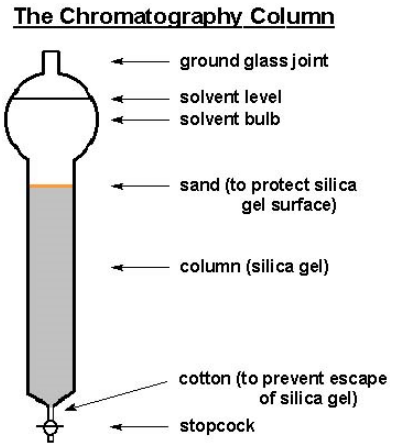
Column Chromatography Protocol

October 15, 2017

Setup



1. Select solvent system (usually by running a test TLC of crude product)
2. Set up column in a hood, secure with 3-pronged clamp
3. Using a fresh glass pipet, tightly stuff a small bit of cotton down in the column
4. In a hood, pour some silica powder into a beaker and dissolve silica in desired solvent system. Use a glass pipet to transfer some of the silica slushy into column so that the packed silica will be about 6-8 fingers long. Open stopcock and use a pipet bulb to push on the column. The silica will begin to compact and be sure to continue pumping so that silica will be tightly packed. Pump on column until a small amount of solvent remains above silica level, then stop. You don't want the silica to be dry.
5. Prepare to load sample by dissolving it in a small amount of solvent system. Gently pipet sample along the glass edge of the column, allowing it to flow down to the silica level. Use the pipet bulb to force the sample into the silica. You want the sample to be all the way in, with the silica on top being just barely dry.
6. Quickly add some sand to the column (about 2 fingers in length). The sand will protect the silica layer (and your sample) from damage when you begin adding solvent.
7. Once the entire sample is loaded, fill the column with desired solvent system, and pump on the column and begin collecting fractions.
   Tip: You have just spent all of this time preparing a nice, even silica level. If you are too forceful when loading your sample, you will damage the silica surface and have difficulty running a nice, even column.

Collecting Fractions

1. Keep an eye on the solvent level, you don't want it to reach the sand. NEVER RUN DRY!
2. You may use the pipet bulb to force the solvent through faster.
3. You can't stop the flow of these columns, so keep an eye on them.
4. TLC while running the column to analyze the product coming out.
5. After TLC, combine same fractions.
6. If stuff in the end doesn't come out for a long time, adjust the solvent system added.

Chromatography Theory

Recall Poutsma's Instrumental Analysis… If the solvent system is non-polar, non-polar stuff comes out first, which is the top guy in the TLC plates. Hex:EA = 5.5:1 (less polar) comes out first in column and it's at the top at TLC plates.

Column Clean Up

1. When you are done, push all of the solvent through with a hand pump.
2. Attach column to a vacuum line and carefully suck off any remaining solvent.
3. Once the silica is dry enough, take the column to the waste hood. Flip the column upside down over the solid waste to remove all sand and silica.
4. Rinse the column with acetone then let dry.