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Column Chromatography Procedures

Columns for chromatography can be big or small, according to the amount of material which needs to be loaded onto the column. Pictured below are three glass columns, two of which are used in the organic chemistry teaching labs at CU.

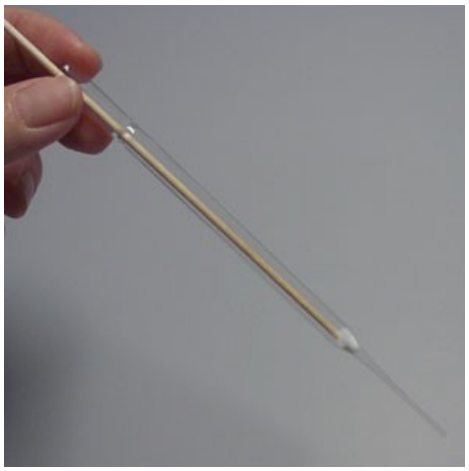
The Pasteur pipet column on the left is used for microscale gravity and microscale flash chromatrography procedures (about 10-125 mg of material); these procedures usually do not require a means of control of gravity-induced solvent flow through the column. In the Organic Chemistry teaching labs at CU, the most frequently used column is the Pasteur pipet. They work well in microscale flash column chromatography procedures because a pipet bulb fits conveniently on top of them to serve as a source of pressurized air (when the bulb is squeezed). Microscale procedures are used at CU Boulder whenever feasible to cut down on waste chemical production.

The middle column is used for gravity column chromatography in a few of the chemistry majors' laboratory courses (chem 3361 and 3381). Note the piece of flexible tubing which has been added to the bottom of the column.To control the flow of solvent, a pinch clamp would be placed on the flexible tubing at the bottom. This column is made from 10 mL disposable glass pipet and can separate about a 1 g of material.

The column on the right is the only one that is actually manufactured as a chromatography column. Note the stopcock at the bottom. This is to control the flow of solvent through the column, important for gravity column chromatography applications. Much larger chromatography columns are available than this. The size employed depends on the amount of material which needs to be separated. Large-scale flash columns look like this column but have a standard taper connection at the top so they can be connected to a source of pressurized air.

Procedure for Microscale Flash Column Chromatography

Microscale flash chromatography is the primary method used in the organic chemistry teaching labs because it is both easy and environmentally friendly. The method is only limited by the fact that it can separate only small amounts of sample. It works best for 25 mg amounts, although we have pushed it to separate 125 mg mixtures if the TLC R_f's of the components of the mixture differ by at least 0.20. In microscale flash chromatography, the column does not need either a pinch clamp or a stopcock at the bottom of the column to control the flow, nor does it need air-pressure connections at the top of the column. Instead, the solvent flows very slowly through the column by gravity until you apply air pressure at the top of the column with an ordinary Pasteur pipet bulb.



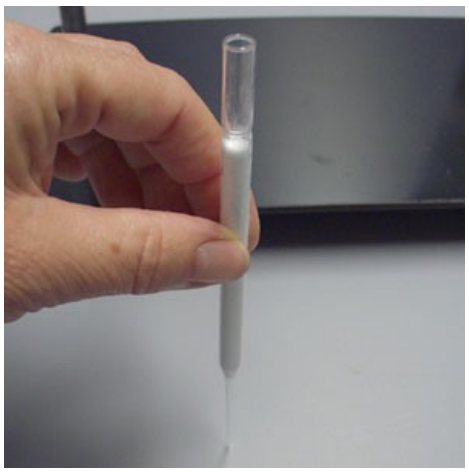
Step 1: Prepare the column

Plug a Pasteur pipet with a small amount of cotton; use a wood applicator stick to tamp it down lightly. 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.

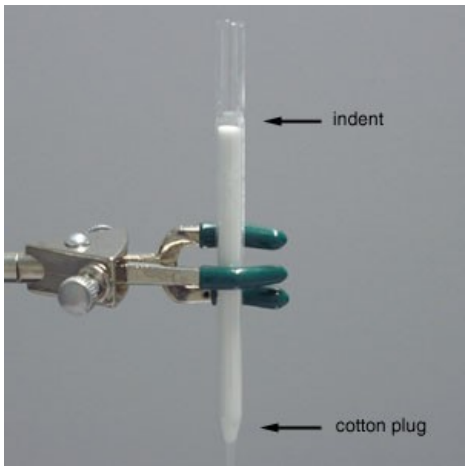
Add dry silica gel adsorbent, 230-400 mesh. Usually the jar is labeled "for flash chromatography." One way to fill the column is to invert it into the jar of silica gel and scoop it out...

...then tamp it down before scooping more out.

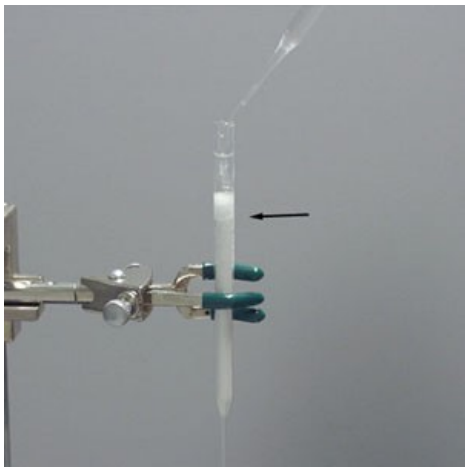
Another way to fill the column is to pour the gel into the column using a 10 mL beaker.



Whichever method you use to fill the column, you must tamp it down on the bench top to pack the silica gel. You can also use a pipet bulb to force air into the column and pack the silica gel.



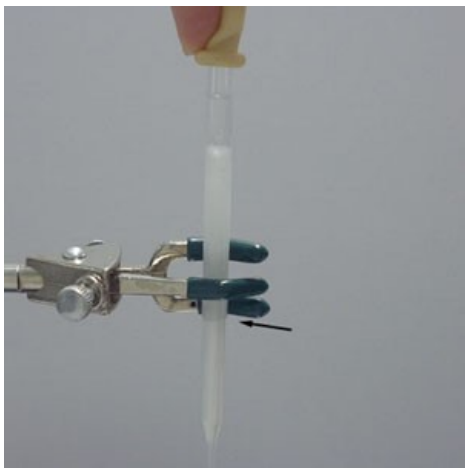
When properly packed, the silica gel fills the column to just below the indent on the pipet. This leaves a space of 4-5 cm on top of the adsorbent for the addition of solvent. Clamp the filled column securely to a ring stand using a small three-pronged clamp.



Step 2: Pre-elute the column

Add hexanes (or other solvent, as specified by the procedure) to the top of the silica gel. The solvent flows slowly down the column; on the column above, it has flowed down to the point marked by the arrow.

Monitor the solvent level, both as it flows through the silica gel and the level at the top. If you are not in a hurry, you can let the top level drop by gravity, but make sure it does not go below the top of the silica. Again, the arrow marks how far the solvent has flowed down the column.



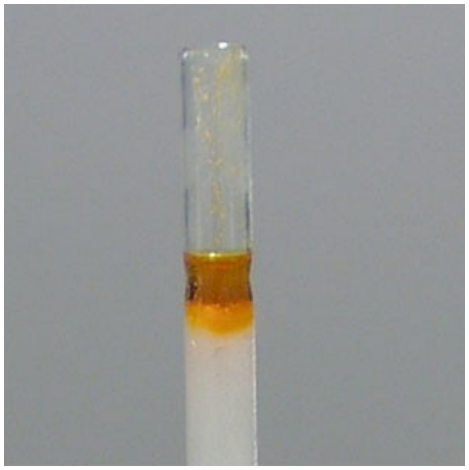
You can speed up the process by using a pipet bulb to force the solvent through the silica gel. Place the pipet bulb on top of the column, squeeze the bulb, and then remove the bulb while it is still squeezed. You must be careful not to allow the pipet bulb to expand before you remove it from the column, or you will draw solvent and silica gel into the bulb.

When the bottom solvent level is at the bottom of the column, the pre-elution process is completed and the column is ready to load.

If you are not ready to load your sample onto the column, it is okay to leave the column at this point. Just make sure that it does not go dry -- keep the top solvent level above the top of the silica (as shown in the picture to the left) by adding solvent as necessary.

Step 3: Load the sample onto the silica gel column

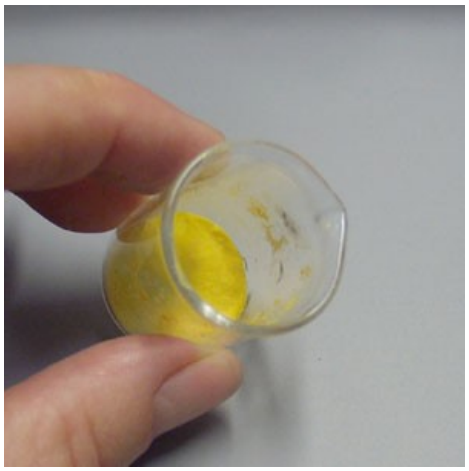
Two different methods are used to load the column: wet and dry. In the **wet** method, the sample to be purified is dissolved in a small amount of solvent, such as hexanes, acetone, or other solvent. This solution is loaded onto the column. Sometimes the solvent of choice is more polar than the eluting solvents. In this case, if you use the wet method of column loading, it is critical that you only use a few drops of solvent to load the sample. If you use too much solvent, the loading solvent will interfere with the elution and hence the separation of the mixture. In such cases, the **dry** method of column loading is recommended.



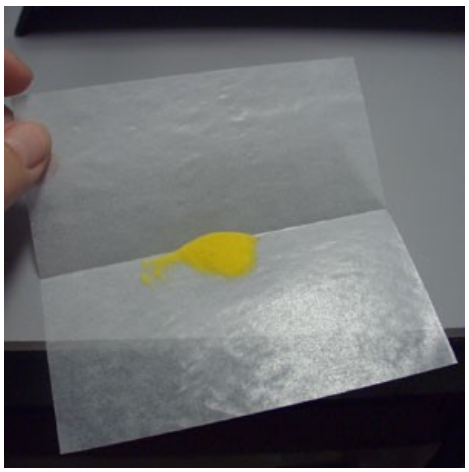
The column at the left is being loaded by the **wet** method.

Once the sample is in the column, fresh eluting solvent is added to the top and you are ready to begin the elution process.

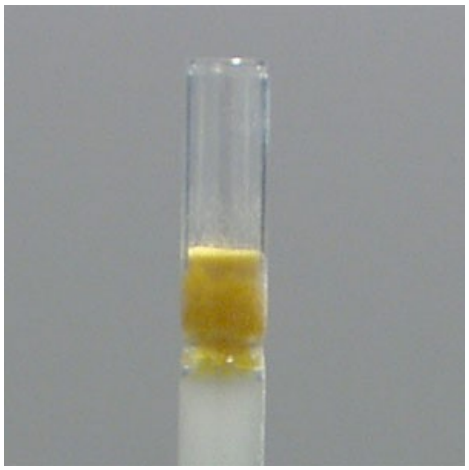
For the **dry** method, first dissolve the sample to be analyzed in the minimum amount of solvent and add about 100 mg of silica gel.



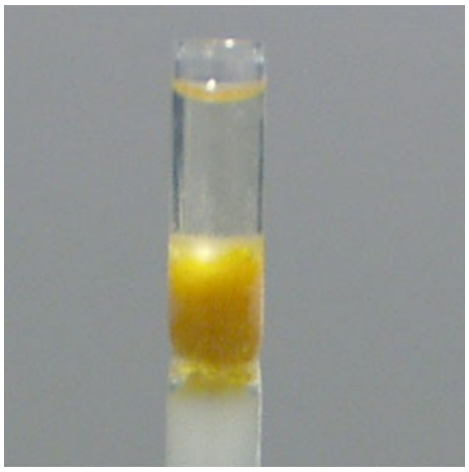
Swirl the mixture until the solvent evaporates and only a dry powder remains.



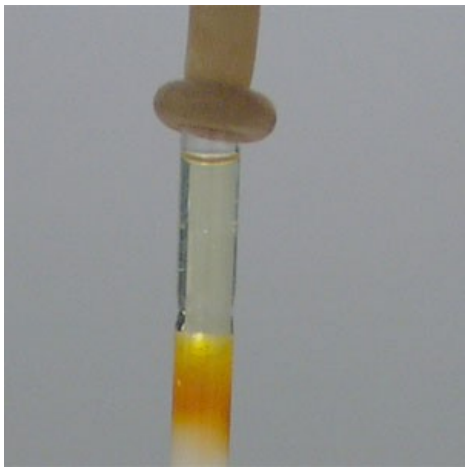
Place the dry powder on a folded piece of weighing paper...



... and transfer it to the top of the prepared column.

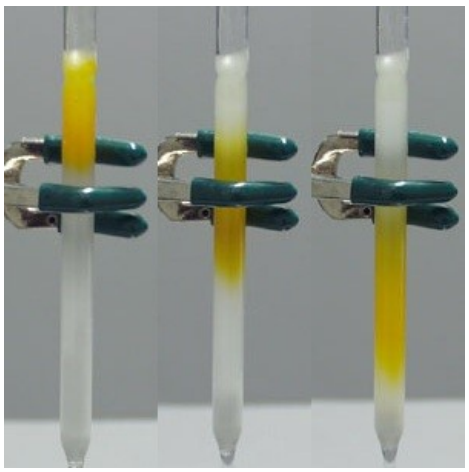


Add fresh eluting solvent to the top. Now you are ready to begin the elution process.

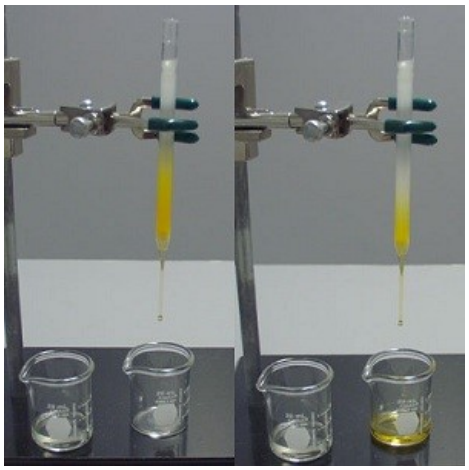


Step 4: Elute the column

Force the solvent through the column by pressing on the top of the Pasteur pipet with a pipet bulb. Only force the solvent to the very top of the silica: do not let the silica go dry. Add fresh solvent as necessary.



The colored bands will travel down the column as the compound is eluted.



As soon as the colored compound begins to elute, the collection beaker is changed. The process is complicated if the compound is not colored. In such experiments, equal sized fractions are collected sequentially and carefully labeled for later analysis.

Step 5 (Optional): Elute the column with the second elution solvent

If you are separating a mixture of one or more compounds, at this point you could change the eluting solvent to a more polar system, as previously determined by TLC. Elution would proceed as in step 4.

Step 6: Analyze the fractions

If the fractions are colored, you can simply combine like-colored fractions, although TLC before combination is usually advisable. If the fractions are not colored, they are analyzed by TLC (usually). Once the composition of each fraction is known, the fractions containing the desired compound(s) are combined.

Procedure for Gravity Column Chromatography

Gravity columns are used only in the majors organic lab courses at CU Boulder (chem 3361/3381). Gravity columns are a lot slower to run than microscale flash columns. They also are more difficult to pack with adsorbent. There are two common methods of packing a gravity column: the slurry method and the dry pack method. Both of these procedures were written for the middle columns shown above; you will need to vary the quantities if you use a different column.

In the **slurry method** of column packing, you mix the adsorbent with the solvent and then pour this slurry into the prepared column. The nature of the slurry is a bit different depending on whether you use silica gel or alumina; some slurries are easier to work with than others. This procedure was written for alumina slurries. The advantage of slurry methods is that they eliminate air bubbles from forming in the column as it packs.

Place a piece of glass wool in the bottom of the column, and gently tamp the glass wool down with a glass rod. Attach the column to a ring stand and make sure that the column is securely fastened in a vertical position. Add a pinch clamp to the bottom of the column and close the clamp. Fill the column about half-way with a non-polar solvent, such as hexanes. Weigh 8 g of alumina into a beaker. Place 15 mL of hexanes in a 125 mL Erlenmeyer flask and slowly add the alumina powder, a little at a time, while swirling. Use a Pasteur pipet to mix the slurry, then quickly pipet the slurry onto the column (you can pour it instead if you prefer). Place an Erlenmeyer flask under the column, open the pinch clamp, and allow the liquid to drain into it. Continue to transfer the slurry to the column until all the alumina is added. Add more hexanes as necessary; the hexanes collected in the Erlenmeyer flask can be re-used to add more alumina to the column. When finished packing, drain the excess solvent until it just reaches the top level of the alumina. Close the pinch clamp. Your column is now packed and ready for use. Sometimes a small amount of sand is added to the top of the column to prevent it from being disturbed when fresh solvent is added.

The **dry-pack method** is easier, but can lead to bubbles in the column. Obtain an empty column, plug it with a small piece of glass wool, and affix a pinchclamp to the bottom of the column. Clamp the column in a vertical position, close the pinchclamp, and fill the column with solvent. Using a dry funnel, sprinkle 8 g of alumina into the solvent, and allow solvent to drain from the column to prevent overflowing. Let the alumina settle and gently tap the column so that the alumina will pack tightly into the column. Drain the solvent until the solvent level is just even with the surface of the alumina.

Loading and eluting gravity chromatography columns: The sample to be analyzed is dissolved in a very small amount of solvent and added to the top of the column. The pinch clamp is opened and the solvent is allowed to drain just to the top of the column. A small amount of the eluting solvent is added and allowed to drain in until the mixture is a little way into the adsorbent, then the column is filled to the top with eluting solvent. The column is now ready to run -- continue adding solvent at the top and collecting fractions at the bottom until the compounds elute at the bottom. If applicable, change the eluting solvent to a more polar solvent during the eluting process. Never let the solvent level drop below the top of the adsorbent. The process is discontinued when the compound(s) desired is (are) off the column.

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