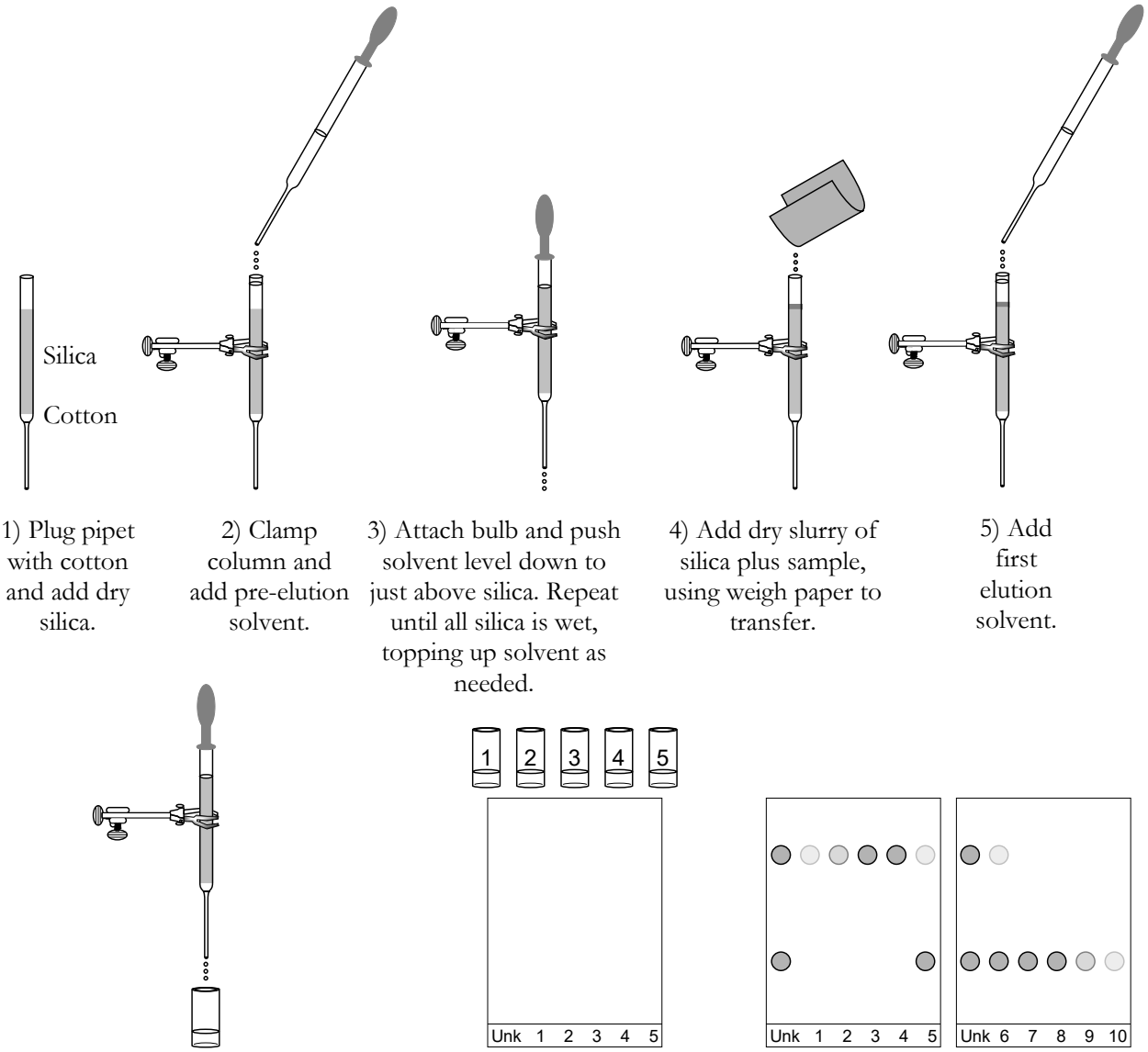


Figure 8-3: The procedure for microscale column chromatography.

1. Prepare the column.

Plug a Pasteur pipet with a small amount of cotton. Take care that you do not use either too much cotton or pack it too tightly. You just need enough to prevent the adsorbent from leaking out.

Add dry adsorbent, usually silica gel 230–400 mesh, to a depth of 5–6 cm. A small beaker works well to pour the adsorbent into the column. Tap the pipet to pack the adsorbent, then apply pressure with a pipet bulb to pack it some more. Recheck the depth and add more silica gel if necessary so that the depth is 5–6 cm. This leaves a space of 4–5 cm on top of the adsorbent for the addition of solvent.

2. Secure the column, then add the pre-elution solvent.

Clamp the column to a ring stand. Fill the upper part of the column with a low-polarity solvent such as hexanes.

3. Pre-elute the column.

Force the solvent through the column until the liquid level is just flush with the top of the adsorbent. To do this, place a pipet bulb on top of the column, squeeze the bulb, and then remove the bulb while it is still squeezed. You must be careful not to allow the pipet bulb to expand before you remove it from the column, or you will draw solvent and adsorbent into the bulb. You will probably want to practice this technique on a "sample column" before you do it on your "real" experiment. Repeat this step, adding more pre-elution solvent as necessary, until the entire column is wet.

4. Load your sample onto the column.

The most common method for getting your sample into the column is by "pre-loading" a small quantity of adsorbent (silica or alumina, depending on what your column is packed with). To use this method, dissolve the compound in any volatile solvent, then add about 150 mg of the adsorbent to make a slurry. Allow the solvent to evaporate completely, until the adsorbent is free flowing. Do not heat the adsorbent mixture, since the solvent will "pop" while evaporating and throw the slurry everywhere. Once it is dry, transfer this adsorbent-sample mixture to the prepared column; the easiest way to do this is by folding a weigh paper in half and using it as a funnel.

Another, less common method is by adding a solution of the sample directly to the column. Weigh out a small amount of sample (typically 25–50 mg). Dissolve it in a small amount of the same solvent you used to pre-elute the column, then add it into the top of the column. On occasion, the solvent used to load the sample is more polar than the eluting solvents. In this case, it is critical that you only use a few drops of solvent to load the sample. If you use too much, the loading solvent will interfere with the elution and hence the separation of the mixture. If the sample does not dissolve readily, you may need to either warm the sample or use a different solvent.

5. Add the first elution solvent.

Always begin the elution with the least polar solvent. The solvent systems for flash column elution may be specified in the experiment, or you may need to determine them experimentally.

6. Elute the column.

Place a labeled vial under the column to collect the eluent, and have ready several more labeled vials with which to collect subsequent fractions. Fill the column with the elution solvent and then apply pressure with a pipet bulb until the level of the solvent is flush with the top of the adsorbent. Change collection vials, then repeat the process several times. You can always pause while running your column to analyze your fractions by TLC – this may help you decide when to swap solvent systems or stop running the column.

If your compounds are colored, you may not need to change vials with each application of eluting solvent. Instead, collect the eluent in a larger container, and change collection flasks as the differently colored compounds elute from the column. With colored compounds, the amount of each eluting solvent used will depend on how long it takes for each compound to elute from the column.

If you are using two eluting solvents, change to the more polar one after your first compound is off the column. If you are using a solvent gradient, you may switch solvents several more times.

7. Analyze the fractions.

Use TLC to analyze the contents of each fraction. Spot 5 or so fractions side-by-side on the same plate, and also spot the plate with your crude mixture. Combine fractions that contain pure samples of the same compound, and then evaporate the solvent (see Chapter 15 on Solvent Removal).

8. When finished, dispose of the column.

Once you have isolated the desired compounds from your sample, push air through the column for a while to remove the solvent. You can then discard the entire column in the solid waste bins.

8.5 Procedure for Large-Scale Column Chromatography

For scales larger than 100 mg, you will need larger, more specialized equipment (Figure 8-4): a chromatography column with attached solvent bulb, a gas inlet adapter, and an air bulb with an attached one-way metal valve. (Any source of compressed air will work, so long as it is constant and not too highly pressurized.) You will also need a green Keck clip to hold the glass items together, and a clear Nalgene hose to carry air from the bulb to the inlet adapter. One more item you may need is a powder funnel, to pour adsorbent and solvents into the column. (A stemmed funnel is normally too narrow and clogs when adsorbent is poured through it.)

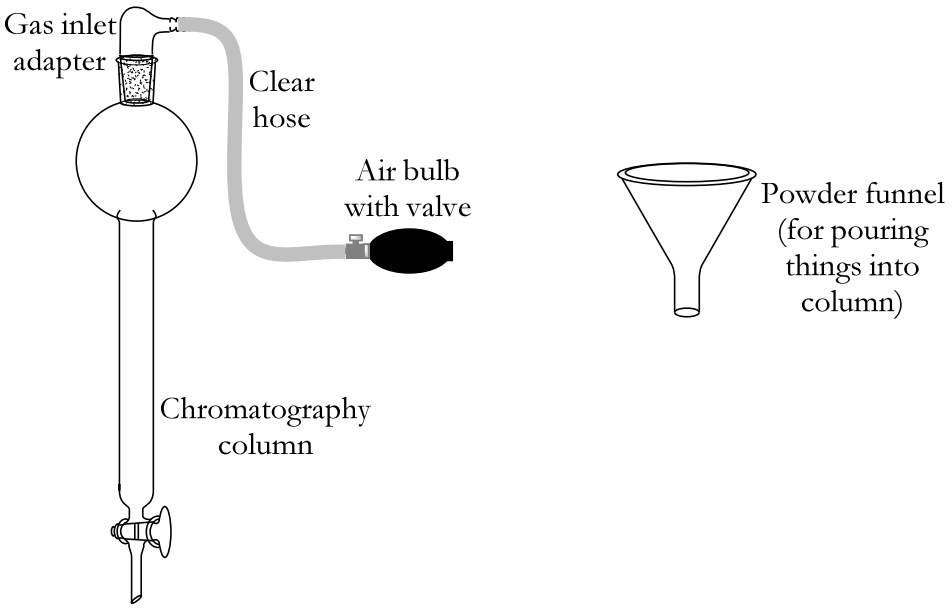


Figure 8-4: Glassware for large-scale column chromatography.

Before placing anything in the column, assemble all the glassware as shown in Figure 8-4 and practice pressurizing and depressurizing the column. Before using the gas inlet adapter, you should grease it lightly so it doesn't become stuck in the column, then hold it on with a green Keck clip so it doesn't fly off and break. With everything assembled and with the stopcock closed, it should take only a few squeezes of the bulb to build up air pressure in the column to a useful level. When you are running a column it is important not to depressurize it too rapidly, to avoid cracking the adsorbent layer. Practice slowly loosening the metal knob on the bulb's valve to release air pressure.

The steps for performing large-scale column chromatography are shown in Figure 8-5.

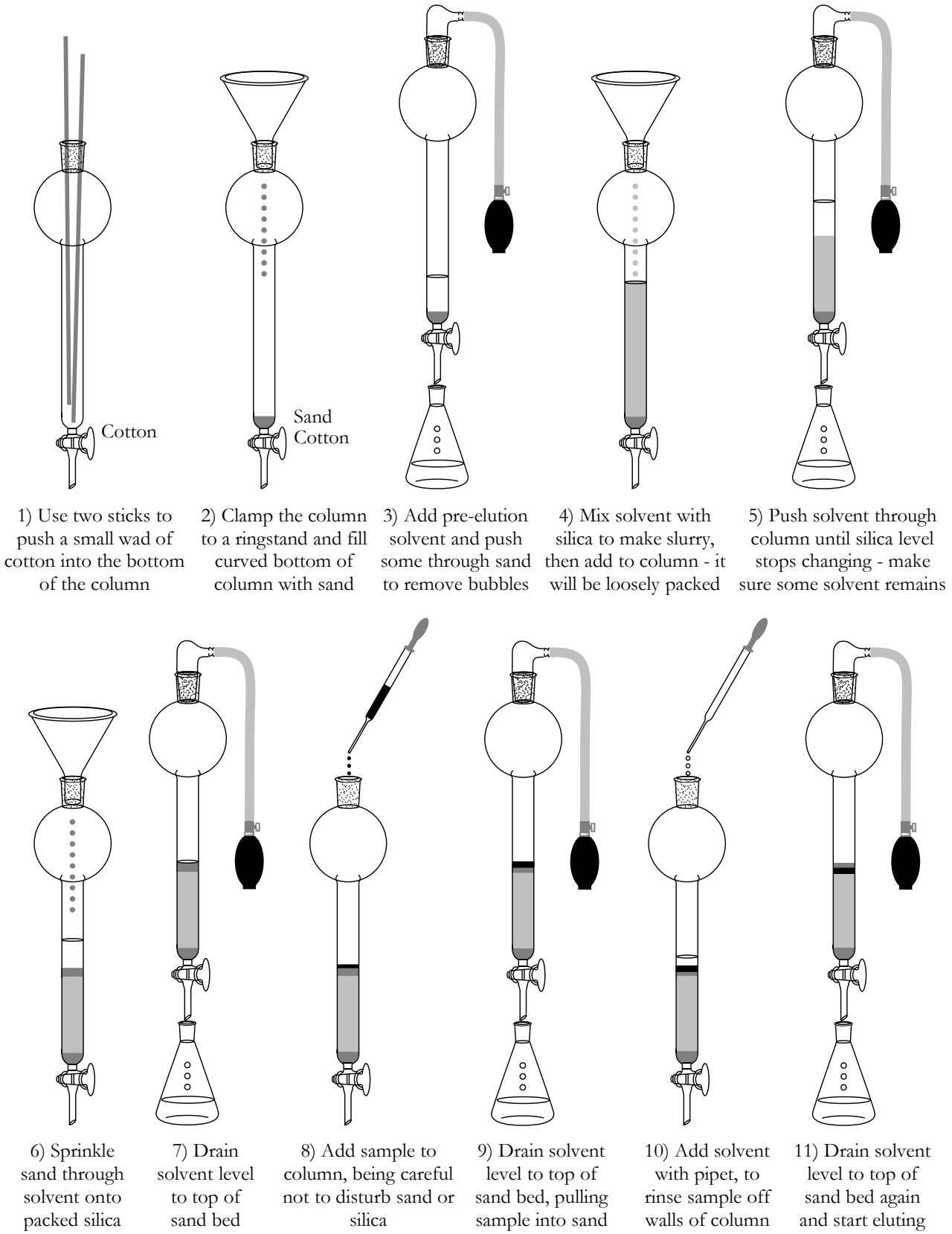


Figure 8-5: Steps for performing column chromatography on a large scale, using solution loading.

1. Pack the bottom of the column with cotton.

Place a piece of cotton in the bottom of the column, and gently tamp it down with a pair of sticks. This step may be easiest if you sit on a stool and hold the column between your knees while pushing the sticks into it. In some cases glass wool may be a better choice than cotton.

2. Secure the column and add sand.

Clamp the column to a ring stand. Use two clamps, one at the bottom and one at the top. Using a powder funnel, pour in enough sand to fill the lower, curved part of the column up to where the sides become vertical.

3. Push some pre-elution solvent through the sand.

Typically you will pre-elute with hexanes, though you might need to use a different solvent as specified in experimental procedure. Add 20-30 mL to the column. Place a large Erlenmeyer flask under the column, reattach the inlet adapter with a Keck clip, and use the rubber bulb to push solvent through the column (remember to open the stopcock at the bottom). Tap the column gently to free any air bubbles and to settle the sand into a uniform layer.

4. Make a slurry of adsorbent and add it to the column.

Put about 100 mL of your pre-eluting solvent in a beaker and slowly stir in about 50 mL of adsorbent (silica or alumina). If the mixture becomes difficult to stir, add more solvent to thin it back out. Gently pour this slurry in on top of the solvent and sand. It will be loosely packed at this point.

5. Pack the silica down.

Reattach the inlet adapter, pressurize the column and open the stopcock. The adsorbent will be compacted downward by the air pressure, leaving a layer of clear solvent on top. (Any clean solvent that gets pushed into the Erlenmeyer flask can be reused by pouring back into the top of the column later.) Once the level of adsorbent has stopped changing, check to see how much of the column is filled. Ideally about half the height of the column (not counting the solvent bulb) should contain packed adsorbent. If not, repeat this step until the column is about half full. This slurry-packing method of filling a large column usually prevents air bubbles from becoming trapped in the adsorbent. Do not allow the solvent level to drop below the surface of the adsorbent at any point!

6. Add more sand.

To prevent the adsorbent bed from being disturbed when you top up the solvent, you will add a layer of sand on top of it. First, make sure there is still a layer of clear solvent at least 3 cm deep above the adsorbent if not, you should add more solvent. Slowly sprinkle sand into the solvent, aiming for even coverage of the surface. If it's uneven, you can gently tap the column to settle it down. Stop once you have a sand layer 1 cm deep.

7. Drain the solvent level to the top of the sand bed.

Reattach the inlet adapter and push the solvent through until the solvent level is at the top of the sand. Be careful not to let it drop any lower than this.

8. Load your sample onto the column.

As for microscale columns, you can either use adsorbent that has been pre-loaded with sample, or you can use a liquid solution of your sample. Both methods are common for large-scale chromatography.

If you are using the solution-loading method, use a pipet to gently drip your compound onto the sand, being careful not to disturb the sand or silica. The best way to do this is to trickle it down the walls of the column, but make sure it is distributed evenly around the walls.

9. Drain the solvent level to the top of the sand bed again.

Reattach the inlet adapter and push the solvent through until the solvent level is at the top of the sand. If you used the solution-loading method, you should see your sample flowing down through the sand towards the silica.

10. Add 5-10 mL of pre-elution solvent to rinse solution off walls

Use a pipet to rinse down the walls of the column with 4-5 mL of solvent. This will wash any stray traces of sample down onto the sand bed as well.

11. Drain the solvent level to the top of the sand bed again, then start eluting.

Push the solvent down, then add your first eluting solvent. From here onwards, the steps will be the same as they are for eluting the microscale column, although the fractions you collect will be larger.

12. When finished, clear out the column.

To clear out the chromatography column, continue to pressurize it without adding any solvent into the top. The adsorbent should slowly dry out under the flow of air. You will be able to tell when it is completely dry, because the adsorbent-filled part of the column will be cold along its whole length. Once this happens, pour the sand and used adsorbent into a solid waste container. Use a small stick to poke the piece of cotton out of the column – this time, you will have to open the stopcock and poke up from the bottom of the column. Alternatively you can connect the air hose to the bottom end of the column (without the inlet adapter) and push the cotton out by air pressure.

8.6 Common Problems Encountered in Column Chromatography

1. The compounds are not separated.

Possibly the eluting compounds were not properly chosen. Try a less polar first solvent and a more polar second solvent. If you used a solution-loading method instead of preloading, you might have used too much solvent, especially if the loading solvent was more polar than the first eluting solvent. Be sure to use as little solvent as possible in this situation.

2. The second compound is not coming off the column.

Try using a much more polar solvent such as acetone or ethyl acetate to force it off the column.

3. The adsorbent in the column is not uniform – it has cracks or bubbles.

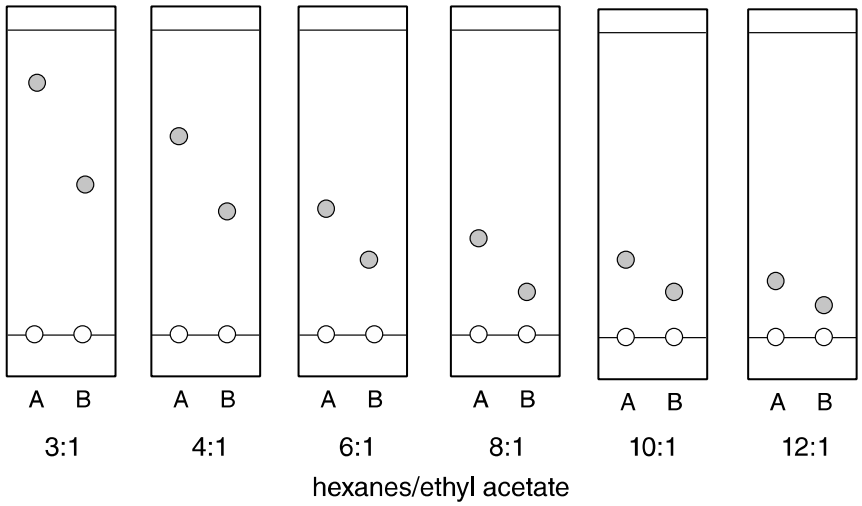
This is most commonly caused by the column being allowed to run dry – air was forced into the column because the solvent layer above the adsorbent ran out. Perhaps you depressurized the column too rapidly at some point, either by releasing the bulb suddenly (for microscale columns) or by failing to secure the inlet adapter to the column (for large-scale).

It can also happen if you switch to a more polar solvent too quickly, such as alcohol. If the temperature in the lab is high enough, low-boiling solvent such as diethyl ether can actually start boiling inside the column, cracking the adsorbent.

In all of these cases, your separation will be extremely poor. If your compounds are still on the column, you will have to flush everything out with polar solvent and start again with a freshly-packed column.

8.7 Study Problems

1. You want to separate compound A from compound B by flash chromatography. The following are the results of running these two compounds in different hexanes/ethyl acetate systems. Which is the best solvent system to elute compound A? Compound B?



2. A student loaded a mixture onto a small flash chromatography column in 1 mL of methylene chloride, then proceeded to elute with hexanes/ethyl acetate 10:1. They found that all of the mixture came off in the first 4 fractions, with no separation. What technical mistake did the student make?

3. You only see one compound coming off the column when you suspect two. Where might the other compound be? How can you recover this compound?