8.9 - Flash Column Chromatography Guide

Overview:

Flash column chromatography is a quick and (usually) easy way to separate complex mixtures of compounds. We will be performing relatively large scale separations in 5.301, around 1.0 g of compound. Columns are often smaller in scale than this and some of you will experience these once you move into the research lab. Column chromatography uses the same principles discussed in the TLC Handout, but can be used on a preparative scale. We are running flash columns since we will use compressed air to push the solvent through the column. This not only helps provide better separation, but also cuts down the amount of time required to run a column.

Reading:

For an excellent description, see LLP pages 205 - 214.

Preparing and Running a Flash Column:

1) Determine the dry, solvent-free weight of the mixture that you want to separate.

2) Determine the solvent system for the column by using TLC (see TLC Handout). You should aim for R_f values between 0.2 and 0.3. If your mixture is complicated then this may not be possible. In complex cases, you will probably have to resort to gradient elution. This simply means that you increase the polarity of the solvent running through the column (eluent) throughout the course of the purification. This technique will be described in more detail later in the handout, but for the TLC analysis you should determine which solvent systems put the different spots in the 0.2 to 0.3 R_f range.

3) Determine the method of application to the column. You have three choices: neat, in solution, or deposited on silica.

Neat: If your compound is a non-viscous oil, it is easiest to apply it neat. You can use a long Pasteur pipet to apply the liquid to the column and then rinse any traces of it into the column using the predetermined solvent system.

In Solution: Neat application can sometimes lead to column cracking, so a more common method for liquids, as well as solids, is to dissolve the sample in a solvent and

apply it to the column in solution. Another option for liquids as well as solids is to dissolve it in a solvent and apply it to the column in solution. In the best case, all of the components of your mixture should have an R_f of zero in this solvent - usually pentane or hexane. In many situations this is not possible, so a solvent that moves only one compound in the mixture can be used, or you can simply use your chosen eluent. Keep in mind that these last two options are risky for difficult purifications.

Adsorption onto Silica: The final technique that is useful for some liquids and all solids is to deposit (adsorb) the compound on silica. *Caution: silica gel is acidic and this procedure can destroy compounds that are acid-sensitive and normally survive on silica gel columns*. First, using a round-bottom flask, dissolve the mixture in dichloromethane and add the silica gel - double the weight of your compound to determine the weight of silica. Concentrate the solution on the rotary evaporator. *Caution: Silica gel is a very fine powder and can easily get sucked into the rotovap*. Therefore plug the opening of the adapter or bump guard with glass wool to prevent "bumping" of the solid. Fast rotation also helps prevent this problem. Once the solid is mostly dry, (you will know it's dry when most of the solid has fallen off the sides of the flask) remove the flask from the rotovap and finish removing the solvent using the vacuum pump - assuming nothing in your mixture is volatile. *Caution: again use a glass wool plug in the vacuum adapter or you will find silica gel (and your compound) throughout your vacuum tubing and manifold*. Once it is totally dry (no more bubbling from the solid) remove the flask from the vacuum line and scrape the sides clean with a spatula. The solid can now be added to the top of the column by simply using a powder funnel followed by a few 1.5-mL rinses with your eluent.

4) Determine the silica gel to compound ratio. Easy separations require ratios between 30-50:1 (by weight), while harder separations call for ratios of up to 120:1. The reading in LLP and discussions with more experienced colleagues can help you make this tough decision.

5) Pick the appropriate column. The amount of silica gel you are going to use determines the size of your column. There is an ongoing debate about whether to use silica columns that are short and wide or ones that are tall and skinny. In 5.301 we will argue that the short, wide columns provide better separation, but this statement may be challenged by some of your future co-workers. When you are first starting, the best way to select the correct column for a given amount of silica gel is to ask other members of your lab which column they would use and record this in your notebook. (This is much

easier than measuring column diameters.) In 5.301 we will only have one size to choose from, so the choice will be fairly straightforward!

6) Pick appropriate collection test tubes. This is also a good time to consult your more experienced colleagues, but a simple guideline is to divide the volume of silica that you used by four and pick test tubes that can accommodate this volume. (200 mL of silica = 50 mL fractions)

7) Once you have selected a column, you need to plug the stopcock end so that the silica will not drain out. This is normally done with a small piece of cotton or glass wool and a long stick or glass rod.

8) Mount the column in your hood - due to the large volumes of volatile solvents used and the health risks associated with dry silica gel, you should never run a column outside of the hood. Check to see that your column is perfectly vertical - crooked columns make separation more difficult.

9) Close the stopcock and add a few inches of your solvent (eluent).

10) Add sand (dried and washed) to the column using a funnel. Your goal is to produce a thin layer of sand (no more than 1 cm) above the plug which will help prevent the silica from ending up in your collection flasks.

11) Measure out the correct volume of silica. The safest way to do this is by volume in the hood. Silica gel has a density equal to 0.5 g/mL so you can use an Erlenmeyer flask to measure it out (100 g = 200 mL). Don't fill the Erlenmeyer more than one third full of silica since we will be adding solvent to the flask as well.

12) Make a slurry of the silica by adding at least 1.5 times the volume of solvent as silica you just measured out. Mix it thoroughly by swirling or stirring vigorously to remove all the air from the silica. (Air bubbles will compromise the effectiveness of your column.)

13) Using a powder funnel, carefully and slowly pour the slurry into the column making sure not to disturb the layer of sand. Stop pouring frequently to swirl the slurry so that the silica is evenly mixed. Once you've finished pouring, rinse the Erlenmeyer several times with the eluent and add the remaining solvent/silica mixture to the column.

14) Using a pipet and your solvent system, rinse any silica stuck to the sides of the top of the column into the solvent layer.

15) Once all of the silica is off the sides of the column, open the stopcock and use the compressed air to pack the column. The silica level in the column will shrink to about half of its original height. Check to make sure that the top of the column is flat. If not, it must be stirred up and allowed to settle undisturbed. As the excess solvent elutes under the applied pressure, you can tap the sides of the column *gently* with a rubber stopper on the end of a pencil. This will improve the packing of the silica particles. Collect all the solvent that elutes from the column and recycle it for use after your compound has been added. *Caution: Never let the solvent level drop below the top of the column.*

16) Once the column is packed, add a protective layer of sand to the top of the silica. This should be relatively level and about 2 cm thick. This will protect the column when you are adding solvent - if you add solvent too fast, it can destroy a flat column (thus hurting separation) unless it is protected by sand.

17) Using the compressed air, lower the solvent level until it is even with the height of the sand.

18) Close the stopcock and put your first test tube under the column outlet.

19) Carefully add your compound to the column - when adding liquids be sure to drip them down the sides of the glass, not directly onto the top of the column. When rinsing the flasks that contained the mixture, carefully add a one pipet-full of the rinse solution to the column at a time. Then open the stopcock and drain the liquid level down to the top of the column and close the stopcock. Rinse the flask three times using this procedure. For mixtures that were deposited on silica gel, an additional 2 cm layer of sand is now added.

20) Carefully fill the column with your eluent. At first, add the solvent via Pasteur pipet. Once 1 cm of solvent has been added, the stopcock can be opened for good. Keep adding the solvent by pipet until a few centimeters of solvent are above the column. Now add the solvent from an Erlenmeyer through a powder funnel—*slowly*—letting it first run down the side of the column. Be patient, you do not want to disturb the top of the column.

21) Once you have filled the column with your eluent, you are ready to "run" the column. Remember that a quick flow rate helps to give good separation. Adjust the air pressure to give a swift flow rate—no fire-hoses, though! So, keep the pressure on and change the test tubes once they become filled. Remember to replenish the solvent in the column frequently.

22) Monitor the column's progress by TLC—this can get a little hectic, trying to run TLCs and collect your fractions, so in the beginning you might want to decrease the air pressure (or remove it entirely) when you are checking the progress of the column.

23) When running a gradient elution column, use your initial solvent system until the higher R_f compounds have come off the column. Once they are safely in your collection flasks, you can begin to increase the polarity of the eluent. *Caution:* Increase the polarity gradually. Drastic polarity changes can "crack" the silica gel - sending fissures through the column like in a bad earthquake movie. This cannot help your separation! Instead, increase the polarity by about 5% for every 100 mL (or more) until you reach your desired solvent system. Then stay with this eluent until your desired compound has eluted. At this point, you can either change eluents again or proceed to the next step.

24) Once you have determined that all of the compounds you are interested in have eluted from the column, you are ready to wrap everything up. First, put a large Erlenmeyer flask underneath the column, and use a green Keck clip to attached your compressed air source to the column. Allow the air to push all of the remaining solvent out of the column and then to dry the silica gel. (It's difficult to remove the silica from the column until it is completely dry.) This will take at least one hour for large columns.

25) While the column is drying, start to combine fractions. Using TLC, determine which test tubes contain your pure compound(s). Combine fractions of similar purity in large round bottom flasks and concentrate them on the rotovap. For longer duration columns, combine fractions while the column is still going to expedite the process.

26) Once the solvent is completely removed, analyze the compounds by NMR.

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