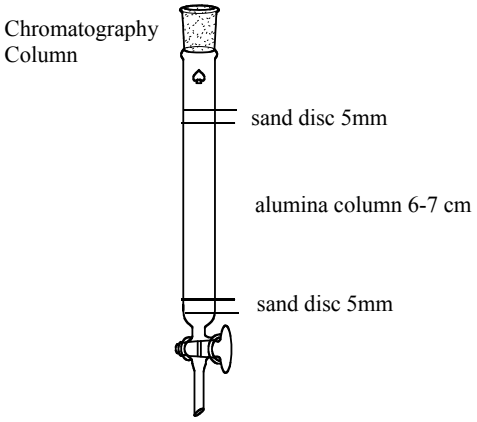
Cerritos Community College

Organic Chemistry 211 Laboratory

Column Chromatography

Objective: To separate methylene blue from fluoresceine via column chromatography.

Procedure: Obtain/set up the following items



1) Ready packed column
    a. Make sure each layer is horizontal before adding the next layer
    b. 5-6 mm disc of pure sand to prevent alumina from falling out
    c. 6-7 cm of alumina
    d. Pack the alumina tightly by gently tapping with spatula
    e. 5-6 mm disc of sand on top that absorbs impact of liquids added to column and serves as a safety buffer to keep the column from running dry.
    f. **Never let the column run dry!!!**
2) 20 ml ethanol
3) 20 ml 3M aqueous ammonia (NH_4OH)
4) 2 dropper bulbs
5) 2 long-stemmed Pasteur pipettes
6) 2 collection flasks (Erlenmeyer)
7) 3 drops of mixture in medicine capped vial.

Solvent systems

a) 95% ethanol b) 3M aqueous ammonia

Once you have the packed column:

8) Wet the packed column with ethanol
9) Using the bulb push the ethanol through the column until the level of ethanol reaches the middle of the column. Wait until the top of the ethanol is at the middle of the top sand disc. At this point gently add 3 drops of the mixture to the top of the sand with the pipette.
10) **Do not let the column run dry**
11) Add the ethanol to elute the first component from the column. Collect in a flask. Wash the first component completely off of the column.
12) Once the first component is washed from the column add NH_3 (aq). Once the second component comes off the column, collect the component in a second flask. Wash the second component completely off of the column.
13) Test each collected sample for UV activity.

For the discussion:

Which compound is more and which is less polar? Give evidence.
What do the observations of each separated compound under UV light lead you to conclude?
Discuss error
Evaluate your technique.