D. Flash Chromatography

General Procedure for Flash Chromatography for 100 to 300 mg of a mixture

1. Obtain a small flash chromatography column and use a 1-mL pipet to push a small wad of cotton or glass wool into the narrow part of the valve stem.

2. Clamp the column high in a ring stand and add about a 1/2” sand bed through a powder funnel. Make up 100 mL of your starting eluant determined by TLC, (for example 10/90 CH₂Cl₂/Hexanes mixture) in a 250-mL Erlenmeyer flask. Mix thoroughly by swirling. Pour 1 cm deep amount of this into a TLC development jar, cap and set aside for later use. Pour enough of this into the column so there is ~ 1” of solvent above the sand.

3. In a 50 mL beaker, obtain 20 mL of 200 mesh silica gel from the blue supply bucket. Fill the beaker to the 40 mL mark with your mobile phase and stir with a wide metal scoopula to make a slurry.

4. With stirring, pour and scoop the silica gel slurry slowly into the column through a powder funnel. Use the scoopula to stir and help transfer the slurry. Tap the column gently with your finger tips to help the silica gel settle. Use additional solvent to rinse any remaining silica gel out of the beaker and into the column.
5. Place the pressure Tee/rubber stopper loosely in the top of the column and connect one hose to the nitrogen supply and turn on. Put a Hoffman screw clamp on the end of the other hose and tighten to pressurize the system.

6. Place the slurry beaker you just emptied under the column and open the stopcock to allow a stream of solvent to flow into it. Allow the solvent to flow out until the liquid level in the column is just at the top of the silica gel bed. To speed up the elution, press the tee/runner stopper into the top of the column to pressurize it and increase the solvent flow. Rinse the sides of the walls with solvent to wash down the silica gel. Drain solvent until it is about 1/2” above the top of the silica gel.

**NEVER LET THE SOLVENT DROP BELOW THE SILICA GEL OR YOU WILL GET AIR INTO YOUR COLUMN AND RUIN IT!**

7. Normally you will use the Wet Loading Method starting at step 9. If you are going to use the Dry Loading Method, place one or two scoops of silica gel into a 25-mL 19/22 RB flask. Dissolve ~250 mg of your mixture in 10 mL CH$_2$Cl$_2$ and add this to the silica gel. Remove the CH$_2$Cl$_2$ on a rotovap or with a stream of nitrogen so that you obtain a dry powder. Carefully pour the dry powder onto the top of the column to obtain a even layer at the top. Using a Pasteur pipet, add eluant by draining it onto the glass wall just above the silica gel until it is just covered. Try not to disturb the silica gel too much.

8. Gently sprinkle about 1/2” of sand through the powder funnel. If necessary, add a little more solvent to cover the sand.
9. **Wet Loading Method** - Dissolve ~250 mg of your mixture in a minimum amount of mobile phase (less than 1 mL of solvent if possible. Using a Pasteur pipet, draw up the mixture solution. Insert the pipet into the column so it is touching the inside wall just above the silica gel surface. Gently flow the solution onto the top surface of the silica gel so that you hardly disturb the surface. Drain until the solvent level is just at the top of the silica gel. Gently sprinkle about 1/2" of sand through the powder funnel. If necessary, add a little more solvent to cover the sand. Drain until solvent level is at top of sand. Apply pressure if necessary.

10. Next, using Pasteur pipet, **very gently** add about 1" of your elution solvent that you collected in the beaker when preparing the column. Let the solvent run down the glass wall so that the sand is disturbed as little as possible. Open the stopcock to allow eluant to drain into the beaker until the liquid level is again just at the top of the sand surface. Gently add more eluant washing down the walls of the column just above the silica. Again drain out just enough to bring the liquid to the top of the surface. Repeat this two more times until you have washed the band of your mixture into the column. You want to get as thin and compact a band of your mixture as possible.

Now pipet in about 3" of solvent in the column, and then pour in more solvent until the column is 3/4 full.

**NEVER LET THE SOLVENT DROP BELOW THE SAND OR YOU WILL GET AIR IN YOUR COLUMN AND RUIN IT!**
11. Place about 8 to 12 numbered test-tubes in a rack and place the rack below the column so that you can collect column eluant in them.

12. Now open the stopcock and pressurize the column. Fill consecutive test-tubes 3/4’s full of eluant. Collect about 5 fractions, then, if necessary, increase your solvent polarity (to 30/70 CH₂Cl₂/Hexanes, for example).

13. Do TLC analysis on each of the fractions, checking spots before development under a UV light to make sure there is enough material to see. Develop in the corresponding eluant solvent and examine under UV light or by iodine to see which fractions contain material. If you do not see any spots, then let the solvent evaporate from your test tubes (Label the rack of tubes with your name and leave in the hood.) You should see material in some tubes.

Combine similar fractions in a flask or beaker, label and put in your hood to evaporate.

14. Clean out the column by blowing out all solvent and leaving upside down in a 5 gallon bucket to dry. In the hood, carefully tap out the silica powder and dispose of in the special used silica waste container.