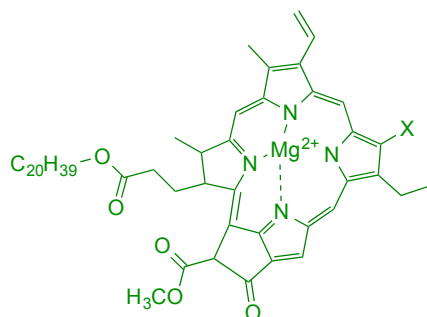
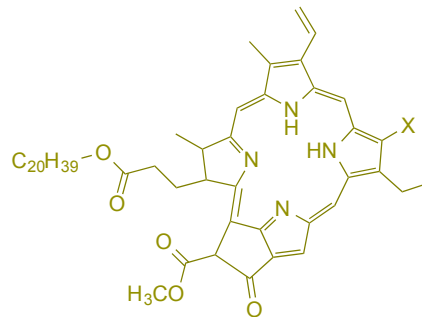


**CHEM 8L, Experiment 3 – Extraction & Thin-Layer Chromatography (TLC) Analysis of Spinach Pigments**

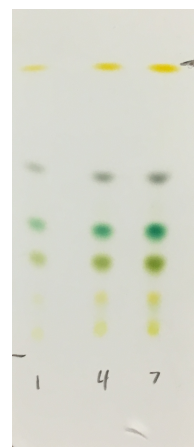
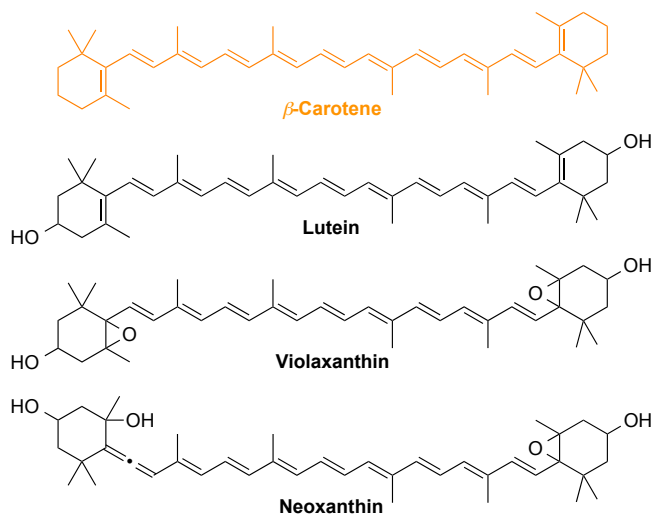
1. **Solid to liquid extraction** of pigments from spinach
2. **Liquid – liquid extraction** of pigments from unwanted water- soluble components
3. **TLC analysis / separation** of individual pigments

**Spinach Pigments – what are they?!**

**Chlorophyll a**, X = CH<sub>3</sub>  
**Chlorophyll b**, X = CHO

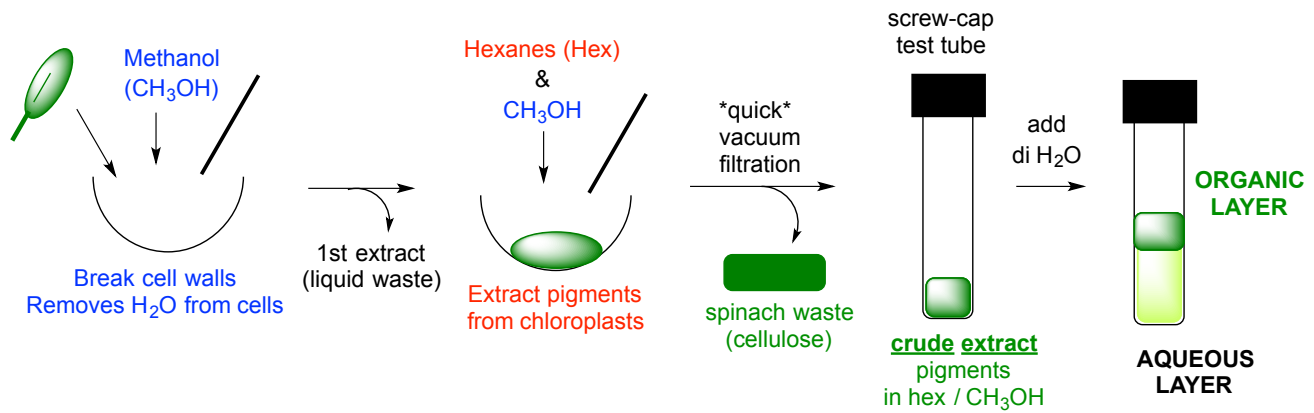


**Pheophytin a**, X = CH<sub>3</sub>  
**Pheophytin b**, X = CHO

**Relative Polarity of Functional Groups**

Functional Group	Structure		Example of Organic Solvent	
<b>Alkanes</b>	R-H	<i>LEAST POLAR</i>	Hexanes	
<b>Alkenes</b>	R-CH=CH-R			
<b>Aromatic Hydrocarbons</b>	Ar-H		Toluene	
<b>Ethers</b>	R-O-R		Diethyl Ether	
<b>Esters</b>	R-COOR		Ethyl Acetate	
<b>Ketones</b>	R-CO-R		Acetone	
<b>Aldehydes</b>	R-CO-H			
<b>Amides</b>	R-CO-NH <sub>2</sub>			
<b>Amines</b>	R-NH <sub>2</sub>			
<b>Alcohols</b>	R-OH		Methanol	
<b>Carboxylic Acids</b>	R-COOH			
<b>Amino Acids</b>	H <sub>3</sub> N <sup>+</sup> -CHR-COO <sup>-</sup>		<i>MOST POLAR</i>	

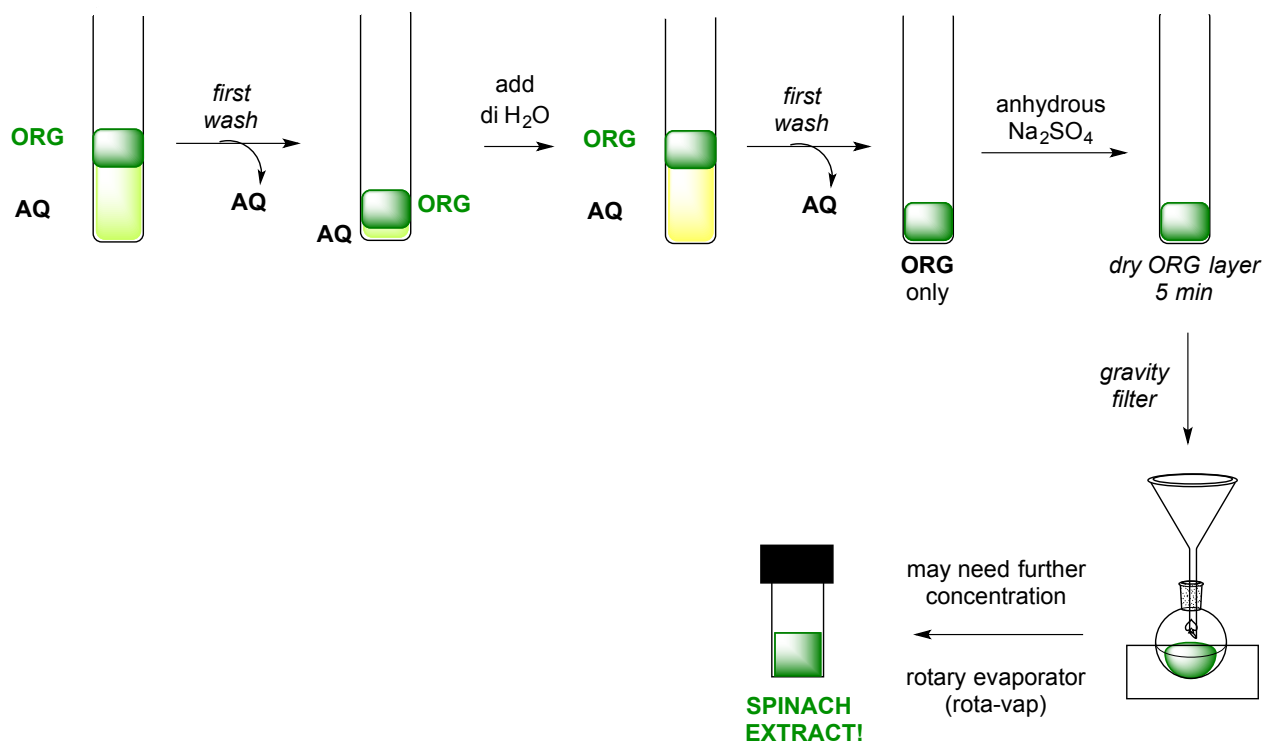
## 1. Solid to liquid extraction



## 2. Liquid – Liquid Extraction, Based on Partition Coefficients ( $K_{H/W}$ )

- Ratio of solubilities of pigments in organic solvent (ex. Hexane) vs. water

$$K_{H/W} = (\text{solubility of pigments in hexane}) / (\text{solubility in water}) \gg 1$$

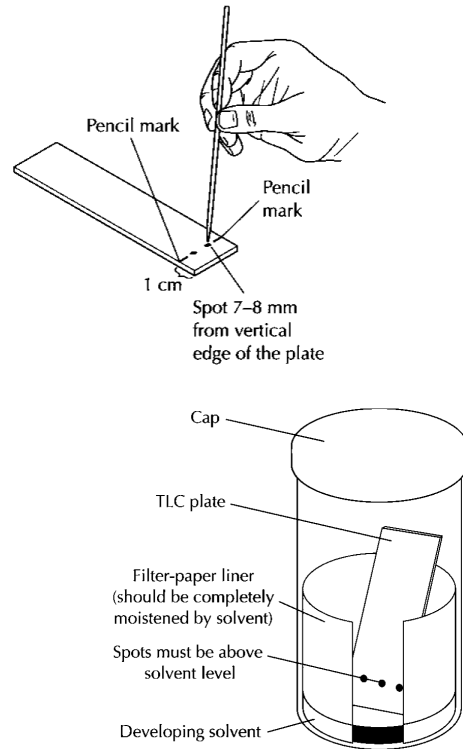
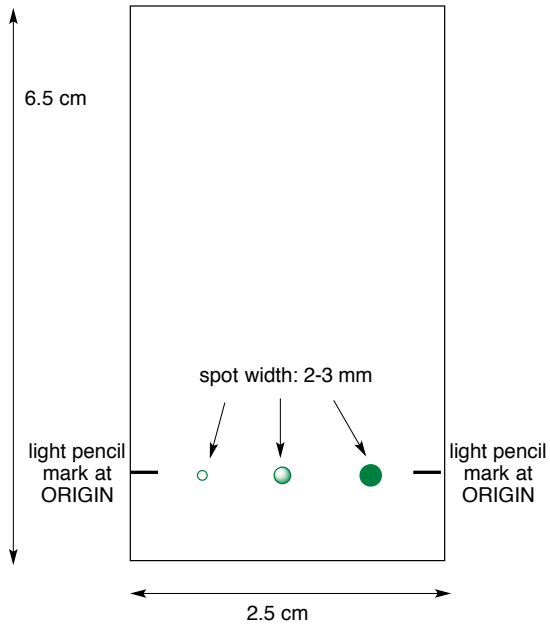


### 3. Thin-Layer Chromatography (TLC) Analysis

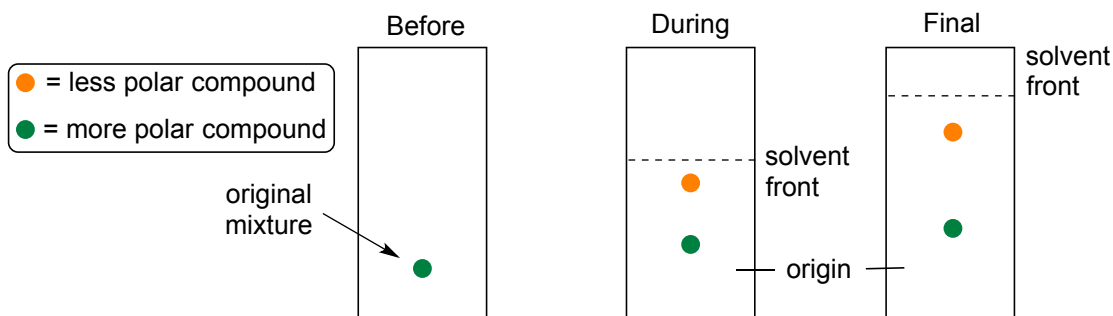
Goal: separate as many pigments as possible

#### Mini-experiment (a) optimal spot application

##### Spotting the Plate



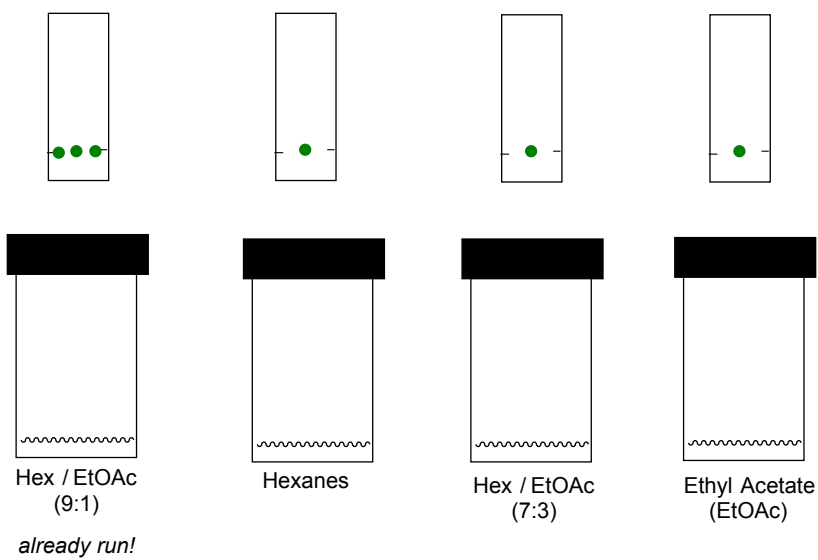
##### Running the plate



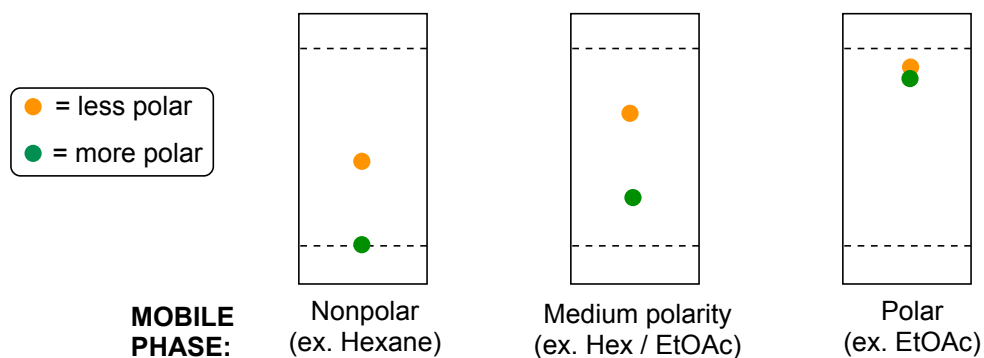
$$\text{Retention Factor (R}_f\text{)} = \frac{\text{distance from origin to spot (cm)}}{\text{distance from origin to solvent front (cm)}}$$

## 3. TLC (cont'd)

## (b) Optimal mobile phase for separation on different plates



## Optimal Solvent for Separation



## Chromatography Overview

	Classification	Nature of Stationary Phase	Nature of Mobile Phase	Property for Separation
GC	Partition			
TLC	Adsorption			