Under construction

Appendix 3

Flash Chromatography[1] (FC).

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Column chromatography allows multiple components to be separated (therefore it is preparative). Unlike with gravity column chromatography, using air pressure to force the solvent through the column reduces the chromatography time, therefore making the column and running the separation could take less than 10-15 minutes. The absorbent has a much smaller particle size (about the same as that on TLC plate). There are many different of kinds of chromatographic columns. If the column contains a porous plate to support packing, no additional support such as cotton, glasswool and sand is necessary.

Safety Guidelines:

1. Silica gel ($SiO_2 \cdot xH_2O$) should be weighed and transferred in the hood to avoid inhalation. **Silica gel is a fine powder and lung irritant**.
2. ----**to be added**----

Step 1. Finding the solvent system.

Find the solvent system that gives an R_f[3] of approximately 0.35 on a silica gel plate.[4] For a general separation it is useful to start with ethyl acetate-petroleum ether (bp 30º C-60º C). For polar compounds use acetone (or methylene chloride) and petroleum ether (bp 30º C-60º C). Dissolve a small amount of the crude material in a minute amount of ether or dichloromethane (1-2% solution, 20-40 mg in 2 mL of the solvent). Make a line at 1 cm from the bottom and another line at 1 cm from the top of 3x7 cm normal-phase silica gel TLC plate. With a micropipet make a spot 1-2 mm in diameter (adding once or twice; overloading causes tailing) on the starting line. Try different solvents until one that results in an R_f of 0.35 is attained. The spot can be easily visualized with a short-wave UV light (254 nm). Carefully circle the spot with a pencil. Calculate the R_f.

Eluotropic series for silica gel (increasing eluting power)

Cyclohexane
Petroleum ether

[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

[3] R_f stands for ***ratio to the front***.
[4] Kodak plastic-backed TLC plate #13181 with fluorescent indicator. CAS number [63231-67-4].

Pentane
Trichloromethane
Ethyl ether
Ethyl acetate
Ethanol
Water
Acetone
Acetic acid
Methanol

Step 2. Selecting the column diameter.

There are several set-ups available for FC.
The recommended length of a FC column is ca 18 (ca. 46 cm) inches long. However, sample size and the difference in R_f (? R_f) between components to be separated dictates the column diameter you should pick to run the FC. For example if you have a sample of approximately 1 g, and the solvent system separated the two components with ? R_f = 0.2 you should choose a column with a 3 cm diameter, collect fractions of 20 mL size and use ca 400 mL of eluent.

Table 1. Guidelines to choose the diameter of a FC chromatography column.[1]

Sample loading (g)		Column diameter (mm)	Fraction size (mL)	Eluent volume (mL)
? R_f > 0.2	? R_f ~ 0.1			
0.100	0.040	10	5	100
0.400	0.160	20	10	200
0.900	0.360	30	20	400
1.600	0.600	40	30	600
2.500	1.000	50	50	1000

Step 3. The column preparation.

To purify ca 1 g of crude product, choose a 50-cm column with a 20 mm inside diameter. The silica gel height should not exceed 5-6 inches (ca. 12-15 cm). Clamp the column in the upright position. Note: **If the column contains a porous plate to support packing omit the cotton and sand support; <u>go to step 3.</u>**

1. Using a glass rod, carefully pack a small piece of cotton in the bottom of the column.
2. Add approximately 1/8" of 50-100 mesh sand.[5]
3. **A.** Close the stopcock and add solvent until the column is approximately 1/3 full. Add ca. 5-6 inches of 230-400 mesh (23-40 μm) silica gel[6] in 100-mL beaker.

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[5] Coarse Ottawa sand

Add 15-20 mL of solvent to the silica gel and make a slurry. Swirl the slurry and add through a long stem glass funnel into the column.

or

B. Pour the silica gel into the column. Tap sides of the column to get the top of silica gel as level as possible. Place a beaker under the column to collect the solvent that will drip out. Next fill the column with the solvent of choice.

4. Add 1/8" of sand to the top of silica gel to protect the sample from disruption when filling the column with solvent.
5. Attach the airline to the plastic T (Y) joint.[7] Insert the T joint into the one hole rubber stopper size #6 and place it firmly into the top of the column.
6. Open the stopcock.
7. And use air pressure to force the solvent through the column so that is dispersed within the silica gel. Do not allow the solvent to drain below the top of the silica gel. Continue to pour solvent (or recycled solvent) **until the silica gel becomes translucent in appearance.** Your column is ready: all the air pockets have become filled with solvent and the column packing is homogeneous.

Step 4. Loading the sample.

- Drain some solvent from the column until the solvent level is just above the top of silica gel.
- Prepare a 20-25% solution of your crude material in the solvent of choice.
- Using a Pasteur pipet carefully add your sample to the top of the surface of the silica gel. Use a few drops of fresh solvent to transfer the remainder of your sample onto the column.
- Open the stopcock. Drain the solvent until the sample is absorbed onto the top of the silica gel.
- **Do not disturb the sand**: add carefully more solvent into the column using a Pasteur pipet by pacing the tip of the pipet inside the surface of the column and letting the solvent to flow gently into the column. After a couple of inches of solvent have been added to the column, fill the rest of the way by pouring solvent into the column by using a glass funnel.

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[6] Silica gel, 230-400 mesh [112926-00-8]; 200-425 mesh: Fischer Scientific, cat #S 733-1.
[7] nalgene

Step 5. Develeoping the column.

- Place small horizontal marks with a marking pen at one inch interval starting at the top of the silica gel upward to the top of the column. These markings will be used to monitor flow rate of solvent through the column.
- Place a 13x100 mm test tube under the column's stopcock. Open stopcock. Use finger to adjust flow rate to approximately 1" every 30 seconds.[8]
- **Never let the column to run dry**. Add more solvent to the column as needed.
- Collect 15-20 fractions of 10 mL each.
- Check every fraction by TLC to find the desired compound.
- Combine all fractions that contained the desired compound into a tared 100-mL round bottom flask. Evaporate the solvent to dryness on a rotary evaporator.
- Record the weight of your pure sample to nearest 0.001 g.

Waste disposal.

Solvent waste should be discarded in the organic solvent waste container. Silica gel should be dried and discarded in a separate labeled waste container.

[8] A faster rate , a slower rate