**Introduction:**

The orange and red pigments in fruits and vegetables such as tomatoes and carrots are hydrocarbons known as carotenoids. The two commonest carotenoids, which incidentally are precursors to Vitamin A, are lycopene and β-carotene, the structures of which are shown below.

![Chemical structures of carotenoids](image)

The carotenoid pigments also occur in the leaves of plants but they are not obvious because of the presence of other pigments.

The green pigments in leaves are principally chlorophyll-a and chlorophyll-b, which have the structures shown below.

![Chemical structures of chlorophylls](image)

Chlorophyll-b differs from chlorophyll-a in that it has a formyl (-CHO) group in place of a methyl group (CH₃), at the position shown in the above structure.
The leaves of plants contain not only the chlorophylls a and b, but also other pigments whose colors are masked by the chlorophylls in live, healthy leaves. The other pigments become visible in the fall when the leaf dies and the chlorophylls rapidly decompose. Among the other pigments are the carotenoids, the two commonest being lycopene and β-carotene, both of which are precursors to Vitamin A.

In this experiment, you will isolate these plant pigments from two foods rich in them, tomato paste and carrots (in the form of baby food). You will use a more modern variant of column chromatography called flash chromatography to separate the lycopene and β-carotene rapidly. Flash chromatography uses a finer adsorbant stationary phase that gives better separations, but the finer packing makes solvent flow very slow. To speed up the flow, solvent is forced through the column by applying nitrogen pressure to the top of the column.

Pre-laboratory Exercise:

Prepare a schematic outline of the experimental procedure for the isolation of the plant pigments.

Cautions:

Do not breathe any of the dust from the silica. Hexanes are flammable.

Isolation:

PART I: Dehydration and Extraction:

Three (3.0) g of tomato paste, 9.0 g of carrot baby food and 30 mL of absolute (100%) ethanol are added to a small beaker. The suspension is then stirred with a glass rod for at least five minutes. The ethanol is removed by vacuum filtration using a Hirsch funnel with a filter paper disc and discarded. The residue is pressed dry and, using a powder funnel, transferred to a 25 mL round bottom flask (blue kit) along with 15 mL of dichloromethane. The flask is fitted with a condenser and refluxed for 3-4 minutes (cooling water is usually not necessary for this short a period of time). The solvent is separated from the solid residue by vacuum filtration, washing the solid with a few mL of CH₂O₂ if necessary, and then the solid residue is returned to the flask. Repeat this extraction procedure two more times with 15 mL CH₂O₂ each time. Using a separatory funnel, wash the combined CH₂O₂ extracts with 20 mL sat'd NaCl(aq.), and dry the CH₂O₂ solution over anhydrous sodium sulfate. Filter into a 50-mL round-bottom flask and evaporate the CH₂O₂ on a rotary evaporator (found at Desk 3 in Lab 215). Add 2 mL of hexanes to the thin orange-brown oil coating the flask.

PART II: Separation by TLC:

NOTE: Proper TLC technique and illustrations may be found in Ch. 7 of the Lab Guide for Chemistry 36.

If you do not have enough time to finish the lab, you need to store your lycopene and carotene until the next period. Flush the flask with nitrogen, quickly stopper with a greased ground-glass stopper, wrap with foil to keep out light, label the flask with your name and place it in a beaker. Store it in the freezer at the south end of room 205 or the north end of room 215.

Using a thin capillary, spot three silica TLC plates (obtained from the stockroom). Spot each plate twice. For spot 1, use three touches to the plate and for spot 2, use six touches to the plate, keeping the spot to 1 mm in diameter. Develop one TLC plate in 100% hexanes, one in 10% CH₂Cl₂/90% hexanes (V/V), and one in 100% dichloromethane. There should be a visible separation of the colored spots. Circle the spots in pencil and tape the plates in your lab notebook.

PART III: Flash Chromatography:

Refer to the Lab Guide Ch. 8, for additional information on column chromatography. In the hood, assemble and clamp a 1 x 15 cm chromatography column (from the microscale kit) on a ring stand. Make sure that the pieces fit together snugly and that the column is as straight vertically as possible. Use a fresh bottom plug from your bookstore Organic Lab Equipment Kit as this will fit snugly. Insert the plastic funnel from your microscale
kit and fill the column with 100% hexanes. Now add dry, fine mesh silica gel powder to a height of 10-13 cm high in the column.

Open the stopcock and push the hexanes through the column by applying nitrogen pressure to the top of the column. This is best done by simply pushing the end of the tygon tube connected to your nitrogen valve into the plastic funnel until pressure builds up. **Don't press the nitrogen tubing too tightly into the funnel, however, or the whole column might be blown apart by the pressure.** Release the pressure before the solvent level reaches the top of the packing. **Do not get gas into the column packing as this will form channels and the chromatographic separation will be poor.** Add more hexanes as necessary and force it through the column until there are no air bubbles present in the column. Then, adjust the hexanes level to just above the silica and add about 1 cm of sand.

Now add the lycopene/β-carotene solution into the sand with a pipet. Force the sample solution into the column with air pressure until the hexanes level is again at the top of the sand. Add 2 mL of hexanes, and force this down to the top of the sand. Make up 50 mL of 10% dichloromethane in hexanes by mixing 5 mL CH₂Cl₂ and 45 mL hexanes in a 125-mL Erlenmeyer flask. Fill the column with this.

Change collection flasks and elute the yellow β-carotene layer by forcing the 10% dichloromethane in hexanes solvent through the column. It should all come off in about 7 mL. Switch collection flasks and force 6 - 7 mL of 100% dichloromethane through the column to elute the orange/red lycopene band. Switch flasks again when the orange band reaches the middle to lower half of the column. This minimizes contamination of the lycopene with b-carotene.

**Electronic Absorption or UV/Vis Spectroscopy**

Irradiation of a compound with electromagnetic radiation of suitable wavelengths can cause transitions of the bonding electrons from their normal (i.e. ground) states to higher excited electronic states. For example, irradiation of lycopene with visible light in the region 400-500 nm causes excitation of the π electrons to π* antibonding levels, and this is evident as an absorption of the exciting radiation. There are actually several absorption peaks in the electronic spectrum of lycopene, but the peak of maximum intensity corresponds to a wavelength near 473 nm. β-carotene exhibits similarly on irradiation, but the peak of maximum absorption is found at shorter wavelengths, around 448 nm, the exact position of the maximum depending somewhat on the solvent.

β-carotene: λ_max at 426, 448, 474 nm  
lycopene: λ_max at 444, 473, 502 nm

The carotenoid pigments separated from spinach, tomato paste or carrots may be identified from their absorption spectra. Record the spectra of your column chromatography fractions. Make sure a blank is run first with the appropriate solvent, 10% CH₂Cl₂ in hexanes or 100% CH₂Cl₂. Since the intensity of electronic transitions in the carotenoid hydrocarbons is very large, only very small quantities are required for an absorption spectrum. If, therefore, your solutions absorb so strongly that they have absorbances greater than 1.5 AU, dilute your samples until the absorption maxima are below this. Then record the spectrum of your sample from 300-800 nm. (This is actually just visible light spectroscopy.) Determine the λ_max of any peaks. Obtain a hard copy of your spectrum to be submitted with your final report. Do the same for both the β-carotene and lycopene column chromatography fractions. Care should be taken not to drop the cuvettes ($160 a piece!)

**Cleaning Up:**

Ethanol solutions can be flushed down the drain. Be sure to dispose of any solutions containing CH₂Cl₂ in the halogenated organic container in your hood.

**Final Report:**

Fasten your TLC plates in your lab report pages and properly annotate them, including R_Fs. Discuss how your UV/Vis spectra compare with the literature. Give a brief description of your observations and give complete sentence answers to the questions below.

1. How many TLC spots are visible, and what are their Rf values? Are they all colored?
2. Is the principal carotenoid component in tomatoes lycopene or β-carotene? Explain your answer.