Separation of ~~the~~ Spinach Leaf Pigments by Column Chromatography

Purpose: Use Column Chromatography and TLC techniques to separate pigments in spinach leaves

Materials:
- 50 mL burette
- 3 disposable pipetes
- 5 test tubes
- Clamps
- Sep funnel
- Erlenmeyer flask

Chemicals:

• Acetone *▽○	3 mL
• Hexanes *▽○	3 mL
• Sodium Sulfate *	1 g
• Alumina δ	
• TLC eluent	

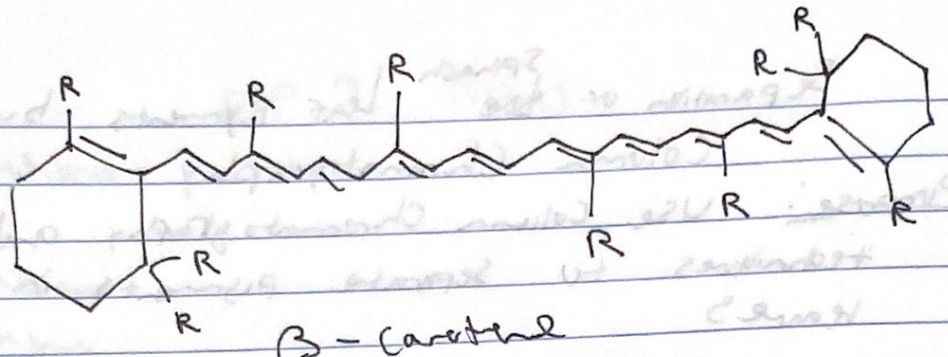
Hazards

* organic waste
▽ Flammable
δ Alumina – respiratory irritant
○ Skin/eye irritant

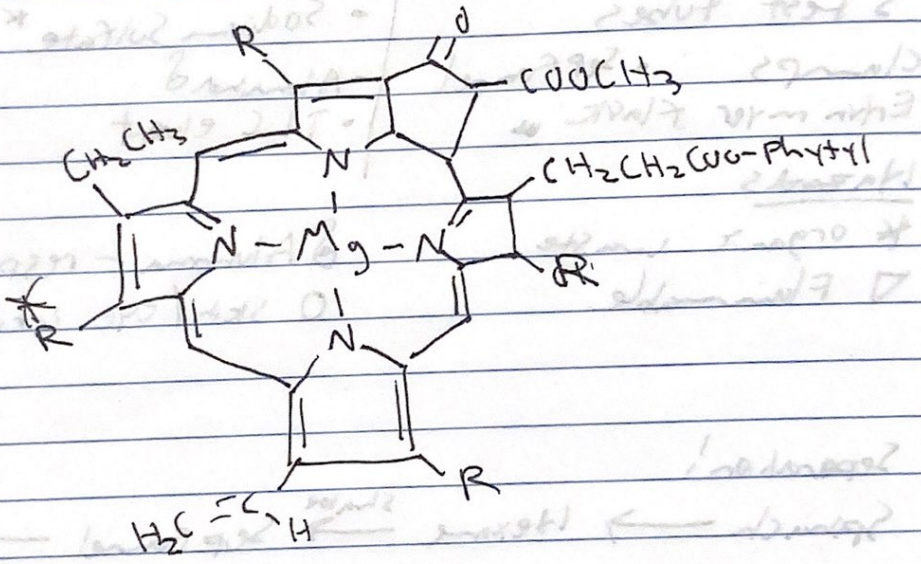
Separation:

Spinach → Hexane —shake→ Sep funnel → discard leaves

Remove ethanol → Remove water → TLC → Prep Column → add Hexane → Pack → add Spinach extract → Collect layers



β-Carotene



Chlorophyll a

Chlorophyll b
- Replace *R with

β-Carotene is non polar so it will elute first from the column and travel farthest on TLC because stationary phase is polar.

Procedure

1) Add 5 mL Spinach and 5 mL Hexane to test tube

2) Shake well

3) Remove upper hexane layer w/ Pipet - add to Sep funnel

4) Add another 5 mL Hexane to Spinach

5) Shake

6) add top layer to Sep funnel

7) Add equal V H_2O to Spinach extract and gently Shake

8) If emulsion: add 2 mL NaCl

9) Separate layers

10) Wash extract with H_2O again

observations

V_{Sp} =

V_H =

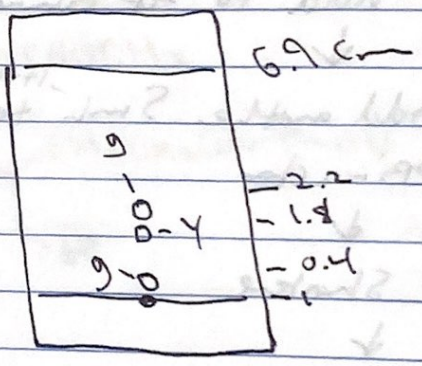
V_H =

Procedure

11) Add extract to erlyn flask
↓
12) Dry w/ Na_2SO_4
- vacuum filter
↓
13) Roto vap
- remove most of the solvent
↓
14) TLC
↓
15) ~~Add extract~~ Add extract to mobile phase
- beaker should have filter -
- dot ~ 1cm from bottom
↓
16) Pack column
↓
17) add cotton and pack to bottom - Sand
↓
18) pour small portions of Alumina in column and ~~pack~~ tap to pack
↓
19) 3/4 height

2.2 cm
1.8 cm
0.4 cm

Didn't separate properly
So had to re separate



Did not spot very well
So saw 3 spots

mason 208 983 5181

Procedure

20) add sand to column top

21) slowly add cyclohexane and continue to pack

22) Dissolve spinach extract in small amount of hexane

23) w/ith hexane at sand layer add 2 drops of extract

24) once extract is at sand level fill column with hexane

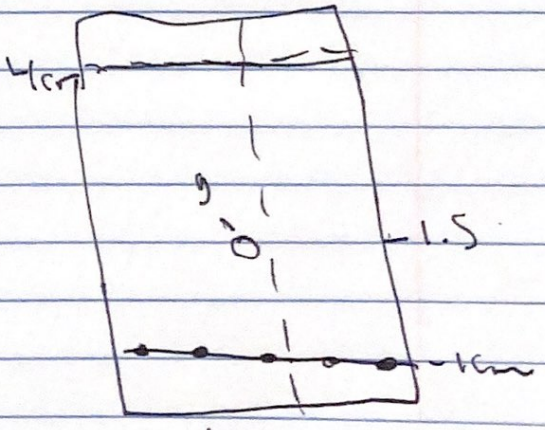
25) Add 15 mL TLC elvent to 5 mL cyclohexane

26) add new mix to column and force with air

27) TLC with different bands
- Compare to TLC of extract

Captured 4 fractions but only 1 dark enough to see on TLC
Yellow - green - green → Yellow

V_{TLC} =
V_{CH} =



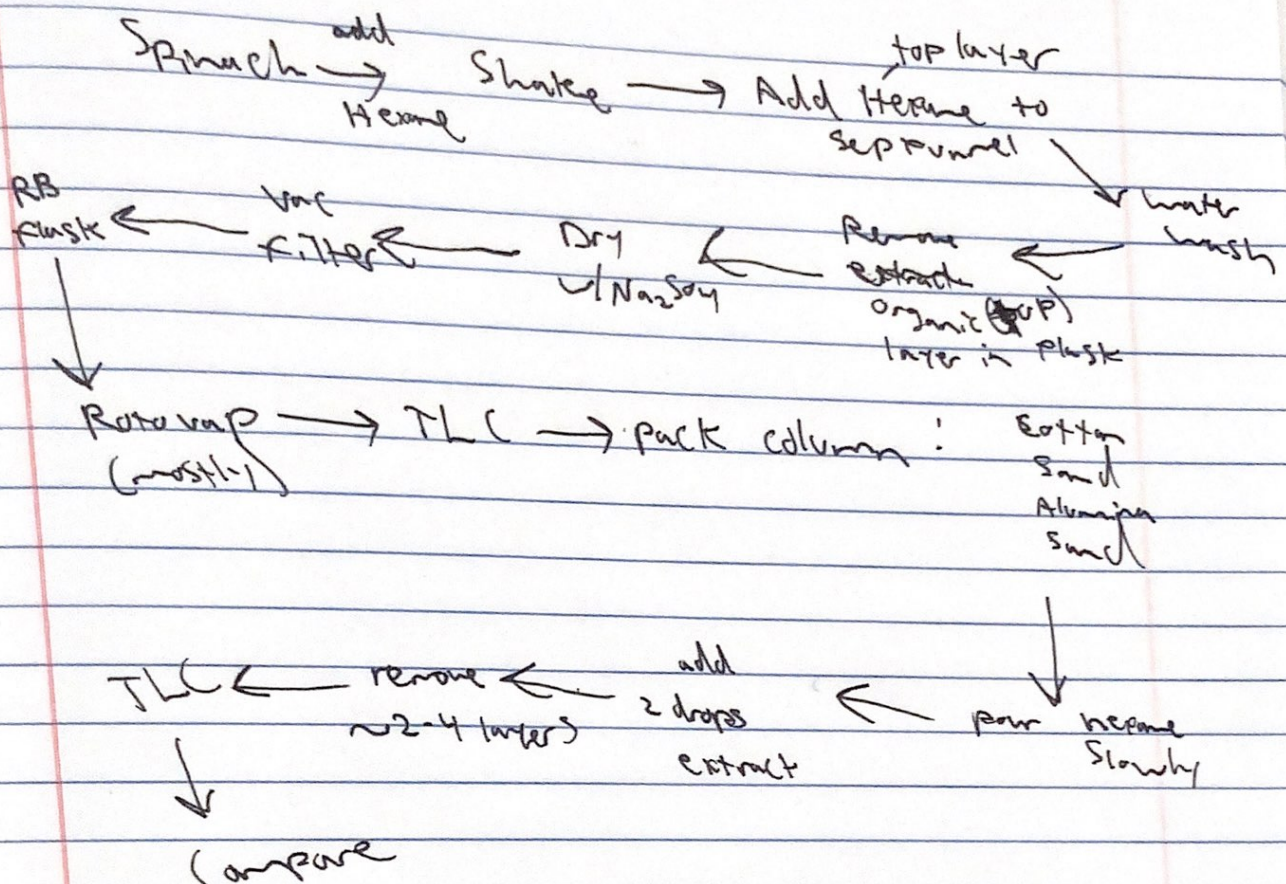
only dark green spot showed

Materials (other)

- Erlenmeyer flask
- RB flask
- Pasteur Pipette
- Clamps
- Sand
- Cotton
- TLC plates
- grad cylinder

Separation:

Spinach → remove cyclohexane layer →


Spinach
add Hexane
Shake
Add Hexane to sep funnel
top layer
water wash
Remove extract organic (top) layer in flask
Dry w/ Na_2SO_4
Vac filter
RB flask
Rotovap (most)
TLC
pack column:
Cotton
Sand
Alumina
Sand
pour hexane slowly
add 2 drops extract
remove ~2-4 layers
TLC
compare

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Experiment 2 “Separation of Spinach Leaf Pigments by Column Chromatography”

The order of color fractions that were captured was yellow, dark green, yellow, light green. Only the dark green fraction was dark enough to appear on the TLC. It traveled 1.5cm with an Rf value of 0.375. This Rf value was comparable to that of the dark green spot seen on the original TLC which traveled 2.2cm but had an Rf value of 0.319. There were two other spots observed on the original TLC. A yellow spot traveled 1.8cm with an Rf value of 0.261 and a light green spot that traveled 0.4cm with an Rf value of 0.058.

Even though the dark green spots on both the original and fraction TLC, the Rf values are still quite different. The higher Rf for the fraction TLC suggests that the compound traveled farther than the original with respect to the total distance traveled by the mobile phase. Since the stationary phase is polar, this indicates that the fraction may have been mixed with a less polar compound.

On the original TLC 3 spots were observed. From smallest to largest Rf, they appeared to be light green, yellow, then dark green. I predicted that beta-carotene would travel the farthest but it should have a yellow/orange color but that did not appear on the TLC. One reason this spot was not observed was because I failed to spot the plate dark enough so only the darkest or perhaps the most concentrated pigments were observed. Properly done however, the TLC could have shown about five pigments. It appears that the yellow spot may have been a xanthophyll because that compound is yellowish and polar due to its OH groups. The yellow spot had a low Rf. The light green molecule may have been chlorophyll b because it has an aldehyde so it is more polar than chlorophyll a, which has an alkane instead of an aldehyde, and it had the lowest Rf. The dark green spot with the highest Rf value is probably from chlorophyll a because it is less polar than chlorophyll b and the procedure suggested it would be more intense (Zharov 3).

At the start of the column separation, there appeared to be two dark bands, one yellow and one green. As the yellow approached the bottom of the column it disappeared and even though the fraction was collected, it was not concentrated enough to appear on the TLC. After collecting the darkest green band, two more bands were observed but as they moved closer to the bottom of the column they disappeared. It is possible that this is due to the use of the house air to try and speed up the process. Using the air increased the flow rate through the column but it is likely that it did not give the fractions a sufficient amount of time to separate and elute separately. Since the only spot that appeared on the TLC had a higher Rf value than expected, it is likely that part of that fraction was the first yellow spot that should have eluted first. For future reference I would recommend patiently waiting for the fractions to elute rather than using force.

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References

Zharov, Ilya, *Column Chromatography: Separation of Leaf Pigments*. September 2021.