

Preparation of Chromatography Columns

- Set up an appropriate number of 5 ml pipets with rubber bands to hold them in the rack. It is a good idea to make 2-3 extra columns in case some are broken or made improperly.
- Place a glass bead in the bottom of each pipet.
- To make the **water trap** mix 9 grams of celite with 3 ml distilled water using a mortar and pestle. Nine grams of celite will make approximately 36 water traps. (The mortar will hold up to 10 grams at a time if more is desired.)
- Mix until you are sure it is homogenous.
- Shake the celite mix into each pipet using a joint adapter/funnel. Fill to about the 3.5 ml mark, then pack firmly to the 4.5 ml mark using a glass rod. Generally, the celite mix packs to about 1/3 of the loose volume.
- To make the **glycol phase** of the columns mix propylene glycol (1,2-propanediol) and ethylene glycol in equal amounts. Add 3 mls of glycol mix to 6 grams of celite and mix well with a mortar and pestle until homogenous. Six grams of celite will make approximately 9 long columns (27 short columns).
- Add the celite mix with the joint adapter/funnel to the 3.0 ml mark and pack down to the 4.0 ml mark. Add again to the 2.5 ml mark and pack to the 3.5 ml mark. Add once more to the 2.0 ml mark and pack to the 3.0 ml mark. The columns are now packed with 1.5 ml celite/glycol mixture on top of 0.5 ml celite/water mixture.
- The columns should be wet with 4 ml isooctane so they do not dry out. An eppendorf Repeater with a 50 ml tip may be used for this.
- Attach the hoses and turn on the nitrogen gas to push the isooctane through the columns. (The flow rate may be as great as desired at this time, but the columns should never be allowed to dry out. If this does occur, the columns must be thoroughly rewet with more isooctane.) Stop when the solvent drops to within one mark of the celite.
- Add another 4 ml of isooctane, then attach the hoses to avoid overnight evaporation. Make sure the valves are closed and the gas is off. (If chromatography is done on the same day that the columns are made this second volume of isooctane can be run through and samples can then be added to columns.)