Preparing a Silica Gel Chromatography Column

The following set of instructions will assist the reader in the preparation of a silica gel chromatography column. A silica gel chromatography column is a device that relies on silica gel to separate the components of a chemical mixture; the separation is accomplished because each component of the mixture has a different polarity. More polar compounds will flow easily through the silica gel, while non-polar compounds will flow more slowly through the gel. Silica gel itself in non-polar, and thus is attracted to other non-polar molecules. The attraction of the non-polar molecules to the silica gel is what causes the non-polar components of a mixture to move slowly through the gel. Chemical separation relies on the relative speeds at which each component travels from the top of the column to the bottom when using a silica gel chromatography column. The length of the column will provide a separation that gives a high purity for each component; each component while drain out of the column and have a sharp ending point. None of the liquid drained from the bottom of the column will have both components of the mixture in it. A full separation (no product is left in the column) of the mixture will also occur within one hour when using the column outlined in these instructions.

Required Equipment

- Glass column with a stopcock
- Pipette, equipped with pipette bulb
- Flat-necked glass funnel
- 250 mL Erlenmeyer flask
- One cotton ball
- 1 Coat hanger, straightened
- 2 500 mL beakers
- 150 mL graduated cylinder
- 2 three-fingered clamps
- Ring stand
- Metal scupula
- Glass stirring rod

Required Chemicals

- 8 grams of reagent-grade sand
- 200 grams of silica gel
- 150 mL of dry diethyl ether

   Warning: Diethyl ether is **extremely flammable**; do not use it near open flame

- 150 mL of cyclohexane

   Warning: cyclohexane is **extremely flammable**; do not use it near open flame

- Mixture to be separated

Procedure

 Warning: Due to the flammability of the solvents (diethyl ether and cyclohexane), the preparation of the chromatography column should be performed in the laboratory's fume hood. Failure to perform the procedure in the fume hood could result in injury and equipment damage. 

All glassware should be **clean** and **dry** before it is used. Failure to clean and dry glassware will result in a poor separation or unwanted side reactions, ruining any previously prepared products.

1. Pour the 150 mL of diethyl ether and the 150 mL of cyclohexane into the 500 mL beaker. Use the glass stirring rod, stirring the solution for two minutes. This solution will serve as your eluent and will be needed for the separation of your compound.

2. Place the two three-fingered clamps on the ring stand. Put the glass column into the three-fingered clamps - placing one clamp just above the column's stopcock and the other clamp roughly three inches from the top of the column. See *Figure 1* for a diagram of the setup.

> Caution: Do not over-tighten the three-fingered clamps, as it could crack or break the glass column.

3. Close the stopcock. Tear the cotton ball in two, and place one of the halves into the glass column. Use the straightened coat hanger to gently push the cotton to the bottom of the glass column.

> Caution: Do not try to compact the cotton. Simply push the cotton plug to the bottom of the column. Compacting the cotton plug may clog the column, preventing it from working.

Figure 1
Clamps on the Glass Column

3. Measure 4 grams of sand and pour it into the column using the scupula. Gently shake the column so that the sand forms a flat surface. See *Figure 2*.

4. Pour the 200 grams of silica gel into the empty 500 mL beaker. Pour roughly half of the mixture prepared in step 1 into the 500 mL beaker that the silica gel is in. Stir the solution gently.

5. Set the flat-necked glass funnel on top of the column. Pour the solution prepared in step 4 through the funnel so that it flows into the column and comes to rest on top of the layer of sand. See *Figure 2*.

> Caution: Pour the solution in slowly and carefully so that the sand layer is not disturbed. If the silica gel touches the cotton at any point, the column may not function correctlv.

6. Leave the flat-necked funnel on top of the column and allow the silica gel layer to settle for five minutes.

7. Pour roughly 5 mL of the remaining diethyl-ether/cyclohexane mixture prepared in step 1 into the funnel.

   Repeat this step until all of the silica gel is cleaned from the walls of the column. The silica gel must form one layer, and not remain on the walls of the column for proper product separation.

8. Remove the funnel from the top of the column and use the scupula to pour the remaining sand onto the silica gel layer. The liquid sitting on top of the silica gel layer will level the sand without shaking or stirring. See *Figure 3*.

9. Place the 500 mL beaker containing the remaining ether-cyclohexane mixture under the spout of the column. Open the stopcock, allowing the liquid in the column to flow out until the liquid's level is slightly above the top of the sand layer. This will eliminate air bubbles from the column. See *Figure 4* for an image of the column after draining the excess solution.

   Close the stopcock when finished.

   Caution: Do not let the liquid level get below the top of the silica gel layer. Allowing the liquid layer to get below the top of the silica gel will ruin the separation power of the column. If the liquid layer gets below the top of the silica gel, you will need to discard the current column and assemble a new one.

10. With the stopcock closed, transfer your sample to the pipette. Pipette the solution into the column. Allow it to run down the side of the column, so that it does not perturb the sand layer. See *Figure 5* for an illustration of proper pouring technique.

    Caution: Be sure to let the sample run down the side of the column. If the sample gets below the sand, separation of your sample mixture will be poor.

Figure 4
Column After Draining Excess Solution

11. Place the Erlenmeyer flask beneath the spout of the column.

Figure 5
A Comparison of Proper Product Introduction Technique
Be sure not to pour the product directly onto the sand. Use the wall of the column to avoid disturbing the sand.

You have produced a chromatography column that will be able to separate all of the two-compound product mixtures produced in the Organic Chemistry Laboratory at Texas Tech. You also have the supply of eluent (the excess liquid prepared in step 1) needed to carry out a separation. Your column should look like *Figure 6*, opposite this paragraph.

Figure 6
Finished Chromatography Column

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Common Column Problems and Precautions

Problem	**Cause**	**Prevention**
When stopcock is opened, the column does not drain.	Cotton is packed too tightly into the bottom of the column.	Do not compact the cotton with the hanger. The cotton ball should still be fluffy when pushed to the bottom of the column.
Eluent drains out of the column, but none of the product flows into the collection flask.	Silica gel is bonding to the product due to part of the gel having dried.	Do not allow the liquid layer to go below the top of the silica gel layer. When the silica gel dries out, it will begin to bond compounds to itself.
The column works, but product separation is poor. The product components are coming out as a mixture, instead of coming out separately.	1. The air bubbles were not removed from the column, causing the product to be moved through the column more slowly. 2. The product penetrated the sand as it was being pipetted into the column.	1. Do not forget to drain the excess eluent from above the sand layer before introducing your product. 2. Do not drop your product into the middle of the column, but pour the product down the side of the column.