Column Chromatography: The Isolation of Plant Pigments from Spinach or other plants

The leaves of plants contain a number of colored pigments generally falling into two categories, chlorophylls and carotenoids. Chlorophylls *a* and *b* are the pigments that make plants look green. Carotenoids are part of a larger collection of plant-derived compounds called terpenes. Carotenoids are tetraterpenes (eight isoprene units). Lycopene, the compound responsible for the red coloring of tomatoes and watermelon, and ® -carotene, the compound that causes carrots and apricots to be orange, are examples of carotenoids. Spinach leaves contain chlorophyll a and b and b -carotene as major pigments as well as smaller amounts of other pigments. Chlorophyll a and chlorophyll b are similar in structure and may not be able to be resolved in this procedure.

The Experiment:

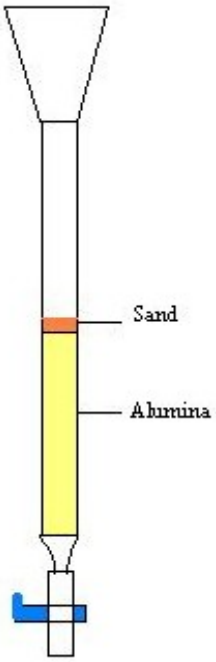
Extracting the pigments:

Blot dry about 5 grams of leaves and place in a mortar. Extract the pigments by grinding the leaves with a pestle with about 5-10 ml of an 80:20 mixture (v:v), petroleum ether (hexane) and acetone. Water is not our friend here so you may have to add some drying agent like $MgSO_4$. Decant the liquid into a 50 ml round bottom flask. Use a quick filtration if necessary. I suggest concentrating the pigments by evaporation under reduced pressure.

Preparing the column.

We will use what is called the "wet pack" method.

1. Assemble you column using the chromatography column (with plastic tip with frit), the one-way stopcock, and the plastic funnel.
2. Fill the column with enough alumina to get the height you want. Pour the dry alumina into a beaker and add hexane (pet ether). Swirl the mixture and then pour into the column. Tap the column gently so air is not trapped as the alumina settles. Add a small amount of sand after the alumina has settled.
3. The column should not contain air bubbles and should be homogeneous. Allow the solvent level to drop to the level of the alumina sand intersection.



Running the column

1. Using a long pipette, add some of the pigment mixture directly onto the sand. Add enough to fill the sand layer with color. Open the stopcock and let the liquid level fall to the top of the alumina. Gently add petroleum ether to fill the sand layer. Open the stopcock and let the liquid level fall to the top of the alumina. Repeat these steps at least three times or until all the colored compounds are in the alumina. Now fill the column with petroleum ether. DO NOT EVER LET THE COLUMN RUN DRY. NEVER LET THE SOLVENT LEVEL FALL BELOW THE LEVEL OF THE ALUMINA!
2. Open the stopcock to allow a drip rate of approximately 1 drop per second. You should see the yellow-orange B-carotene elute first. As the yellow-orange colored product elutes, collect it in a test tube.
3. When the B-carotene has eluted, we can speed up the elution of the chlorophylls by using a more polar solvent. Let the solvent level fall to the the top of the alumina. Gently fill the column with either pure acetone or a petroleum ether acetone combination. By using a 70% petroleum ether: 30 % acetone combination, you might be able to separate chlorophylls *a* and *b*. Collect the chlorophylls in a separate test tube.
4.

Post Lab Questions.

1. Carotenoids are terpenes. Please draw B-carotene and identify the eight isoprene units in the skeleton. You can do this by circling the isoprene units or by using a highlighter. (If you use a highlighter, do not highlight the bonds between isoprene units.)(See reference one for structures.)
2. Which is more polar, B-carotene or chlorophyll? What functional group(s) make it more polar?
3. Which is more polar, chlorophyll *a* or *b*? What functional group(s) make it more polar?
4. How can we improve the resolution of chlorophylls *a* and *b*? Would this make the experiment take a longer or shorter (time)?