Column Chromatography

Purpose: Separate a mixture of fluorene and 9-fluorenone by column chromatography.

Preparation: Mayo, Technique 6 Chromatography including the following:

- Column Chromatography (pg 119)
- Packing the Column (pg 120)
- Sample Application (pg 121)
- Elution of the Column (pg 122)
- Fraction Collection (pg 123)

Look up and record the structures and melting points of fluorene and 9-fluorenone. View the "Column Chromatography" DVD in the Vasche Library prior to this experiment.

Background:

Like thin layer chromatography, silica gel is used as a stationary phase, and an organic solvent (less polar than the silica gel) is used as the mobile phase. Column chromatography is carried out in a glass tube clamped vertically with the initial mixture placed at the top. Organic solvents run past this mixture on their way down the column. Because of silica gel's stronger affinity for the more polar components, the components with the lower polarity will descend ("elute") first through the column. During the chromatography, the polarity of the mobile phase can be slowly increased by varying the solvent mixture used. As a result, increasingly polar components will elute.

As in any other chromatography it is important to introduce the mixture in as concentrated volume as possible to avoid overlapping of the components as they elute. Below the column, pre-weighed flasks are used to collect the solvent with the various components ("fractions"). Normally, flasks are changed when the solvent mixture is changed. Each flask is then warmed to evaporate the solvent, then reweighed, and the amount of component in the fraction is recorded.

In the procedure below a two-component mixture will be separated. Ideally the two components will end up in separate flasks, preferably with an empty flask in between. This blank fraction is akin to having a baseline separation of peaks on a gas chromatograph chart or a clear space between spots on a TLC. Success in a separation requires good organization and planning, a concentrated mixture at the beginning, and as few interruptions of dripping from the column as possible after the chromatography is started.

Procedure:

Work in teams of two. It is imperative that you and your partner divide the work and work efficiently to complete the entire experiment in a timely manner.

Preparing Glassware

1. Obtain six 50 mL Erlenmeyer flasks and/or beakers. Clean the flasks/beakers **only if visibly dirty**:

- Use a brush to wash each with soapy water.

- Thoroughly rinse each flask with distilled water then a small amount of acetone.
- Shake out the acetone and then warm each briefly on a hot plate.
- The flasks should be clean and dry.

2. Label each flask/beaker numbering them 1-6.

3. Weigh each to the nearest milligram. Record each mass in notebook.

**Take note if you weighed each flask with/without a label, as your tare must be consistent at the end of the experiment.

Preparing the Column

The prepared column is shown to the right (Figure 1). Follow the steps below to prepare the column yourself.

1. Obtain a clean 5 mm x 20 cm column with a fritted disk. If you do not have a fritted disk, then you must put in some glass wool (Figure 1a) or else your silica will slip out. Fill the column with dry silica gel (~18-20 cm high, **USE A FUNNEL!**). Pour the silica gel back out into a 100 mL beaker.

2. Add a small amount (~1 cm) of dry sand to the column on top of the glass wool plug (Figure 1b).

3. Fill the column ~1/4th full with petroleum ether, and shake the column to remove air bubbles from the sand and glass wool.

4. To the beaker containing silica gel, add 20 mL of petroleum ether. Stir the contents of the beaker to remove bubbles and thoroughly wet all the silica gel.

(Note: petroleum "ether" is really a mixture of alkanes whose boiling point is 30-60 °C. Do not confuse this with diethyl ether, $CH_3CH_2OCH_2CH_3$, that is normally used as a solvent.)

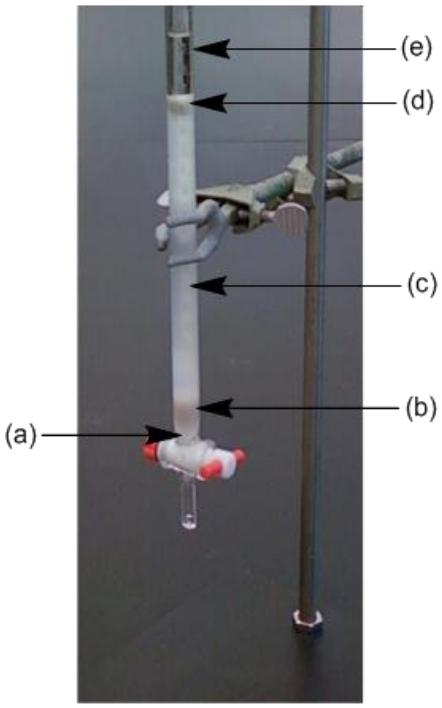

Figure 1. Prepared chromatography column
(a) small wad of glass wool
(b) sand
(c) stationary phase - silica gel
(d) sand
(e) mobile phase

5. Place a funnel at the top of the column and quickly pour the silica gel slurry back into the column. You will likely have to stir while pouring, as the silica gel "falls out of solution" quickly. If necessary, open the column stopcock and drain some of the petroleum ether into the silica gel beaker to re-slurry the silica, and pour again. Repeat this process until most of the silica gel has been transferred to the column (Figure 1c).

6. With a Pasteur pipette and petroleum ether, rinse the sides of the column, washing down all the silica gel.
7. Pack the column by repeatedly hitting/smacking the side of the column with your hand. You should observe the level of silica gel falling slightly. Once the silica level ceases to drop, the column is packed and you may proceed to the next step. Properly packing the column is imperative to the success of your separation.
8. Very gently add ~1 cm of sand to the top of the silica gel (Figure 1d). If you packed the column properly, the sand will sit on top. If the column is poorly packed, the sand will sink into the silica.
9. Open the stopcock and drain the excess petroleum ether (Figure 1e) until the level reaches **just to the sand**.

Running the Column

This section will test the teamwork and communication between you and your partner. Once the column has started, it should not be stopped, or allowed to run dry for any reason! One partner should monitor the column and collect fractions, while the other should obtain the necessary eluting solvent **well in advance of its use**.

1. Weigh approximately 500 mg of the fluorene/9-fluorenone mixture to the nearest milligram into a 25 mL Erlenmeyer flask.
2. Add enough chloroform, drop-wise, to completely dissolve the mixture using **minimal** solvent.
3. Carefully transfer the solution directly to the top of the sand layer in the column (not down the side of the glass tube).
4. While one partner is performing Step **3**, the other partner should simultaneously obtain 10 mL of **9:1** (v:v) **petroleum ether:chloroform**.
5. Open the column stopcock and allow the fluorene/9-fluorenone solution to pass through the sand and enter the silica gel. At this point, you should not close the stopcock for any reason!
6. Once the fluorene/9-fluorenone solution level has gone into the sand, add a few drops of 9:1 pet ether:chloroform. Allow these few drops to enter the sand and silica. Add a few more drops and repeat the process until all the fluorene/9-fluorenone mix has visibly been washed out of the sand and into the silica gel (the sand is no longer yellow).
7. Carefully pour the remaining 9:1 pet ether:chloroform into the column. Make sure to not disturb the silica layer when adding the eluant.
8. Place flask/beaker #1 under the column, collecting the eluate (the column drippings). Allow all 10 mL of 9:1 pet ether:chloroform to pass through the column. This is fraction #1.
9. Once the level of the initial 10 mL has reached just above the sand, carefully add 10 mL of **4:1 pet ether:chloroform** to the column as before. Collect another 10 mL as fraction #2.
10. Repeat the process, adding 10 mL of **1:1 pet ether:chloroform** (fraction 3), followed by 2 × 10 mL of **100% chloroform** (fractions 4 and 5), and 10 mL of **100% acetone** (fraction 6).

11. If a significant yellow color persists in the silica gel, and if time permits, continue to collect 1-2 more fractions (fractions 7 and 8) with 100% acetone.

Thin Layer Chromatography (TLC): Each group has to perform two TLC plates.

1. This can be done while waiting for column chromatography fractions. Place and develop three spots on a silica gel TLC plate:

a) pure fluorene
b) pure 9-fluorenone
c) fluorene/9-fluorenone mix

} use available standard solutions

Use a developing jar with a 9:1 mixture of hexane:chloroform as the developing solvent. Repeat until a good TLC plate is obtained.

2. For the second plate, spot all the fractions that you collected above and the fluorene/9-fluorenone mix. Calculate and report the R_f values for **ALL** spots on **ALL** plates. From the TLC plate, indicate the composition of each fraction and which fractions are pure in your results and discussion.

Submit your TLC plates according to the directions on the Report Form.

Once you have spotted each fraction and obtained an adequate TLC analysis, remove the solvent from each fraction by gentle evaporation on a hotplate. Be careful not to vaporize or burn your fractions! Let each flask cool, and reweigh to obtain a mass for each fraction.

In your notebook, tabulate the results as follows:

- Fraction Number
- Solvent
- Mass
- Remarks (such as "crystals, light yellow, fluorene, 9-fluorenone, mix, nothing, or not enough to analyze").

Each group should choose their most “pure” fluorene and their most “pure” 9-fluorenone fraction and obtain melting points for the **TWO** fractions. Using the melting point data, check the two fractions for contamination from the other compound in the mixture. Does the purity as shown by TLC match the purity as shown by melting point?

You should calculate an overall percent recovery for the column chromatography separation. While writing your Discussion, ask yourself, “Was the purpose of the column chromatography separation to achieve a high percent recovery?”. What was the purpose, and did you achieve it? What data shows this? Use your data (R_f values, melting point) to back up your claims.

References:

1) Mayo, D. W.; Pike, R. M.; Trumper, P. K. *Microscale Techniques for the Organic Laboratory*, 2nd ed.; John Wiley & Sons: New York, 2001.

CHEM 3012 - Spring 2009
Organic Chemistry Laboratory I

Name _____

Partner _____

Column Chromatography – Report Form

Raw Data

mass of crude mix: _____ g

Complete the following table.
"No data" entries should be denoted by entering a dash (–).

fraction	solvent	mass[a] (g)	remarks	m.p.[b] (°C)
1				
2				
3				
4				
5				
6				
7				
8				

[a] Mass of the isolated, dried eluate.

[b] Analyze one "pure" fluorene fraction and one "pure" 9-fluorenone fraction.

The partner in possession of the TLC plates must submit them in *separate* sealed bags, with all bags stapled to the front, upper, left-hand corner of this page.
Sketch the TLC plates below.
Label each plate "lane" as "fluorene standard", "fraction 1", etc.

Results

Complete the following table. Only report the data which you have. If you only have one spot by TLC, only report one R_f value; if two spots, report both R_f values, no spot, no R_f value. "No data" entries should be denoted by entering a dash (–). All calculations of R_f values must be shown in your notebook.

		R_f 1st spot	R_f 2nd spot	fraction ID
standards	fluorene			none, fluorene, 9-fluorenone, or mix
	9-fluorenone			
	mix			
fractions	1			
	2			
	3			
	4			
	5			
	6			
	7			
	8			

total mass of eluate: _____ g

overall % recovery (show calculation):

Discussion (Must be duplicated in notebook. Limit yourself to the available space.)

Conclusion (Must be duplicated in notebook. Limit yourself to the available space.)

Questions (Answer in complete, full sentences. Limit yourself to the available space.)

1. Predict the order of elution of a mixture of triphenylmethanol, biphenyl, benzoic acid, and methylbenzoate from a silica gel column. Explain your answer.

2. From the "standards" TLC plate, were the relative R_f values of fluorene and 9-fluorenone as you expected? Explain. Draw structures of both fluorene and 9-fluorenone to support your explanation.

3. Once the chromatographic column has been prepared, why is it important to allow the level of the liquid in the column to drop to the level of the sand/silica before applying the solution of the compound to be separated (the fluorene/9-fluorenone mix)?

4. What would have been the result if the column was run with acetone as the only eluting solvent? Thoroughly explain your answer.