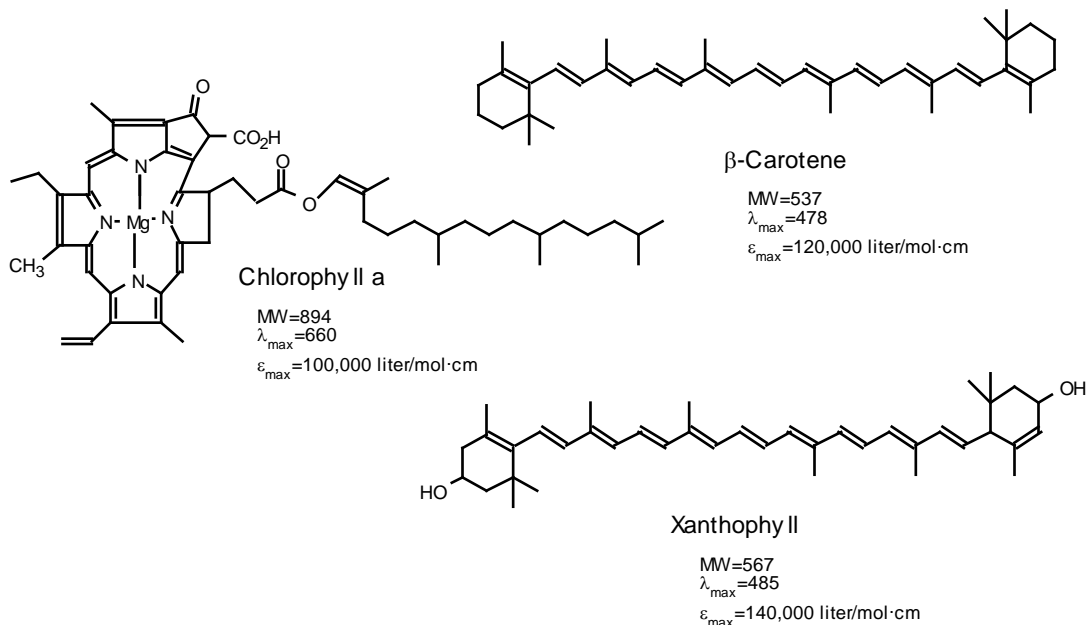


Spinach Separation

Introduction:

In plants, photosynthesis take place in organelles known as chloroplasts. Chloroplasts contain a number a light absorbing components (chromophores), categorized as carotenoids, chlorophylls and xanthophylls. To isolate the chromophores from spinach, you will dehydrate a spinach sample, extract the colored components with an organic solvent and then use thin layer and flash chromatography to separate the components in the following order: β -carotene, chlorophyll and xanthophyll.



Week 1: Isolation & Thin Layer Chromatography

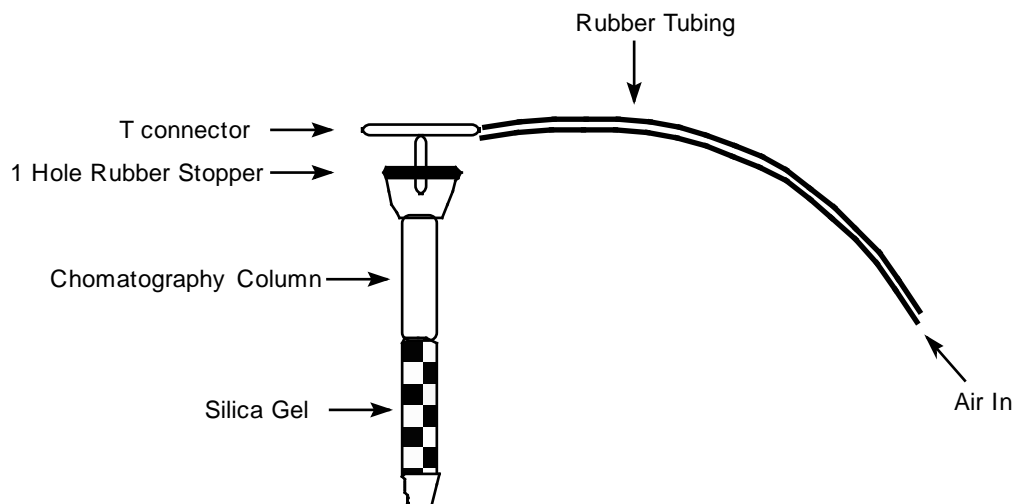
Obtain 2.5 grams of spinach leaves and tear them into small pieces. Crush them in a mortar and pestle along with 10 mL of ethanol. Vacuum filter the mixture and allow the spinach to dry for a few minutes in the Buchner funnel. Discard the filtrate and transfer the dehydrated spinach to a small beaker or flask. Add 25 mL of hexane and vigorously stir the mixture to extract the colored components. Decant the spinach extract into a small flask. Concentrate the solution on the steam bath to a volume of 1-2 mL.

Prepare four TLC plates, each containing one spot of concentrated spinach extract. Develop each plate with a different elution solvent: 100% hexane, 90% Hexane:10% Ethyl Acetate, 70% Hexane:30% Ethyl Acetate or 100% Ethyl Acetate. Determine which separation is the best and calculate R_f values for that separation.

Tightly seal the flask containing your spinach extract with a cork and save it in your drawer for next week.

Week 2: Flash Chromatography

As discussed in your text, flash chromatography is a modified form of column chromatography in which the time it takes the solvent to run through the column is sped up by using air pressure to force the solvent through the column. The diagram below depicts the flash chromatography apparatus that you will use. You will use a *plastic* column that is connected to a source of air pressure using a t-shaped connector. You will press on the open end of the t-connector with your thumb to control the amount of pressure.



Obtain a column from the front bench and clamp it in an upright position. Place a beaker under the column to collect the solvent that will drip out. Weigh out 5.5 grams of silica gel (**do this in the hood: silica gel is a fine powder and a lung irritant!**). Add about 20 mL of hexane to the silica gel to make a slurry. Pour the slurry onto the column. Use extra hexane to make sure that all the silica gel has been transferred to the column.

Using air pressure to force the hexane through, drain some solvent from the column until the solvent level is just above the top of the silica gel. Pipet your spinach solution on to the top of the column using a disposable pipet. Use air pressure to force the hexane through until again the solvent level is just above the top of the silica gel.

Obtain about 30 mL of 90% Hexane: 10% Ethyl Acetate. Fill the column with 90% Hexane: 10% Ethyl Acetate and force the solvent through using air pressure, making sure not to let the solvent level go below the silica gel. You will notice that the colored components will begin to separate on the column. Keep refilling and forcing the solvent through until the first colored band nears the bottom of the column. Place a large, clean test tube underneath the column and collect the first colored band. After you have collected the first colored component, switch your eluting solvent to 70% Hexane: 30% Ethyl Acetate and collect the second colored component in a test tube. Then switch to 100% Ethyl Acetate and collect the third colored band.