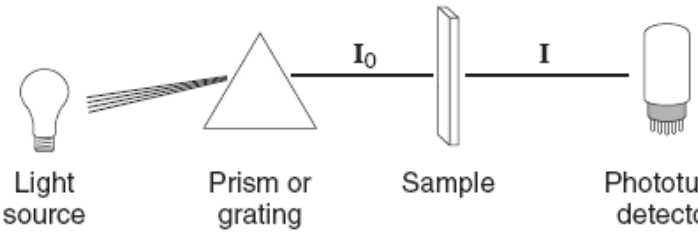
#22 Visible Spectrum of Chlorophyll from Spinach

Purpose: Chlorophyll is extracted from spinach. From a spectrum of the solution produced, the ratio of chlorophyll a and b present is estimated.

Introduction: A spectrum is a recording of the wavelengths absorbed by a sample. Colored compounds, such as the green chlorophyll in this experiment absorb in the visible.



The transmittance, %T, is the ratio of the intensity of the incident light ($\mathbf{I}_0$) and the intensity of the emerging light (**I**), or %T = **I** / $\mathbf{I}_0$. Absorbance, A, is the logarithm of 100 divided by %T :

$$A = \log \frac{100}{\%T}$$

If %T = 50, then, A = log 100/50 = log 2 = 0.30

$$0.30 = \log \frac{100}{50}$$

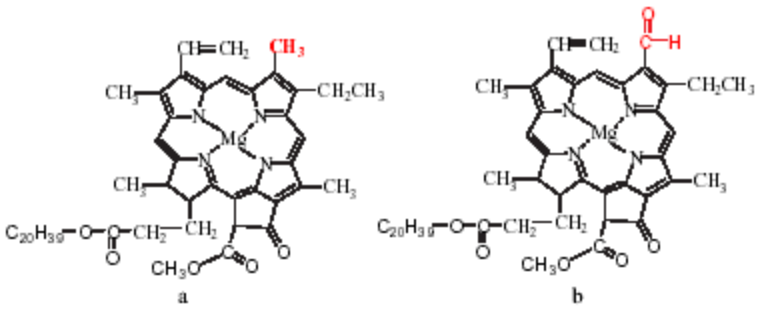
Find 50% T on the meter scale and notice that it corresponds to 0.30 on the Absorbance scale. The % T scale is linear and increases from left to right. The A scale is logarithmic and increases from right to left. It is more accurate to read % transmittance rather than absorbance, so record %T values and convert to A.

Extraction

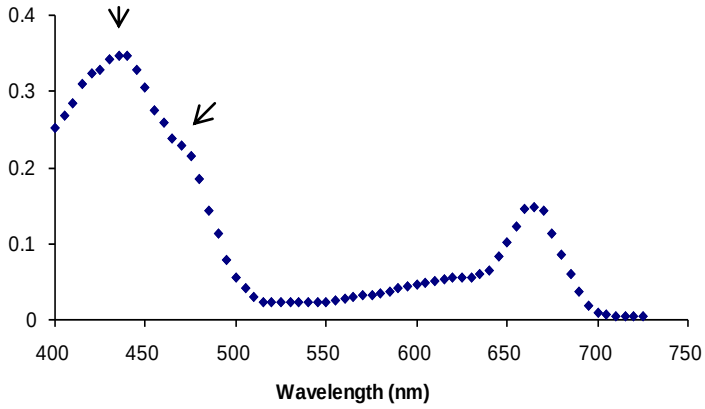
In solid extraction, a liquid is used to remove one or more substances from a mixture of solids, often those that occur in nature. In this experiment the solid mixture is spinach, the liquid solvent is ethanol, CH_3CH_2OH, and the substance to be isolated is chlorophyll.

Chlorophylls

Plants synthesize carbohydrates (sugars and starches) from CO_2 and H_2O through a complex set of reactions called photosynthesis. The first step is the absorption of sunlight by chlorophyll molecules in plants. There are two chlorophylls with closely related structures. The 'a' compound has a methyl group, CH_3, where the 'b' form has an oxygen containing group, CHO. These are in bold in the formulas below.



The visible absorption spectra of the two chlorophylls are different. The plot on the right is a spectrum of spinach extract. The peak at 430 nm is due to chlorophyll a. The shoulder at 460 nm is from chlorophyll b.
Note that both chlorophylls have a second weaker absorption between 600 and 680 nm.



Apparatus
The Spectronic-20 will be used to take a spectrum of the chlorophyll solution. To use the instrument refer to the operating instructions attached.

Procedure
Part A: Extracting Chlorophyll from Spinach
1. Shred a medium-sized leaf of spinach and mix with 10 mL of ethanol in a 50 mL beaker. After approximately 5 minutes, the solution should have a green tint.
Note: If the solution looks yellow, the extraction is not finished.

2. Filter the mixture of leaves and ethanol into another 50-mL beaker, using a funnel with a fluted filter. Discard the spinach.

Part B: Spectrum of Chlorophylls
1. Fill one cuvette with ethanol (the blank) and another with the solution of chlorophylls (the sample). First, check the absorbance at 440 nm. The reading there, where both chlorophylls absorb to some extent, should be somewhere between 0.3 and 0.8. If the solution is too concentrated, pour the sample into a graduated cylinder and add an equal amount of alcohol to dilute the solution by half. Check the absorbance at 440 again. Dilute further if needed. Record the dilution needed.

2. Set the wavelength dial to 400 nm and measure the % transmittance of your sample. Continue to measure the % T every 10 nm between 400 nm and 700 nm, remembering to adjust the instrument to full scale at each new wavelength. You can take readings every 5 nm between 420 and 480 to make it easier to observe the absorption due to both chlorophylls.

3. Convert % T to A. Plot absorbance vs. wavelength by drawing a smooth line through the points (do not "connect the dots").

4. Compare the spectrum with the one above to find evidence for presence of both chlorophyll a and b. Estimate the relative amounts of a and b in your sample.

Data and Results (Chlorophyll)

Name(s)_____

Part B: Spectrum of Chlorophylls

Dilution(if required): Vol. Leaf Extract _____mL Vol. Alcohol added: _____ mL

λ nm	%T	A	λ nm	%T	A
400			530		
410			540		
420			550		
425			560		
430			570		
435			580		
440		.	590		
445			600		
450			610		
455			620		
460			630		
465			640		
470			650		
475			660		
480			670		
490			680		
500			690		
510			700		
520					

Estimated amount of chlorophyll a/chlorophyll b _____

Questions:

1. Why is it necessary to shred the spinach before extraction?

2. How do plants benefit from the presence of both chlorophyll a and b?

_____

Instructor's Guide
(#22 Chlorophyll)

Data and Results (Chlorophyll)

Part B: Spectrum of Chlorophylls

Dilution(if required): Vol. Leaf Extract _____mL Vol. Alcohol added: _____ mL

λ nm	%T	A	λ nm	%T	A
400		*0.252*	530		*0. 0223*
410		*0.284*	540		*0. 0223*
420		*0.323*	550		*0. 0223*
425		*0.328*	560		*0.0269*
430		*0.342*	570		*0.0315*
435		*0.347*	580		*0.0339*
440		***0. 347***	590		*0.041*
445		*0.328*	600		*0.0458*
450		*0.305*	610		*0.0506*
455		*0.276*	620		*0.0555*
460		*0.260*	630		*0. 0555*
465		*0.237*	640		*0.0655*
470		*0.229*	650		*0.102*
475		*0.215*	660		*0.146*
480		*0.184*	670		*0.143*
490		*0.114*	680		*0.0861*
500		*0.0555*	690		*0.0362*
510		*0.0292*	700		*0.0088*
520		*0.0223*			

Questions:
1. Why is it necessary to shred the spinach before extraction? Ans: *More surface area comes into contact with the ethanol, speeding up the process of extraction.*
2. How do plants benefit from the presence of both chlorophyll a and b? Ans: *Since the two chlorophylls absorb strongly at different wavelengths, 430nm and 460nm, plants can capture more sunlight.*

Instructor's Guide (cont'd)
(Chlorophyll)

Equipment and Materials:

Items	Am't/gp	Comment
Spinach	1	Fresh spinach is best
Ethanol	10 mL	
Funnel	1	
Fluted filter paper	1	
50 mL beaker	2	Extraction and filtering steps
Spectronic-20	1	
Cuvettes/tubes	2	1 for sample and 1 for blank
10-mL graduate	1	For dilution
Safety glasses	1 per student	
Rubber gloves	1 box per class	

Ideas/Information

Spinach is used in this experiment because its pigments are primarily chlorophyll. Red, yellow and orange pigments called carotenoids are present in other leaves. These absorb strongly in the blue and violet, producing a broad band in that region, making it difficult to see the chlorophyll a and b peaks at 430nm and 460nm. The colorful carotenoids cannot be seen until autumn when the chlorophyll begins to break down.

In most plants the amount of chlorophyll a is about 3 times that of chlorophyll b.

Variations

1. Try other leaves to notice the additional absorbance due to carotenoids.
2. In the autumn, compare green leaves with the same kind of leaves that have turned color.
3. Try other very dark green leaves.