Separating Compounds by Column Chromatography

Column chromatography is similar to TLC in that a mixture of compounds can be separated by being carried by a mobile phase through a stationary phase. However, instead of just giving us information about the mixture, column chromatography is used to actually separate larger amounts of the compounds.

The stationary phase in column chromatography is a glass column filled with alumina or silica gel. The mobile phase is a solvent (or a mixture of two solvents) which is pushed down through the column, carrying compounds through at different rates. The solvent is collected as it comes out the bottom, and separate compounds can be obtained.

It is very important that the stationary phase be uniformly packed so that the compounds will flow evenly down the column. There are several ways to achieve this – we will use the dry pack method.

Procedure:

- Place a small wad of cotton at the bottom of a buret (about 1/3 of a cotton ball) using a long glass rod. Don't break my rod – it is the only thing in the lab that is long enough for this task! Also, don't put too much cotton or you will make it difficult to push the solvent through. All the cotton needs to do is keep the solid from getting through the hole.

- Place the buret in a buret clamp on a lab bench (they don't fit in the hoods). Try to get it to stand up at straight as you can.

- To pack the column, one person will pour a steady stream of alumina or silica gel through a funnel on top while the other person taps the buret with his/her fingers to pack it evenly as it falls. Stop when the solid reaches about the 25 ml mark. Tap the column some more until the solid doesn't settle any further. As you run the column, you will be able to see how well you did this part – hopefully the bands will run down all sides of the column at the same rate. If it runs faster down one side, then your packing wasn't very even.

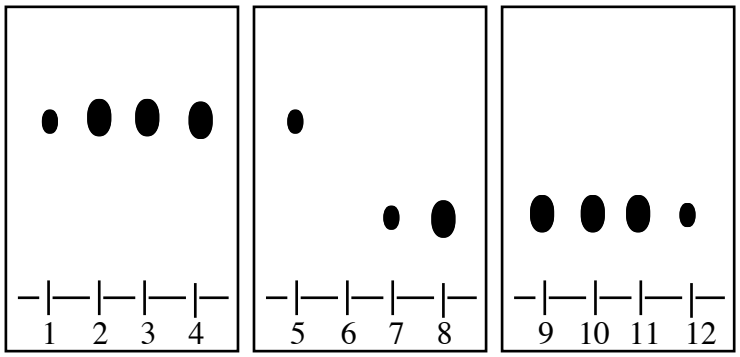
- To prepare the sample you will be separating, add about 500 mg of the same material that you put on the column to a solution of the sample and rotovap it. This coats the compound onto the solid rather than the sides of the flask so that you can more easily remove it. Add this sample to the column by pouring it in the top of the funnel, scraping it out of the flask as best you can. If the sample sticks to the sides of the column, rinse it down with a little bit of hexanes.

- To start eluting the column, fill the rest of the column with the first solvent that you are going to use. Put a beaker or flask underneath the column; then open the stopcock and start forcing the liquid through the column with a bulb (shove the pointed part in the top of the column, squeeze, and pull it out without letting go). Try not to let the column run dry – always stop before the top of the liquid reaches the top of the solid.

- In both of the columns we will run in the lab, the compounds will be colored so that you can see them coming down the column. As you see a band or bands coming down, switch beakers when the band is about an inch from the bottom (it actually moves faster in the middle of the column

than on the outside where you can see it). Continue pushing solvent through until all of the solvent containing that band is in the container. Then switch to the next solvent, as directed in the instructions for that lab. You will always be going from a low polarity solvent (or solvent mixture) to a more polar solvent, so that the less polar compounds will have a chance to be eluted before the more polar compounds begin to move. If you weren't told what solvents to use, you could figure it out by first running some TLC plates to see how far different solvents moved each of the spots.

- If the compounds were not colored (as is usually the case), you would collect the solvent in test tubes and test each one by TLC to see what compounds it contained. If the separation went well, the TLC's would look like the ones shown below. Test tubes 1-5 contain the first compound, and test tubes 7-12 contain the second.



- Once all of the bands have been eluted from the column, you can test each one by TLC to see how good your separation is. Then pour them into tared round bottom flasks and rotovap them. They can now be weighed, and you can calculate the % recovery or the % yield for each one.

- When you are finished with the column, force all of the liquid out of it, and then leave it on my desk with the stopcock open. After the solvent has dried, it will be easy to remove it from the column.

- Miniature chromatography columns can also be run in a glass pipet – this sort of column is usually used for purification rather than actual separation. Push a very small piece of cotton down in the end with a plastic pipet, then fill it half way up with silica gel or alumina (as directed). Add the solution to be purified and push it through with a piece of rubber tubing (plug the top with your fingers and squeeze). The desired compound will be eluted out, while the impurities will remain on the column (they will be more polar).