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.

References

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