

Separation of Beta Carotene and Chlorophyll from Spinach Leaves by Column Chromatography

Introduction

In this experiment, you will use column chromatography to separate and isolate two colored compounds from spinach leaves. The first is beta carotene, which is the precursor to vitamin A, and has a yellow color. The second is chlorophyll, which plants use in the process of photosynthesis, and has a green color.

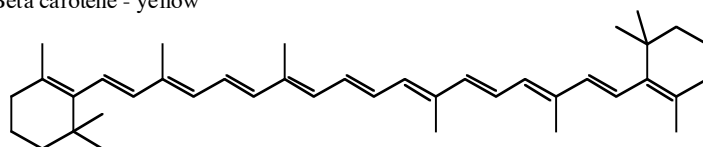
Column chromatography operates on the same principles as TLC, but instead of using a plate coated with silica gel, you will use a buret filled with alumina. Solvent will travel down the column instead of up a plate. The same concepts of polarity apply.

Beta carotene is a hydrocarbon, making it quite nonpolar. Chlorophyll contains very polar bonds to magnesium as well as a few polar functional groups. This large difference in polarity makes this separation very effective. The beta carotene moves much more easily down the column than the chlorophyll.

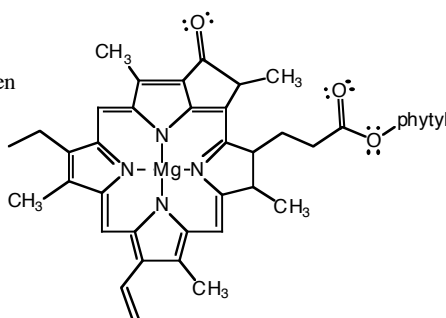
Both beta carotene and chlorophyll are colored, which will make it easy to observe their movement down the column. The color comes from light being absorbed by the compounds because of their numerous C=C bonds.

Since we are isolating naturally occurring compounds from a mixture, we will calculate a % recovery for each one.

Beta carotene - yellow



Chlorophyll a - green



To prepare for this lab, please read "Separating Compounds by Column Chromatography" and "Evaporating a Solution." Then read all of the instructions for the lab, and do the on-line questions. Write an introduction in your notebook.

Procedure

Separate the beta carotene and chlorophyll from the spinach leaves:

- Obtain 10-14 spinach leaves. Pinch off the stems and wash off any dirt. Pat them as dry as you can with paper towels. Then add leaves to your pile until you have at least 10 g of leaves.
- Place the leaves in a large mortar and add about 20 ml of dichloromethane and 20 ml of petroleum ether. Mash the leaves carefully with a pestle for about 5 minutes to extract all organic-soluble components.
- Carefully pour off the organic solvents into a 100 or 50 ml round bottom flask, leaving any water (which will be at the bottom) behind.
- Add about 500 mg of alumina to the flask. Rotovap to dryness, as described in "Evaporating a Solution."

Separate the beta carotene and chlorophyll from each other:

- Prepare a column as described in "Separating Compounds by Column Chromatography."
- Pour the sample plus alumina in the top of the column and wash it down with a few ml of hexanes if it sticks to the sides.
- Prepare a solution of 10% ethyl acetate in hexanes (start with 100 ml and prepare more as needed).
- Fill the column with solvent, open the stopcock, and begin pushing it through with the bulb. At this point a yellow band should appear and move down the column – this is the beta carotene.
- Collect it in a separate flask as it comes out the bottom (start collecting when the yellow band is about an inch from the bottom). When the yellow band has been completely eluted, push out any extra solvent.
- Prepare 100 ml of 10% methanol in dichloromethane.
- Add the new solvent to the column and begin pushing it through – a green band should now move down the column.
- Collect this band in a separate flask as it is eluted from the column.
- Force all liquid from the column and leave it on my desk. (It is very difficult to clean it out until it dries.)

Obtain and characterize the final products:

- Clean and weigh 2 round bottom flasks (use three decimals!).
- Transfer the yellow and green solutions to the flasks and evaporate them on the rotovap.
- Observe the appearance of each compound
- Subtract the mass of the flask to obtain the mass of each compound. Calculate the % recovery of each.
- Write a conclusion in which you discuss the appearance, mass, and % recovery of each compound.

Questions

- 1) What is the purpose of putting a solution on the rotovap? Explain in your own words how this process works.
- 2) Which is more polar, beta carotene or chlorophyll? If you didn't have access to their structures, how would you know this from the experiment?
- 3) Which solvent system is more polar, 10% ethyl acetate in hexanes, or 10% methanol in dichloromethane? How can you tell from the experiment?
- 4) If you took a TLC of the original solution containing both β -carotene and chlorophyll, what would happen if you used the first eluting solvent as the developing solvent? What if you used the second eluting solvent? Draw a picture of each TLC plate as you think it would look.
- 5) What property of the compounds causes them to be colored? How is this related to what causes compounds to absorb UV light?
- 6) It would be impossible to calculate a % yield for this experiment. Why?
- 7) If the materials we wanted had been UV active instead of colored, how do you think could we have known when the first one was finished so that we could switch solvents? (Hint – you can't see UV light unless you use a fluorescent indicator, like on the TLC plates. Also, UV light won't go through glass.)