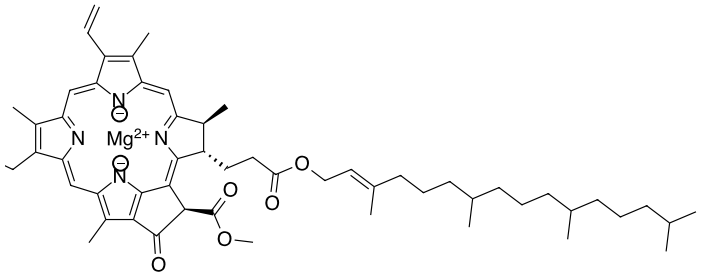
CHM 3342

Analysis of Plant Pigments by Chromatography

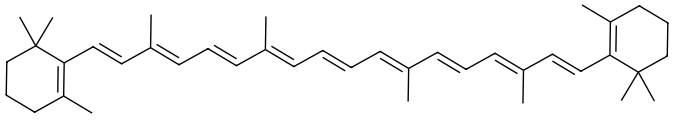
TUD Department of Chemistry

[1]Ultraviolet-visible spectroscopy is a useful tool for studying the electronic structure of unsaturated molecules and their conjugation. The electronic absorption spectra can generally reveal the degree of delocalization of the conjugated π system. The electronic transitions between bonding and anti-bonding orbitals in organic molecules are large and normally require higher energy. As the number of π molecular orbitals increases in conjugated systems, the energy gaps between the filled and unfilled orbitals decrease. Lower energy is needed to promote electrons into an excited state, resulting in molecules that can absorb in the visible region.

The pigments found in spinach are good examples of highly conjugated molecules that can absorb in the visible region. Spinach contains green pigments known as chlorophylls and yellow pigments known as carotenoids, both of which are involved in the photosynthesis process. There are several types of chlorophyll with chlorophyll a and b being the most common. The difference between the two chlorophylls is that a methyl side-chain in chlorophyll a is substituted with a -CHO group in chlorophyll b. Carotenoids are a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls). The yellow color due to the carotenoids is obscured by the chlorophyll pigments. Structures of chlorophyll and β-carotene are shown below.



Chlorophyll



β-carotene

[1] Adapted from: Organic Chemistry with Vernier, Vernier Software and Technology, 2012.

Part I Extraction

1. Weigh 1.0 g of plant material. (If available, try kale, turnip greens, etc. in addition to the spinach)
2. Place the plant material in a mortar. Add 3 mL of acetone and 3 mL of hexane and grind the mixture for 3—5 minutes.
3. Create a filtering pipet with a cotton plug and a disposable Pasteur pipet. Filter the green solution into a centrifuge tube. Try to avoid transferring the solids.
4. Wash the solution in the centrifuge tube:

   Add 2 mL of saturated aqueous sodium chloride solution to the centrifuge tube.

   Place a stopper in the top and shake the centrifuge tube.

   Immediately vent the centrifuge tube by removing the stopper.

   Place the stopper back in the centrifuge tube, shake, and vent.

6. Place the centrifuge tube in the centrifuge tube rack and allow the layers to separate.
7. Remove the bottom aqueous layer and discard. Add a spatula of anhydrous sodium sulfate to dry the organic phase.
8. Transfer the liquid to a suction flask.
9. Evaporate the solvent to approximately 0.2—0.3 mL by evacuating the flask using the water aspirator.

Part II Thin-Layer Chromatography

Spot a TLC plate with a 5μL of the extract and develop in 50:50 hexane:acetone.. Note the colors and Rf values for spots seen under room lighting and under UV illumination.

Part III Column Chromatography

Carefully read pages 172-177 (Column Chromatography) in Making the Connections and we will discuss the details of separating the spinach pigments before we do the separation.

Part IV Visible Spectroscopy

We will collect two fractions from the column chromatography separation and obtain the visible spectrum of each of those fractions.