TLC Determination of Carotenoids

In this experiment we will look at the antioxidant, beta carotene

Isoprenoid

Steroids       Terpenes

Carotenoids

Carotenes   Xanthophylls

- Carotenoids have repeating isoprene units.
  
  C
  |   
  C-C=C-C
  Isoprene Unit

- Beta-carotene is conjugated hydrocarbon of eight repeating isoprene units.

Functions of beta-carotene in the body.

1. Precursor to Vitamin A. Two vitamin A molecules are generated from 1 molecule of beta-carotene.
Retinol $\leftrightarrow$ Retinyl ester

beta-carotene $\rightarrow$ retinaldehyde (retinal)

retinoic acid

Beta-Carotene

Retinal

Retinal

C. Ophardt, c. 2003
A. Retinaldehyde (Retinal)
- Retinal is needed for proper vision.
- It functions as an active component of the visual cycle. Retinal is bound to the protein opsin forming the complex rhodopsin. When stimulated by visible light, the rhodopsin complex undergoes isomerization to the 11-cis-retinal from the all-trans retinal. This is reversed in “dark” reactions to return to the all-trans configuration.

![11-cis-retinal](image)

B. Retinoic acid
- Retinoic acid is necessary for maintenance of healthy epithelial tissue. In the epithelial cells, the hormone-like action of vitamin A is the most important controller of cell size, shape, number and differentiation.

C. Retinol
- Retinol functions in the synthesis of certain glycoprotein and mucopolysaccharides necessary for mucous production and normal growth regulation.
- Cellular binding proteins complex with vitamin A and bind to DNA to trigger the transcription of key growth factors and other important control compounds.
Retinyl ester formation and storage in stellate cells of the liver both withdraws excess retinol from circulation and provides a reserve of vitamin A for future use during periods in which dietary vitamin A might be reduced.

Mechanism for activation of oxygen

- Diatomic oxygen is biradical form of oxygen $\cdot O-O\cdot$. It is also called triplet oxygen and is in a ground state because the unpaired electrons have parallel spins.
- If the triplet oxygen absorbs enough energy to reverse the spin of one electron form the singlet state, $O-O$: where the two electrons will have opposite spins.
- This activation overcomes the spin restriction and singlet oxygen can consequently participate in reactions involving transfer of two electrons.
- Since paired electrons are common in organic molecules, singlet electron is much more reactive than the triplet oxygen.
2. Antioxidant function of beta-carotene
   a. Quenches singlet oxygen and dissipating the energy as heat.
   b. Reacts with lipid peroxidation products to terminate chain reactions.
   c. Reacts with triplet oxygen to prevent the formation of singlet oxygen.
   d. The quenching process does not destroy beta-carotene. The mechanism is physical rather than chemical.
   • Excess energy of the singlet oxygen, $^{1}\text{O}_2^*$, is transferred to the carotenoid’s electron-rich structure.
• The carotenoid is excited by this added energy into the “triplet” excited state, $^3\text{Car}^*$, and then relaxes into its ground state, $^1\text{Car}$, losing the extra energy as heat.

$$^1\text{O}_2^* + ^1\text{Car} \rightarrow ^3\text{O}_2 + ^3\text{Car}^*$$

$$^3\text{Car}^* \rightarrow ^1\text{Car} + \text{heat}$$

• A single molecule of beta-carotene can arrest up to 1000 molecules of singlet oxygen.

Procedure:
Saturate TLC Developing Chamber
Extract beta-carotene from samples
NaCl
Carrots
Sweet Potato
Spinach
Hexane
Separate layers
Hexane layer  aqueous layer
Drops left for TLC  Discard
Thin Layer Chromatography

- The stationary phase is silica gel spread over either plastic or glass.
- The mobile phase is solvent which is mixture of 60:40 hexane:acetone.
- Samples are spotted on the line of origin. Standards and samples are spotted on the plate.
- The spotted plate is placed in a saturated developing chamber and allowed to run until the solvent line reaches 2 cm from the top of the plate.
- The solvent front is marked.
- The samples are identified by comparing the distance traveled by the standard to the distance traveled by the samples.
- TLC is a qualitative determination. It does not quantify the concentration, it only identifies what is present in a sample.
- Rf values is a mathematical representation of the ration of the distance the spot traveled over the distance traveled by the solvent.

<table>
<thead>
<tr>
<th>Table 32.2</th>
<th>Plate #1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot #</td>
<td>Substance</td>
</tr>
<tr>
<td>1</td>
<td>carrots</td>
</tr>
<tr>
<td>2</td>
<td>sweet potatoes</td>
</tr>
<tr>
<td>3</td>
<td>spinach</td>
</tr>
<tr>
<td>4</td>
<td>β-carotene (tt#1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 32.2</th>
<th>Plate #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot #</td>
<td>Substance</td>
</tr>
<tr>
<td>1</td>
<td>β-carotene (tt#1)</td>
</tr>
<tr>
<td>2</td>
<td>retinol (tt#2)</td>
</tr>
<tr>
<td>3</td>
<td>retinoic acid (tt#3)</td>
</tr>
<tr>
<td>4</td>
<td>β-carotene (exposed)</td>
</tr>
</tbody>
</table>
• The Rf values of the standards are compared to the Rf value of the sample.
• The Rf value for each spot is calculated.

Spotting
1. Use the stretched capillary tubes.
2. The smaller the spots, the better the resolution.
3. Concentrating the spots is done by spotting over the same spot, making sure that the spots are dry before spotting over it.

Running of sample
1. Saturate the developing chamber by adding the solvent and covering the chamber for several minutes. The solvent does not dry as it moves up the plate when the chamber is saturated, decreasing the running time.
2. Make sure that the line of origin is above the solvent line.
3. The spots will move up as the solvent rises up the plate. The distance traveled by the spot is dependent on it’s attraction to the solvent and silica gel.
4. Mark your solvent line.
5. Use the uv light to visualize spot.
6. Circle the spots and determine the Rf value of each spot.