Photosynthesis - Exercise 6

Objectives

The purpose of this lab exercise will be to examine several factors involved in photosynthesis.

-The effect of the intensity of light (# of photons per time) on the rate of photosynthesis and know how to calculate the effect of light intensity on the rate of photosynthesis

-Isolate and identify the photosynthetic pigments found in the chloroplasts of spinach leaves - the separation of chloroplast pigments by paper chromatography. Know how three factors affect capillary action: Molecular weight, Solubility, and Affinity for the chromatography paper.

-Know what pigments travel the farthest up the paper chromatography: Carotenes, Xanthophyll, chlorophyll-a, and Chlorophyll-b

-Calculate the Rf of the Paper Chromatography.

-Know how to operate the spectrophotometer.

-Construct an absorption spectrum of chloroplast extract from spinach leaves - absorption spectrum of chloroplast extract and know what wavelengths are the least and most strongly absorbed.

- **The overall process of photosynthesis is summarized by:**

$$6\,CO_2 + 12\,H_2O + LIGHT \rightarrow C_6H_{12}O_6 + 6\,H_2O + 6O_2$$

- Notice photosynthesis *uses energy* (light energy) to make a more complex compound (glucose). Therefore, photosynthesis is an anabolic and endergonic reaction.

- **Cellular Respiration**: The process by which the energy stored in food is converted to ATP and heat. The overall process of cellular respiration is summarized by:

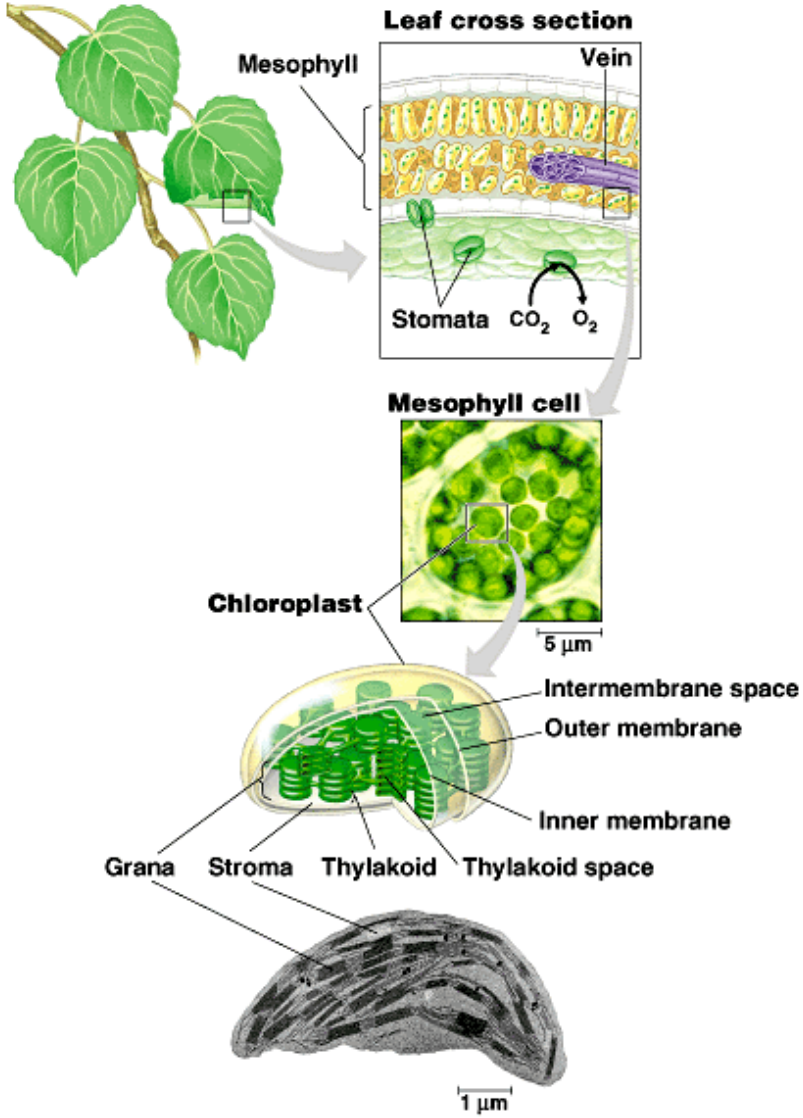
- Notice cellular respiration *creates energy* (ATP and heat) by breaking down a complex compound (glucose). Therefore, cellular respiration is a catabolic and exergonic reaction.

- Plants use special organelles called **chloroplasts** to absorb light. Chloroplasts contain special pigments called **photosynthetic pigments**. A single plant may have different photosynthetic pigments - each pigment absorbs a specific wavelength (color) of light.
- In plants, what absorbs energy? **Chlorophyll**
- **Chlorophyll is in chloroplast.**

Photosynthesis

$$6\,CO_2 + 12\,H_2O + LIGHT \rightarrow C_6H_{12}O_6 + 6\,H_2O + 6O_2$$

- Photosynthesis
  - Light energy from the sun is captured and converted into chemical energy (sugar)
  - Occurs in chloroplasts in mesophyll cells found in the tissue of plant leaves
  - **Chlorophyll is the green pigment of plants that absorbs light energy**
  - CO_2 enters and O_2 exits through small pores called stomata



- **Photosynthesis: Is the process by which light energy is converted into chemical energy. It occurs in all plants, algae, and some bacteria. Photosynthesis provides oxygen to the atmosphere.**

- In this process, plants use light energy to convert carbon dioxide and water into organic molecules and oxygen. In this process, plants use light energy to convert carbon dioxide and water into organic molecules and oxygen. **The reactions of photosynthesis occur in specialized structures called <u>chloroplasts</u>.**

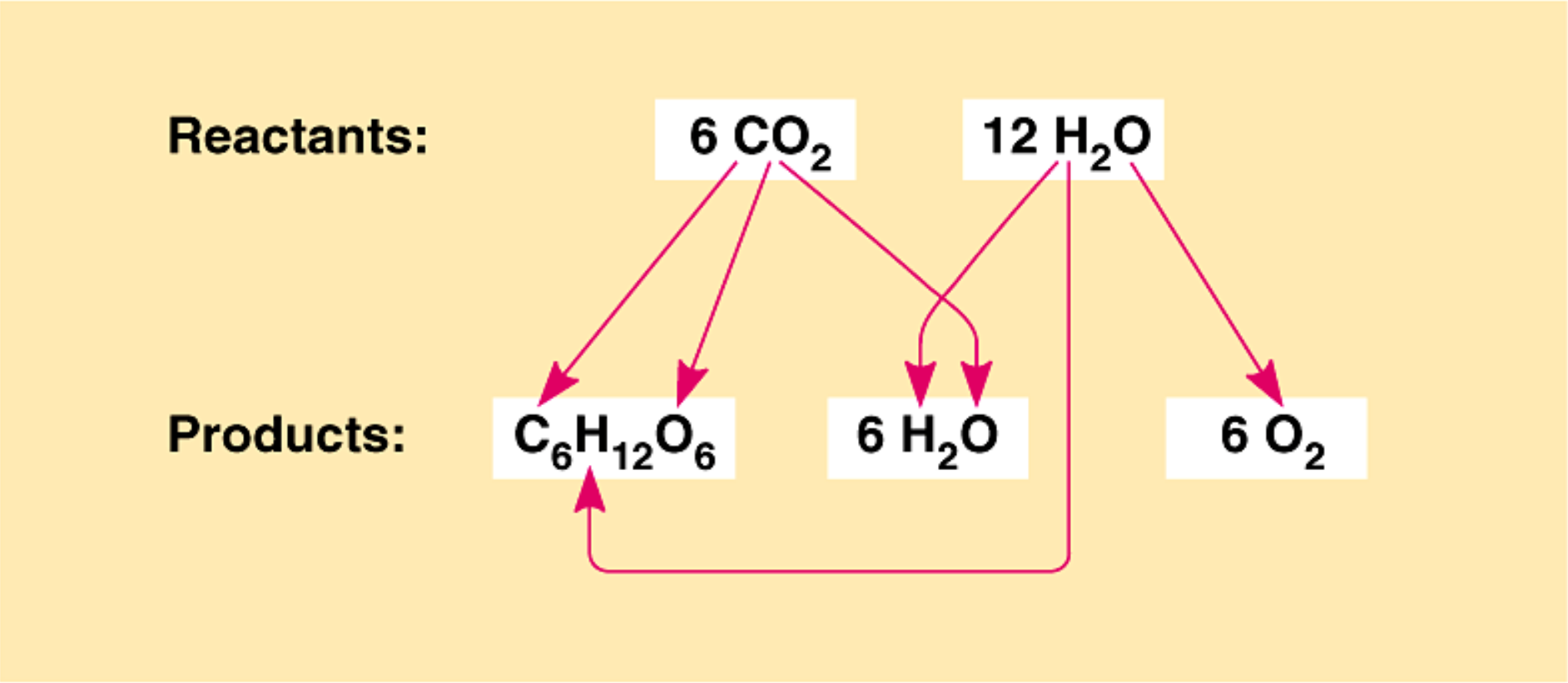
- Light is radiated in very packets called **photons**, which travel at specific **wavelengths**. We can distinguish among the different wavelengths as differences in color. A prism is often used to separate white light into its component colors.

Tracking atoms through photosynthesis

Photosynthesis splits water: using heavy oxygen (18)

$$CO_2 + 2\,H_2O \rightarrow CH_2O + H_2O + O_2$$

$$CO_2 + 2\,H_2O \rightarrow CH_2O + H_2O + O_2$$



Effect of light Intensity on the Rate of photosynthesis

One of the products of photosynthesis is molecular oxygen (O_2). Oxygen is only slightly soluble in water, so in a saturated solution, the excess oxygen will form gaseous bubbles. The rate of oxygen evolution by a plant immersed in water can be a reasonable measure of the rate of photosynthesis. We will set up a system that can measure the amount of oxygen gas released from plant leaves. We will use the system to determine the effect of carrying light intensity (using light bulbs of differing wattage) on the rate of photosynthesis.

Elodea plant

- -The tube was filled with 3% sodium bicarbonate solution. This solution provides the carbon dioxide source necessary for photosynthesis.

- Turn on the lamp. As photosynthesis proceeds, oxygen (O_2) will be produced. This increased volume of oxygen gas emitted by elodea leaves into the tube will force the liquid up and out of the test tube in the glass tubing. **The amount of movement of the liquid in the glass tubing should be proportional to the amount of oxygen gas released.** When repeating the experiment keep light at the same distance from beaker. If you change distance of light you added another variable to the experiment.

- The volume between the two marks equal 0.1 ml.

- **Calculate the rate of photosynthesis as the ml of oxygen produced per hour.**

- Repeat procedures 4-8 two more times, each time replacing the low wattage bulb with a medium wattage bulb, then, on the last trail, replacing the medium wattage bulb with a high wattage bulb.

- **Look at increasing light intensity on rate of photosynthesis.**
- Sodium Birom - source of carbon dioxide.

Effect of Light Intensity on the Rate of Photosynthesis

Formula

$$\frac{.1}{\text{Elapsed Time}} \times 60 = \text{ml } O_2/\text{hr}$$

Position the test tube in the holder or in a test tube rack in front of the low wattage lamp. Place a large beaker of water between the lamp and the test tube to act as a heat filter. Place the lamp and beaker as close to the test tube as possible.

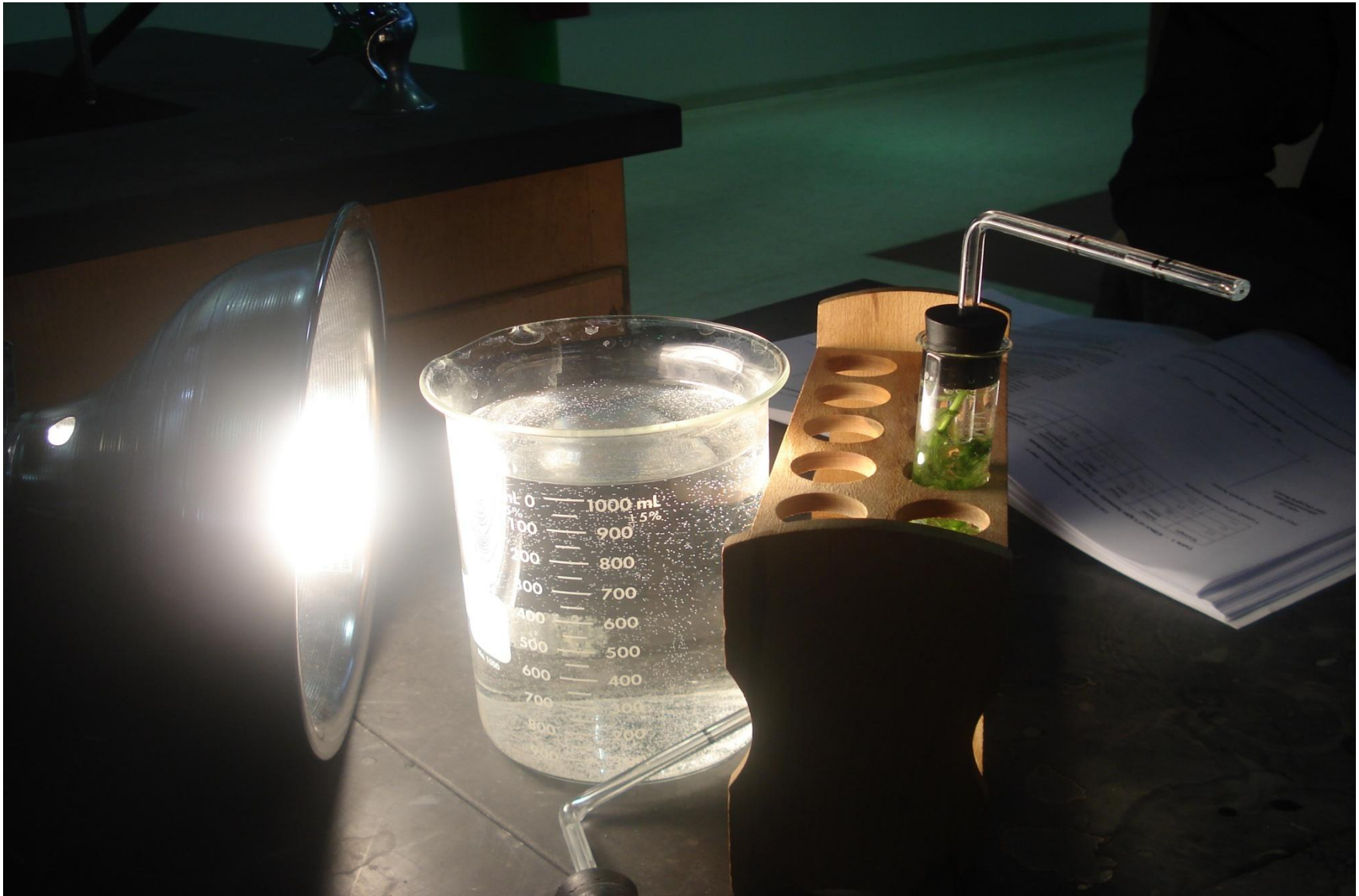


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Table 1: Effect of Light Intensity on the Rate of Photosynthesis

(round all times to the **nearest whole minute**)

Wattage	Initial time	Final time	Elapsed time	mL O_2	mL O_2/hour
				0.1	
				0.1	
				0.1	

Calculating the Rate of Photosynthesis: The procedure is much the same as the one you used to calculate the metabolic rate (page 74). Divide the mL O_2 released (0.1 mL) by the number of minutes (elapsed time) it took, then multiply that by 60 minutes/hour, as shown in this **example**:

$$\frac{0.1 \text{ mL oxygen}}{8 \text{ minutes}} \times \frac{60 \text{ minutes}}{\text{hour}} = .75 \text{ mL oxygen/hour}$$

Plot the rate data on the graph below:

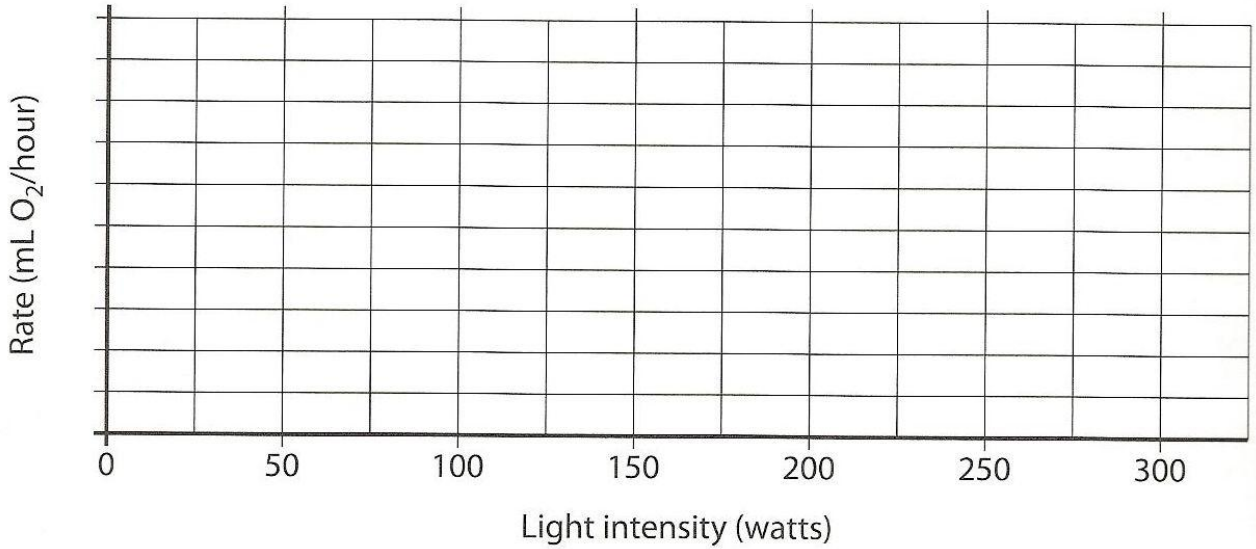

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Table 1: Effect of Light Intensity on the Rate of Photosynthesis

(round all times to the **nearest whole minute**)

Wattage	Initial time	Final time	Elapsed time	mL O_2	mL O_2/hour
60	10:16	10:28	12	0.1	.5 ml/hr
100	10:37	10:45	8	0.1	.75 ml/hr
200	10:48	10:51	3	0.1	.2 ml/hr

Calculating the Rate of Photosynthesis: The procedure is much the same as the one you used to calculate the metabolic rate (page 74). Divide the mL O_2 released (0.1 mL) by the number of minutes (elapsed time) it took, then multiply that by 60 minutes/hour, as shown in this **example**:

$$\frac{0.1 \text{ mL oxygen}}{8 \text{ minutes}} \times \frac{60 \text{ minutes}}{\text{hour}} = .75 \text{ mL oxygen/hour}$$

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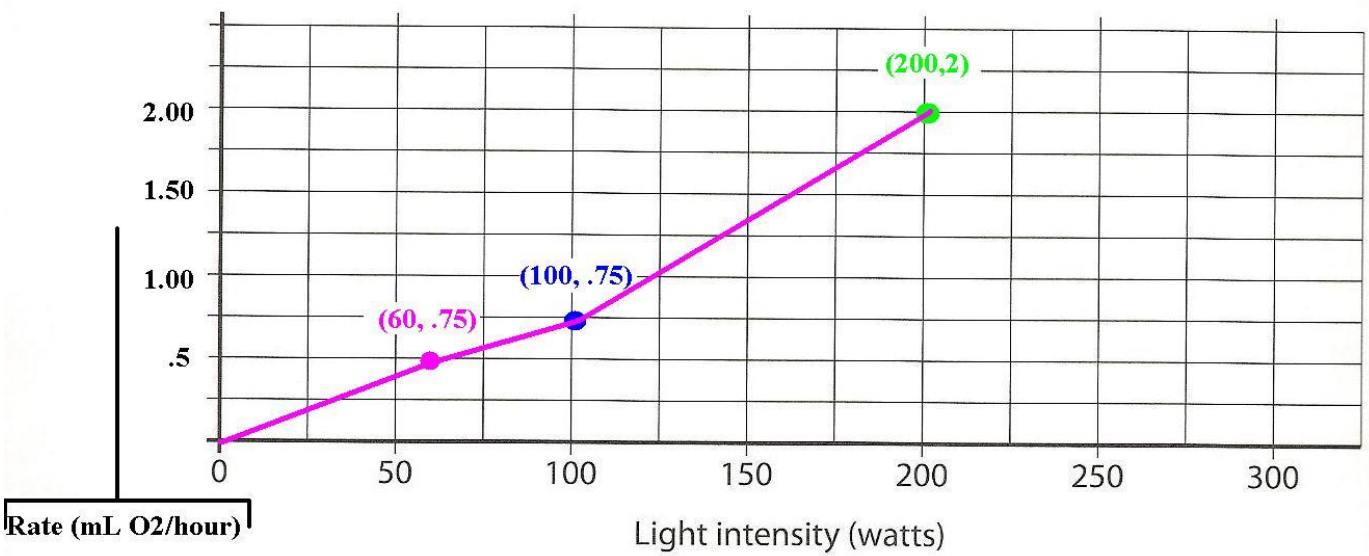


Why did you put a beaker of water in front of the lamp, what purpose did that serve?

Questions

1. Complete the following table and plot a graph with the data (Wattage: Y axis Vs. rate oh photosynthesis X axis)

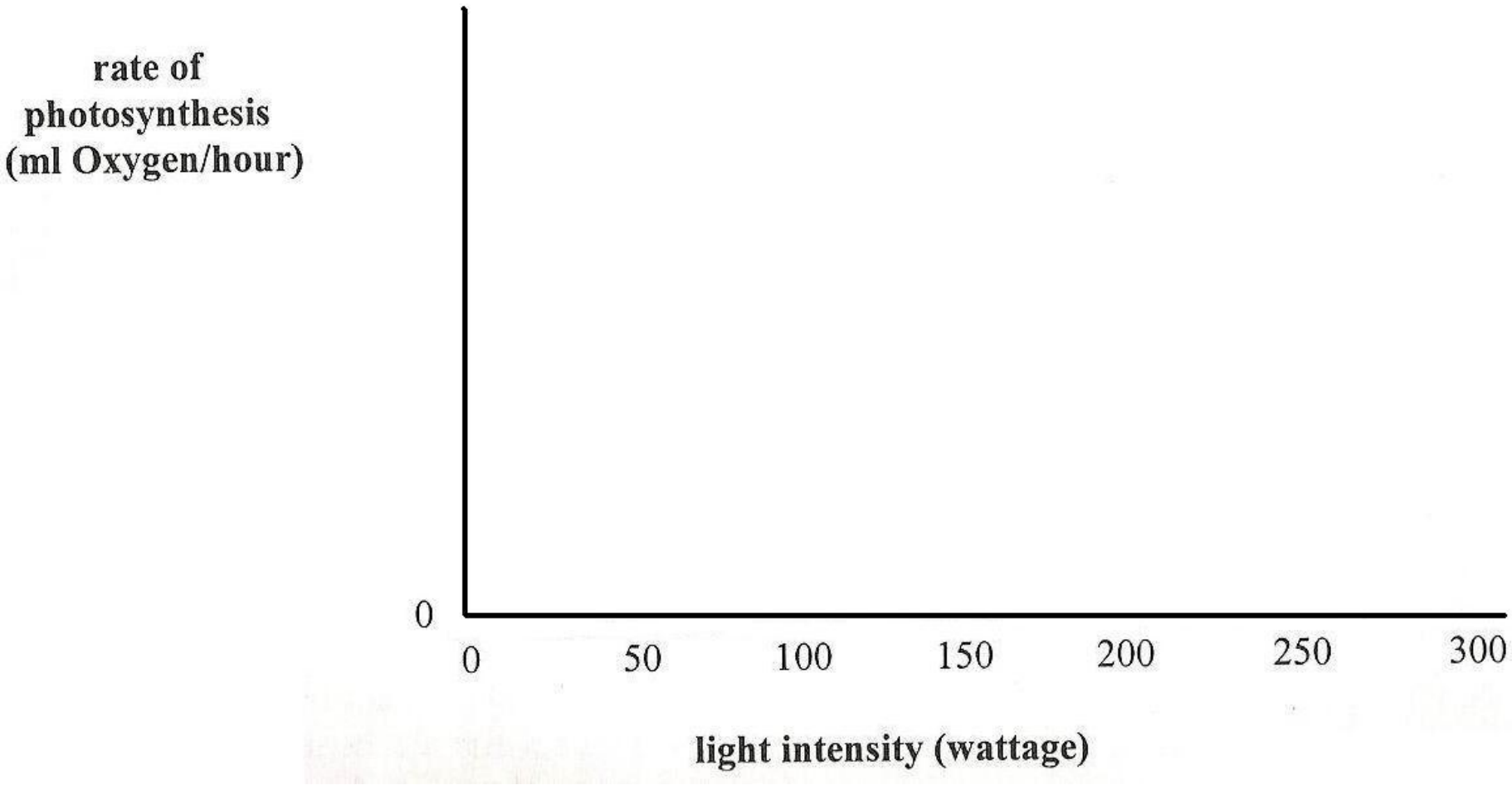
Wattage	Total Time Elapsed	Amount of Oxygen Produced	Amount of oxygen Produced in 1hr **(photosynthesis rate)**
50	30 min	0.1 ml	
100	15 min	0.1 ml	
200	7 min	0.1 ml	
300	8 min	0.1 ml	

Questions

1. Complete the following table and plot a graph with the data (Wattage: Y axis Vs. rate on photosynthesis X axis)

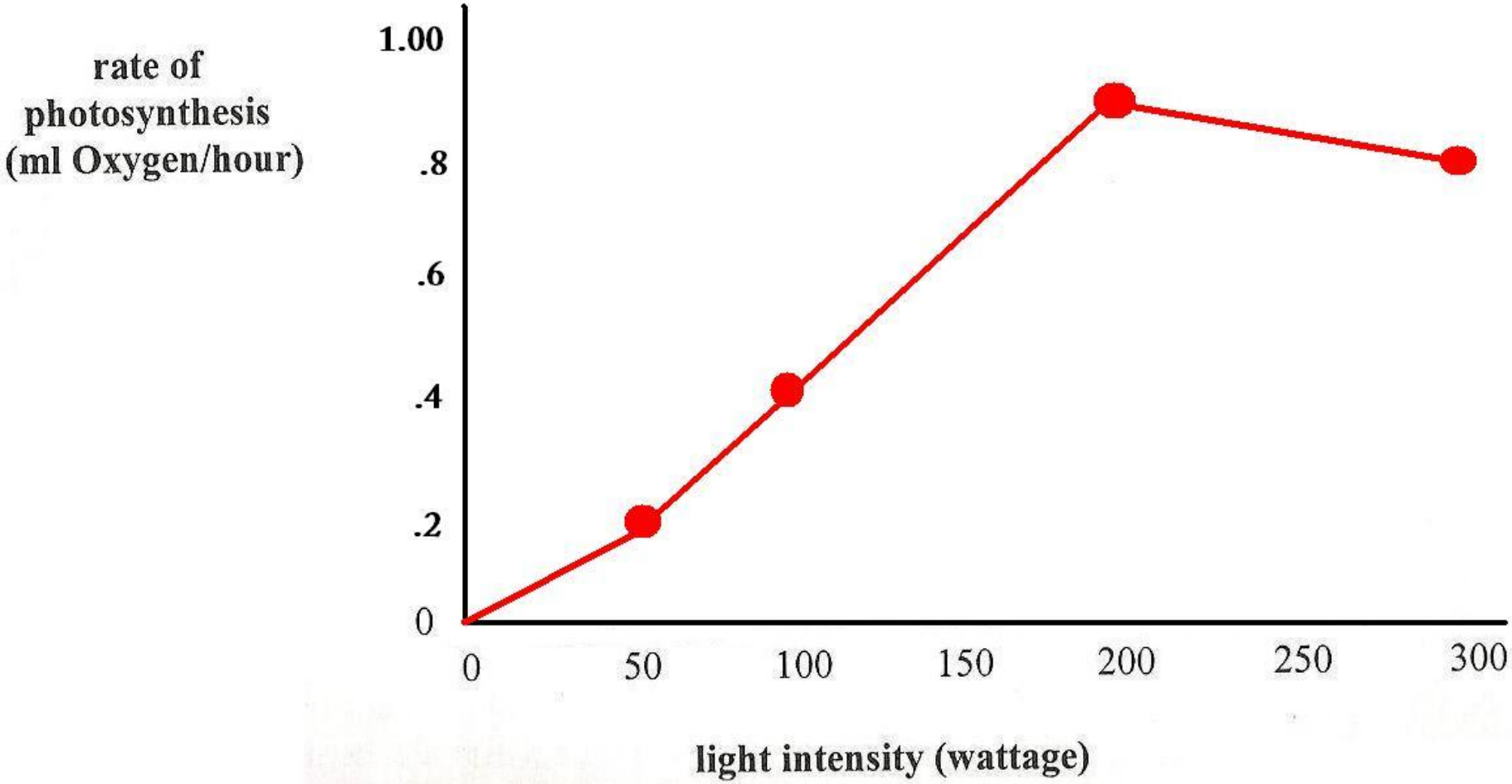
Wattage	Total Time Elapsed	Amount of Oxygen Produced	Amount of oxygen Produced in 1hr **(photosynthesis rate)**
50	30 min	0.1 ml	**.2 ml/hr**
100	15 min	0.1 ml	**.4 ml/hr**
200	7 min	0.1 ml	**.9 ml/hr**
300	8 min	0.1 ml	**.8 ml/hr**

Questions



2. Why does this graph make sense?

Questions



2. Why does this graph make sense? **As wattage increases more oxygen is being release by the plant, but at a certain point photosynthesis has to stop because a plant simply just give up oxygen for infinity of time, that's why the graph is going down, or maybe the plant is small in structure.**

Separation of Chloroplast Pigments by Paper Chromatography

- The primary photosynthetic pigment is **chlorophyll a**, however, other accessory pigments (e.g., xanthophylls and carotenes) are also typically present in the chloroplast, albeit in smaller quantities. We are separating different pigments in this experiment. In this exercise, the separation will be accomplished by **paper chromatography**. When a solution of these pigments is placed on a strip of filter paper, the pigment molecules adsorb onto the cellulose fibers of the paper. When the tip of the strip of paper is then immersed into a suitable solvent, the solvent is absorbed by the paper and moves up the paper by **capillary action**.

- In order to capture the energy from light, it must first be absorbed by the plant. This is accomplished by the organelles called **chloroplasts**, which contain special pigment compounds called **photosynthetic pigments**. These pigments are capable of absorbing photons having the wavelengths of light which are used in photosynthesis. The principal photosynthesis pigment is chlorophyll-a, while the secondary (accessory) pigments are chlorophyll-b (also c and d in certain organisms), the carotenoids, and the xanthophylls. Since the pigments do not absorb all wavelengths of light equally, they have characteristic colors. Chlorophyll is responsible for the green color of plants because chlorophyll reflect green wavelengths of light. The carotenoids reflect much of the orange and red wavelengths; thus, they provide coloration for plant organs such as tomatoes, carrots, pumpkins, and the striking colors of the autumn leaves.

- Chlorophyll a will always have the same Rf no matter how big piece you run it on.

Seeing interaction of three different things going on. The more it likes the solvent The pigment molecules are generally unable to move as fast as the solvent, but some of them some move faster than others. **The differential movement of the pigments is caused by three main factors that differ:**

- **a) Molecular weight: The higher molecular weight, the lower the pigment will travel and vice versa.**

- **b) Solubility in the specific solvent: The more soluble the pigment, the higher up the paper it will travel and vice versa.**

- **c) Affinity for the paper: How attracted the pigment is to the paper, so the higher the affinity of the pigment the lower it will travel on the paper and vice versa.**

- **The distance traveled by a particular pigment relative to the distance traveled by the solvent is specific for any given pigment and set of conditions. This relationship is called the Rf.**

- The Rf for any given substance is constant in a specific solvent-separation system; this, it can be used to characterize the pigment molecules. The number is unique for that material in this solvent system, use in science a lot to say what weigh is that.

- Put less than a drop on the chloroplast extract on the paper between the notches. Build up the spot (called the origin) for about 20 times.

- If the leading edge of the solvent, which is called the **solvent front,** reaches within one centimeter of the top of the paper, you should remove the paper. Don't let the solvent to migrate up over the top of the paper.

- **Bright yellow – Carotenes**
- **Yellow – Xanthophyll**
- **Blue-Green – Chlorophyll-a**
- **Yellow-Green – Chlorophyll-b**

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- Measure the distance from the origin spot to the solvent front and from the origin in the approximate center of each of the pigment bands.

- One important calculation that you will make today is the Rf of different photosynthetic pigments (separation of chloroplast pigments). A pigment's Rf tells you about its **A) molecular weight, B) Solubility in the solvent used, C) Affinity for paper.**

Paper Chromatography - Rf

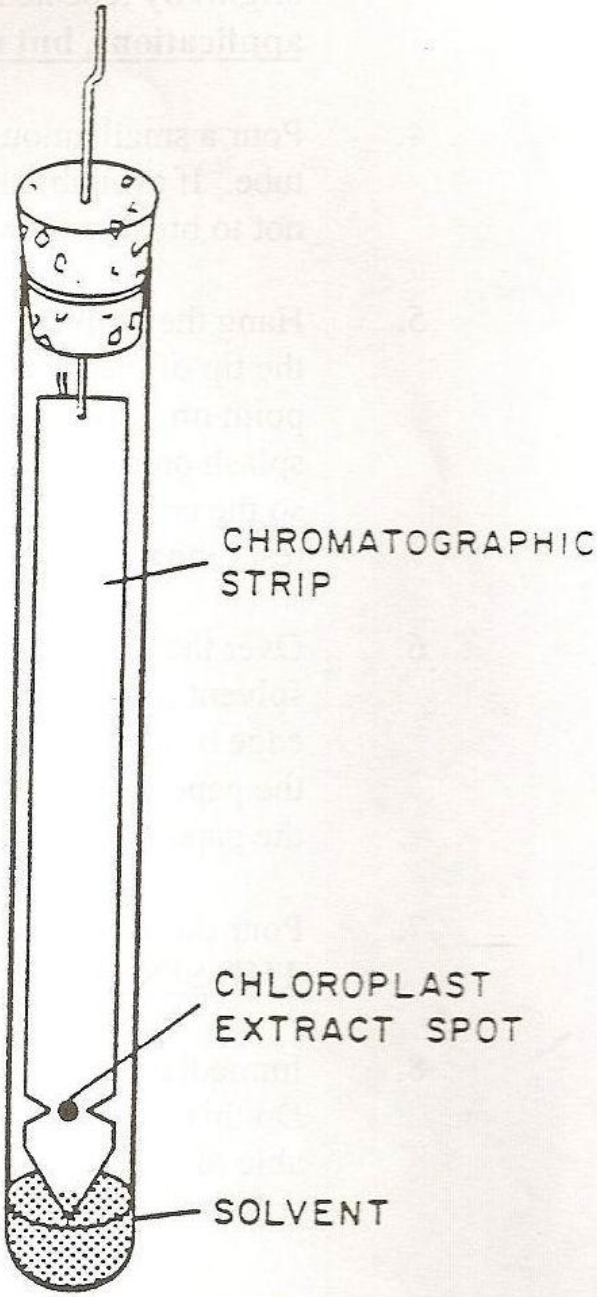
Formula

Rf of substance = distance from origin to substance / distance from origin to solvent front.

$$R_f = \frac{\text{distance moved by pigment}}{\text{distance from pigment origin to solvent front}}$$

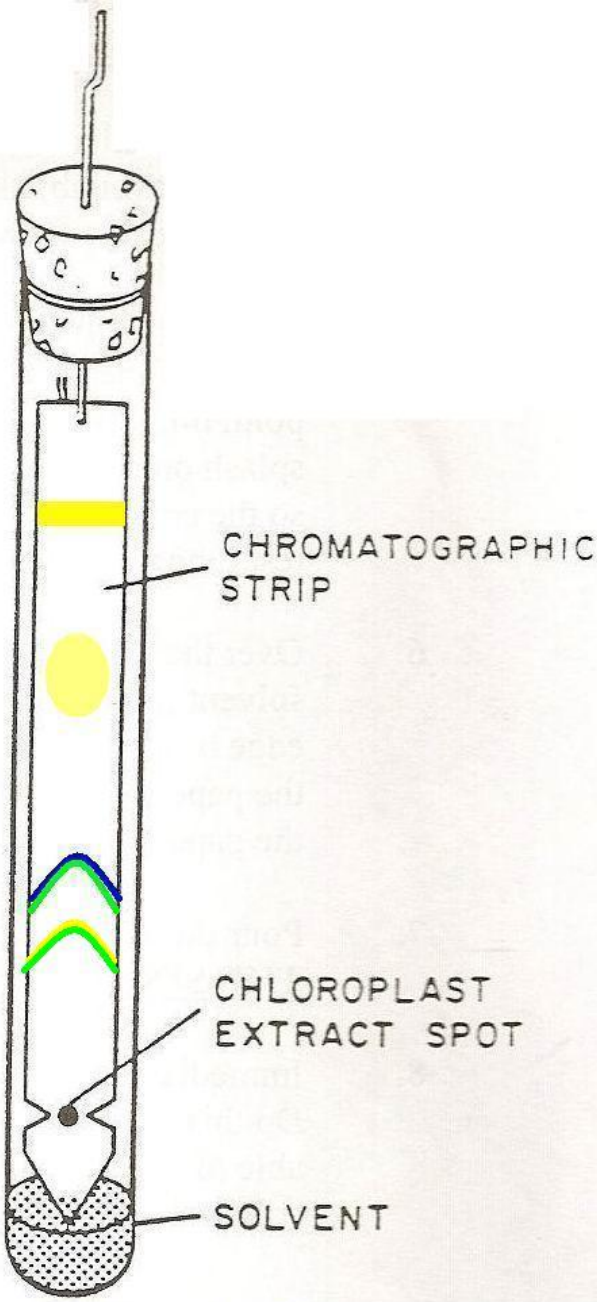
Note: cm or mm, depending on your professor, but the KEY POINT… If you get a number that's higher than "1." You got the wrong answer. Flip the numbers, and you should get the right answer.

Units: cm or mm



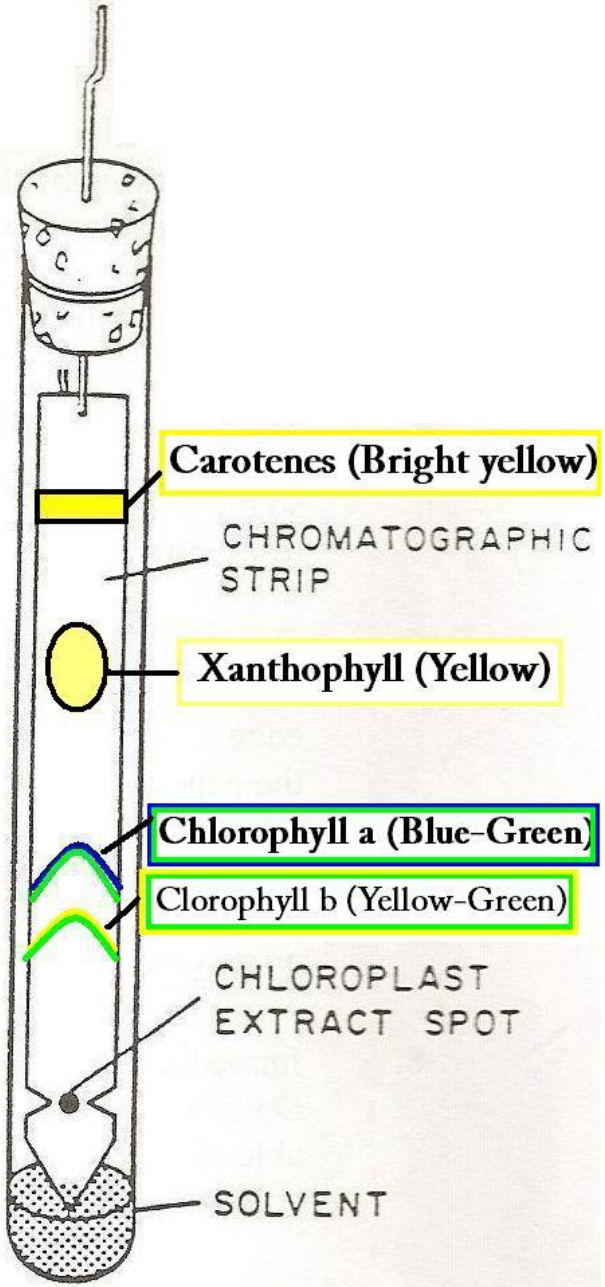
Separation of Chloroplast Pigments

by Paper Chromatography



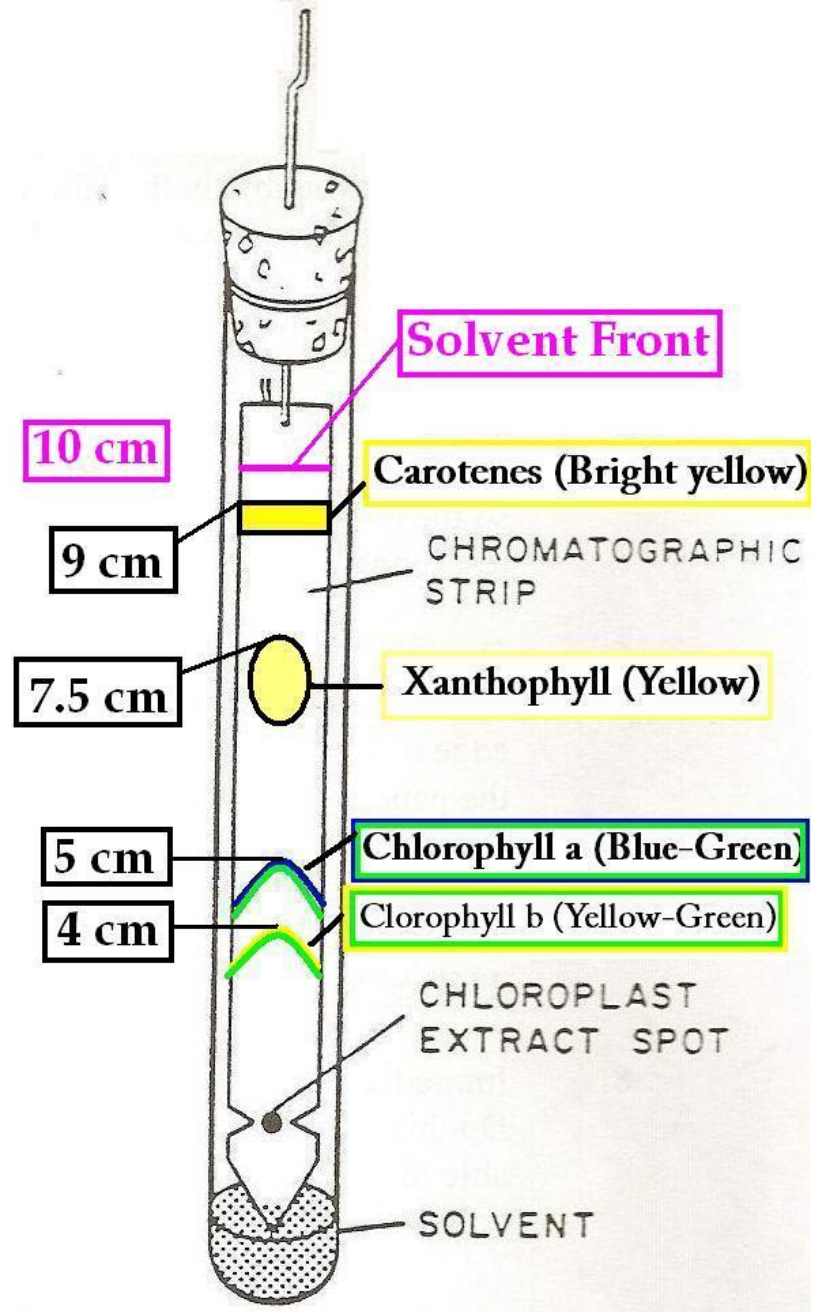
Separation of Chloroplast Pigments

by Paper Chromatography



Separation of Chloroplast Pigments

by Paper Chromatography



Separation of Chloroplast Pigments by Paper Chromatography

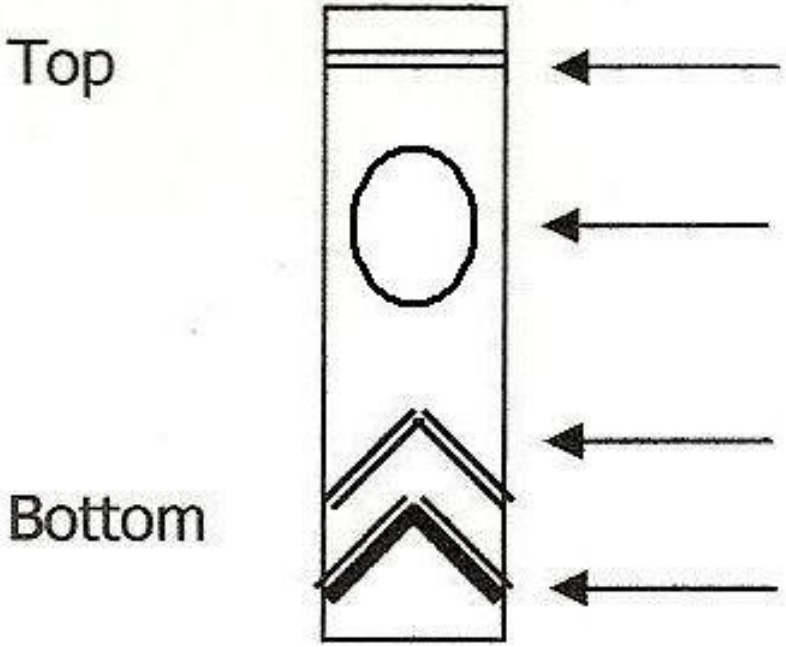
Rf Must be a # less than "1"

Put the smaller number over the bigger number.

$$Rf = \frac{7.5}{10} = .75 \text{ mm or cm}$$

Questions

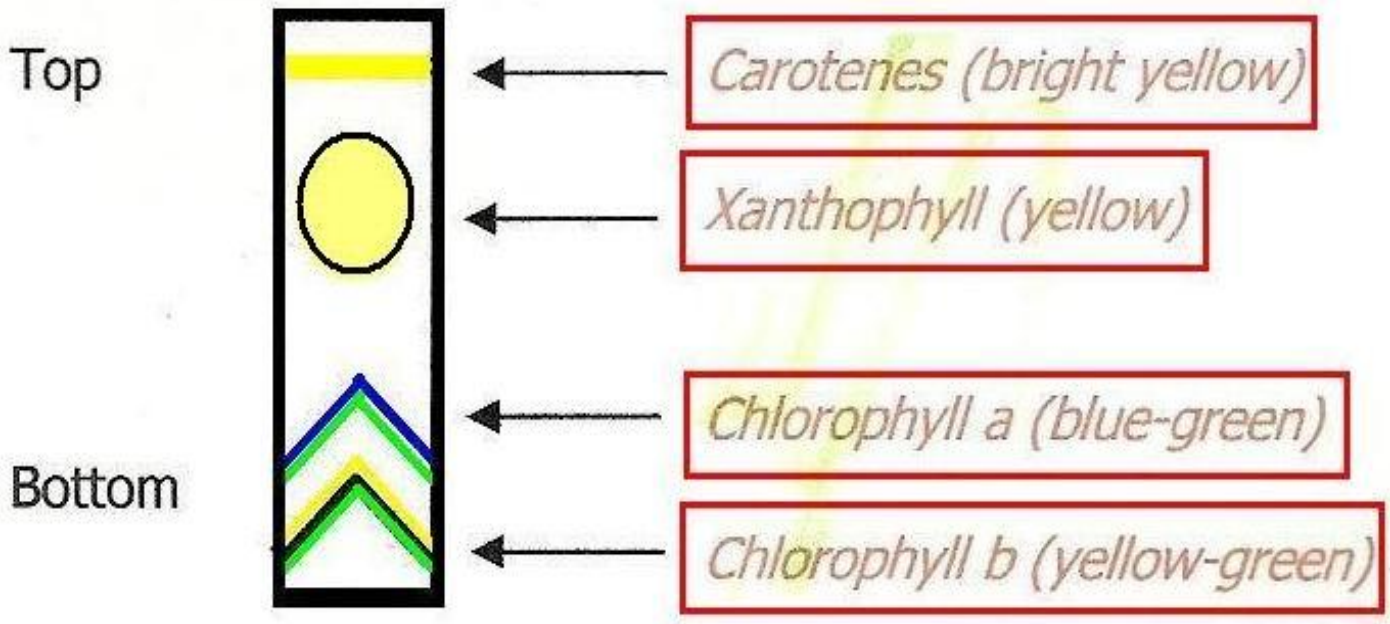
1. Labe the pigments on the chromatograph:



2. A paper chromatography was done using acetone as a solvent. At the end of the chromatography it was found that the solvent traveled 20 cm and a pigment molecule traveled 17 cm from its origin. Calculate the Rf value of the pigment molecule.

Questions

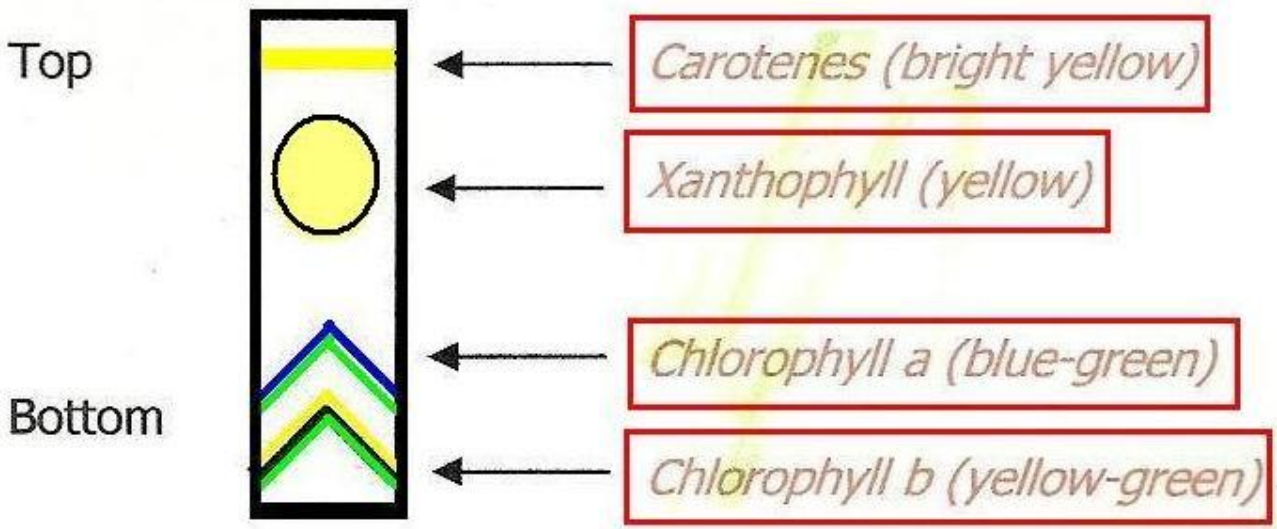
1. Labe the pigments on the chromatograph:



2. A paper chromatography was done using acetone as a solvent. At the end of the chromatography it was found that the solvent traveled 20 cm and a pigment molecule traveled 17 cm from its origin. Calculate the Rf value of the pigment molecule?

17/20 = .85 cm

Questions

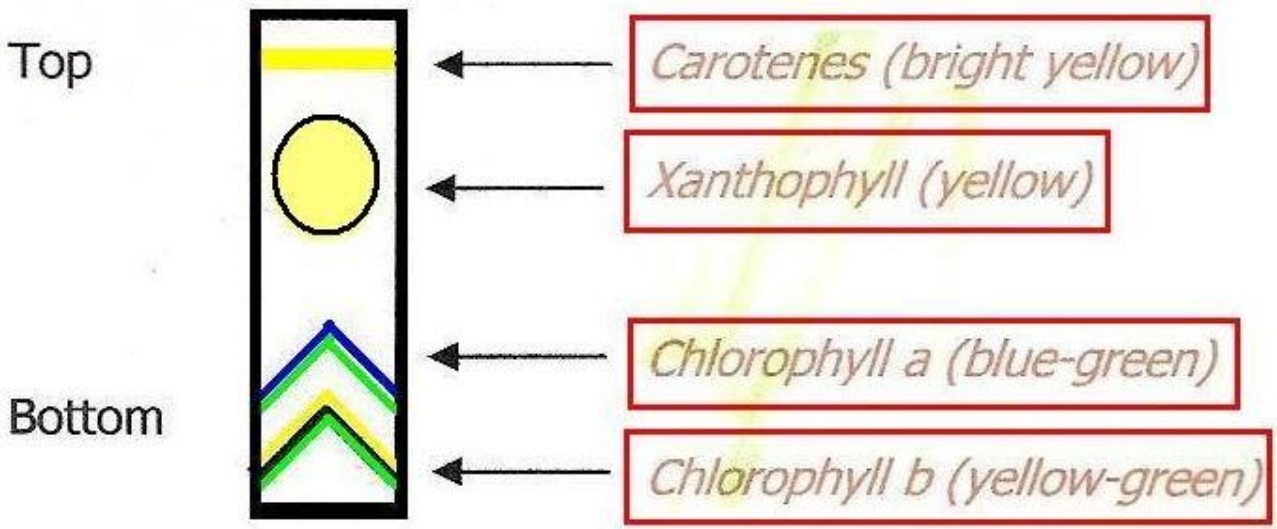


- 3. Which pigment has the second highest molecular weight?

- 4. Which pigment is the most soluble, lowest molecular weight, and has a low affinity?

- 5. Which pigment has the highest affinity and molecular weight?

Questions



- 3. Which pigment has the second highest molecular weight? **Chlorophyll-a**

- 4. Which pigment is the most soluble, lowest molecular weight, and has a low affinity? **Carotenes**

- 5. Which pigment has the highest affinity and molecular weight? **Chlorophyll-b**

Absorption Spectrum of Chloroplast Extract

- An instrument called **Spectrophotometer** can be used to pass light of specific wavelengths through a sample of chloroplast pigments dissolved in a solvent. **The degree of absorption of each of the wavelengths of the light by the pigments can then be measured**.

- One important graph you will draw today is an **absorption spectrum** (chloroplast pigments). **The absorption spectrum tells you which wavelengths of light are strongly absorbed by the photosynthetic pigments. Peaks in your absorption spectrum indicate the wavelengths of light that are strongly absorbed - these wavelengths generate the highest rates of photosynthesis.** Valleys in your absorption spectrum indicate the wavelengths of light that are least absorbed - theses wavelengths generate the lowest rates of photosynthesis.

- Pass wavelengths through a sample of chloroplast pigments and produce an absorption spectrum

- <u>Know what wavelengths are more strongly absorbed.</u>

Photosynthetic Pigments: The Light Receptors

- Pigments
  - Are substances that absorb visible light
  - Reflect light, which include the colors we see
- The visible light spectrum
  - Includes the colors of light we can see
  - Includes the wavelengths that drive photosynthesis

The Nature of Sunlight

The light reactions convert solar energy to the chemical energy of ATP and NADPH

Light is a form of electromagnetic energy, which travels in waves

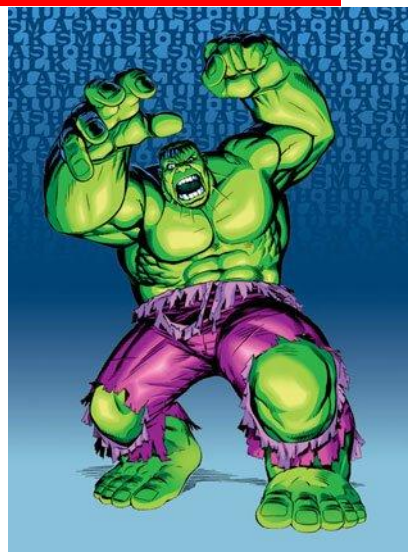
Wavelength
Is the distance between the crests of waves
Determines the type of electromagnetic energy

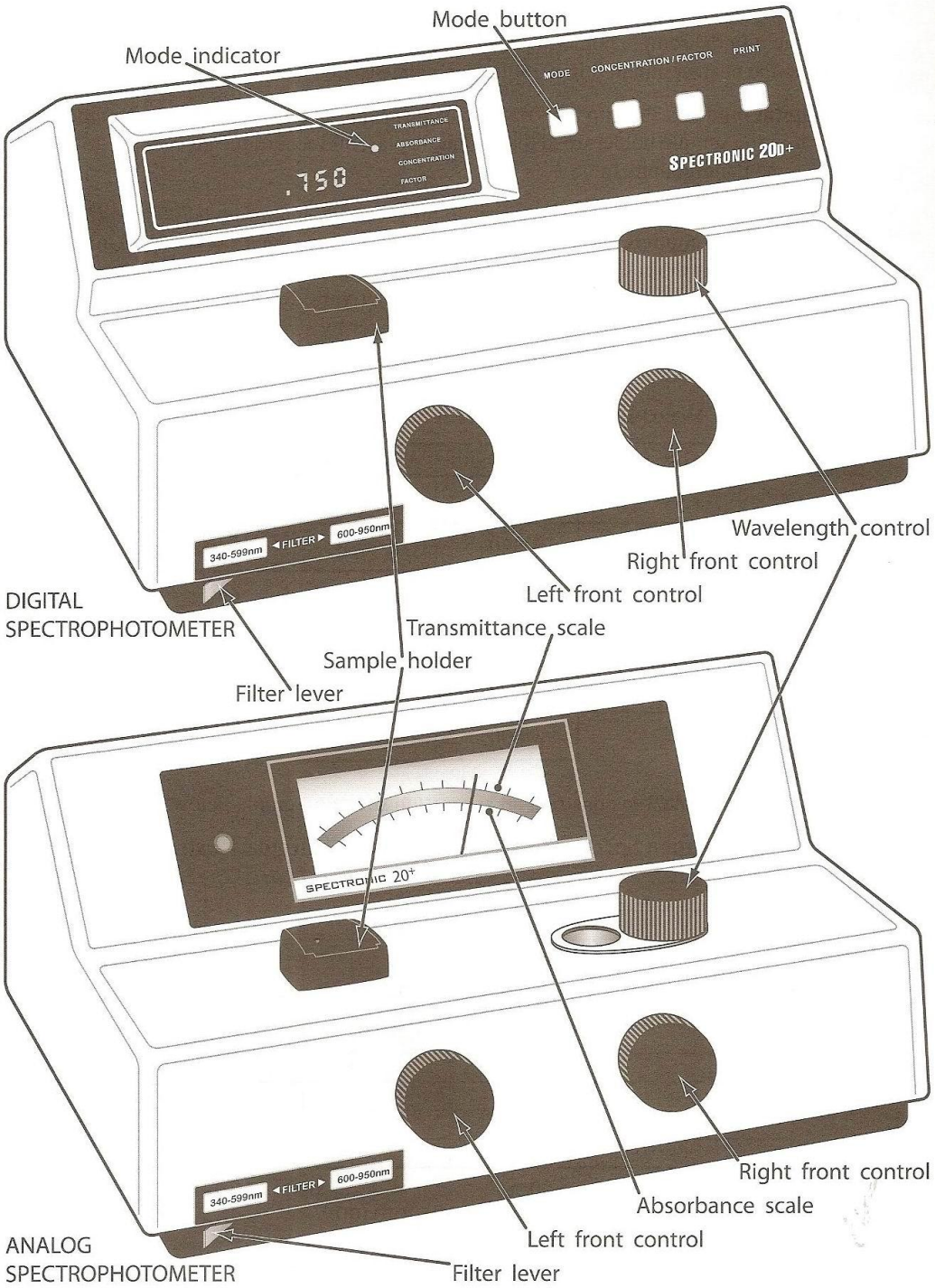
The electromagnetic spectrum
Is the entire range of electromagnetic energy, or radiation

Light is radiated in packets called **photons**, which travel at specific **wavelengths**. We can distinguish among the different wavelengths as differences in color. A prism is often used to separate white light into its component colors.

The energy of light is inversely proportional to the wavelength, i.e., the shorter the wavelength, the greater the energy in each photon.

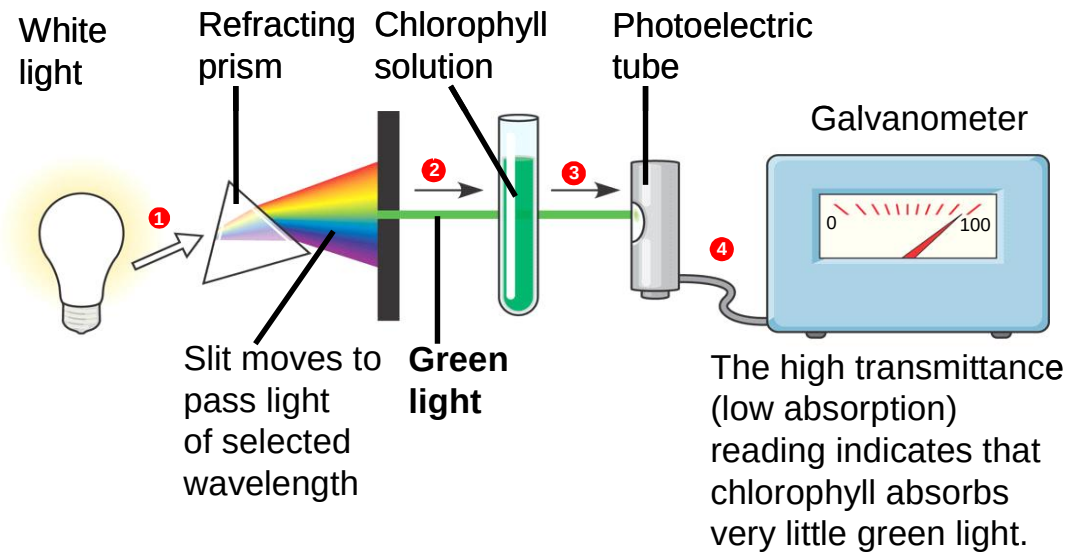
For example, radio waves are long and harmless. Gamma waves are short and harmful.

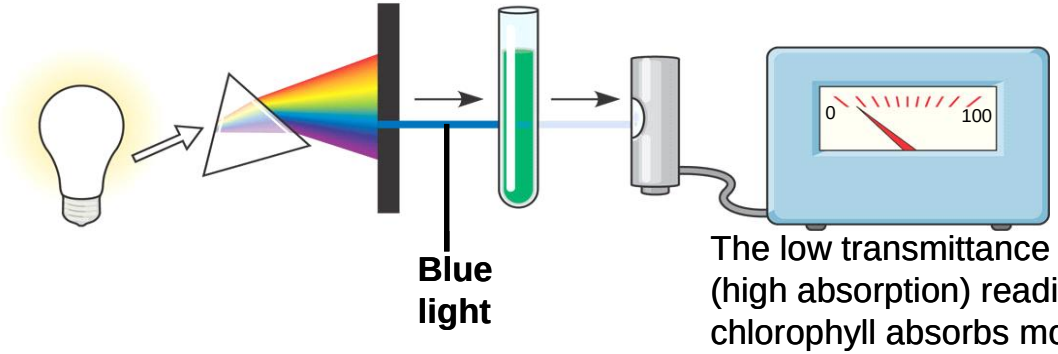



Mode button
Mode indicator
MODE
CONCENTRATION / FACTOR
PRINT
TRANSMITTANCE
ABSORBANCE
CONCENTRATION
FACTOR
.750
SPECTRONIC 20D+
340-599nm
FILTER
600-950nm
Wavelength control
Right front control
Left front control
DIGITAL
SPECTROPHOTOMETER
Transmittance scale
Sample holder
Filter lever
SPECTRONIC 20+
340-599nm
FILTER
600-950nm
Right front control
Absorbance scale
Left front control
ANALOG
SPECTROPHOTOMETER
Filter lever

An absorption spectrum

Plots light absorption vs wavelength





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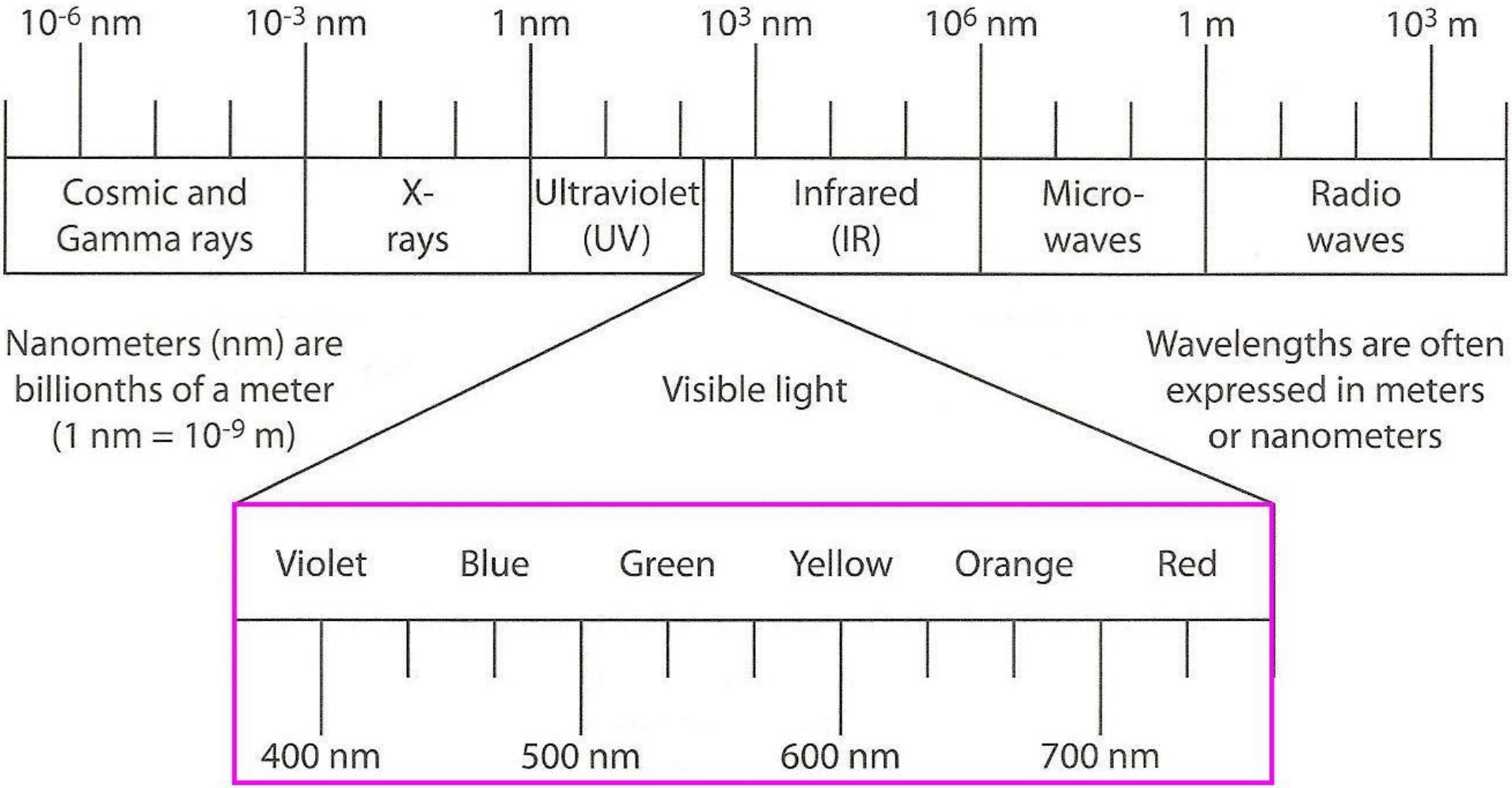

THE VISIBLE LIGHT SPECTRUM AS PART OF THE OVERALL ELECTROMAGNETIC SPECTRUM

The electromagnetic spectrum

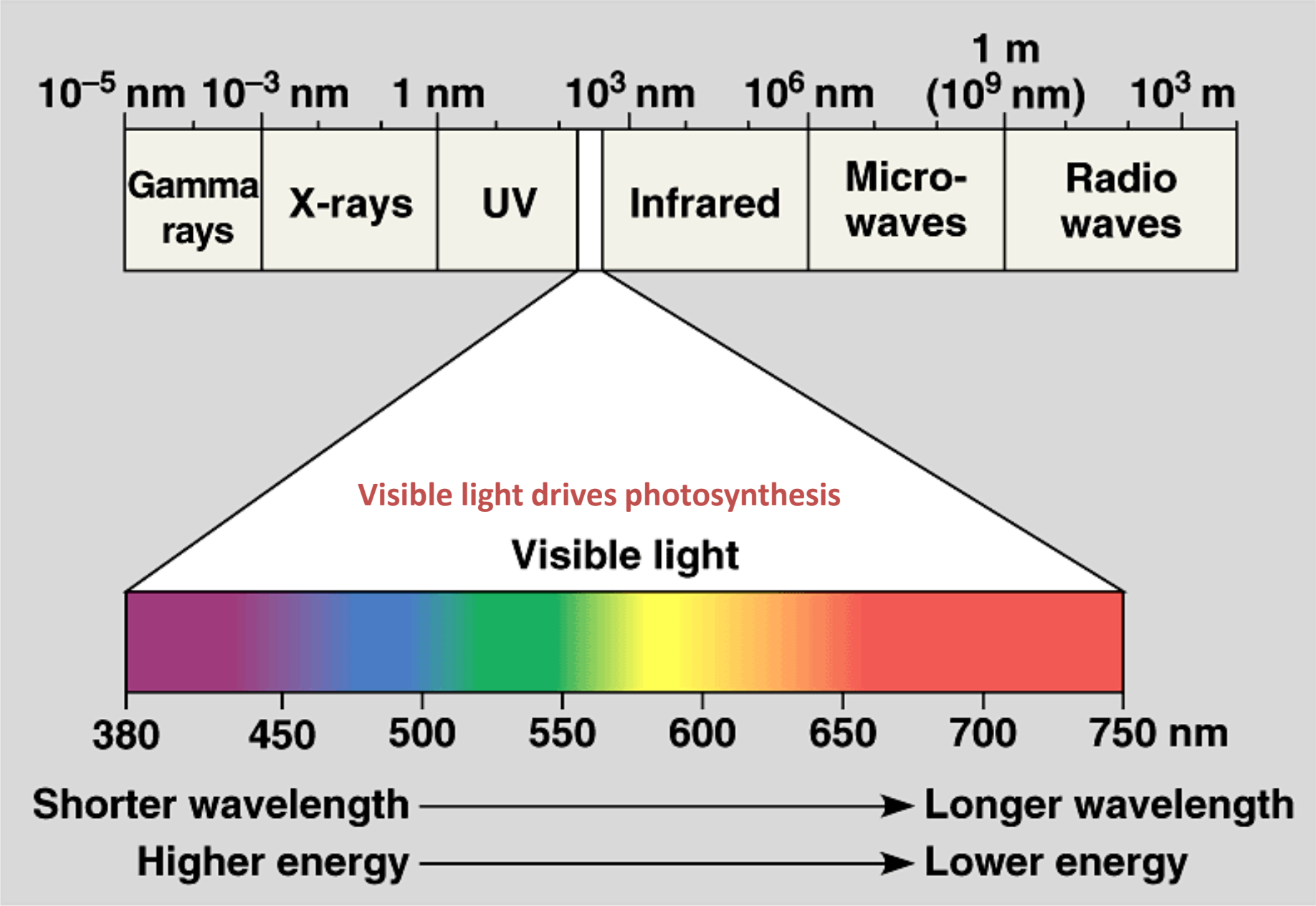

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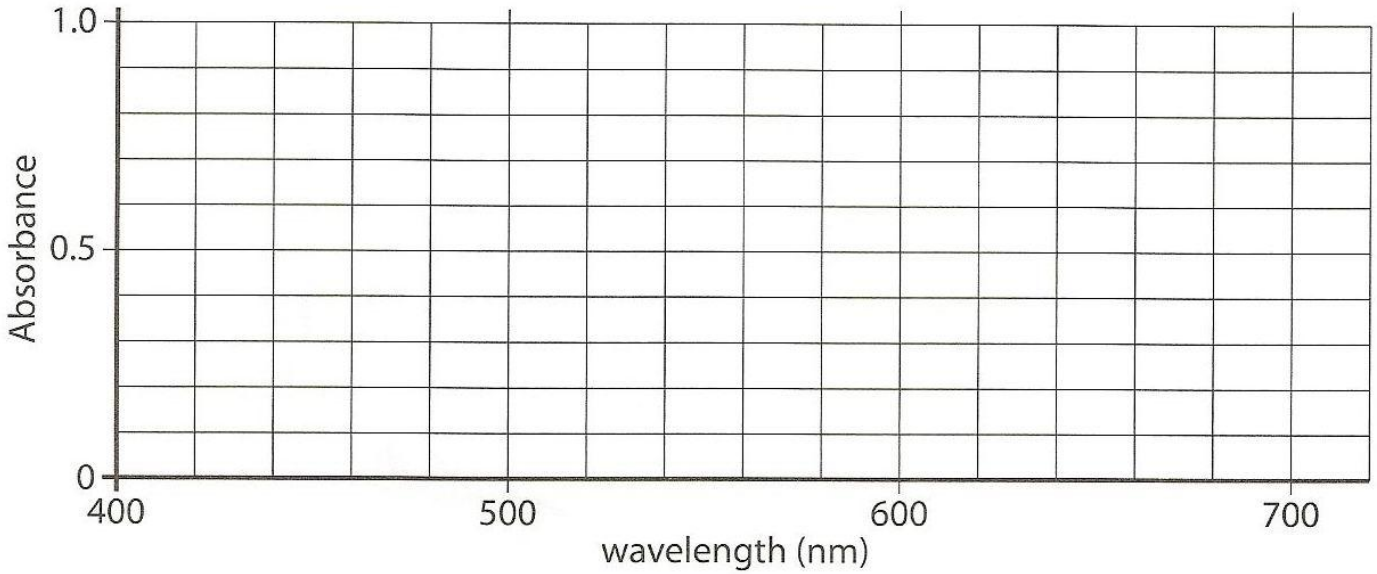
Table 3: Absorption Spectrum Data for Spinach Chloroplast Extract

Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
400		500		620	
420		520		640	
440		540		660	
460		560		680	
480		580		700	
		600			

Use the data in Table 3 to construct an absorption spectrum graph, below.

Absorption Spectrum Graph of Spinach Chloroplast Extract



Graph: Absorption spectrum of the pigment mixture

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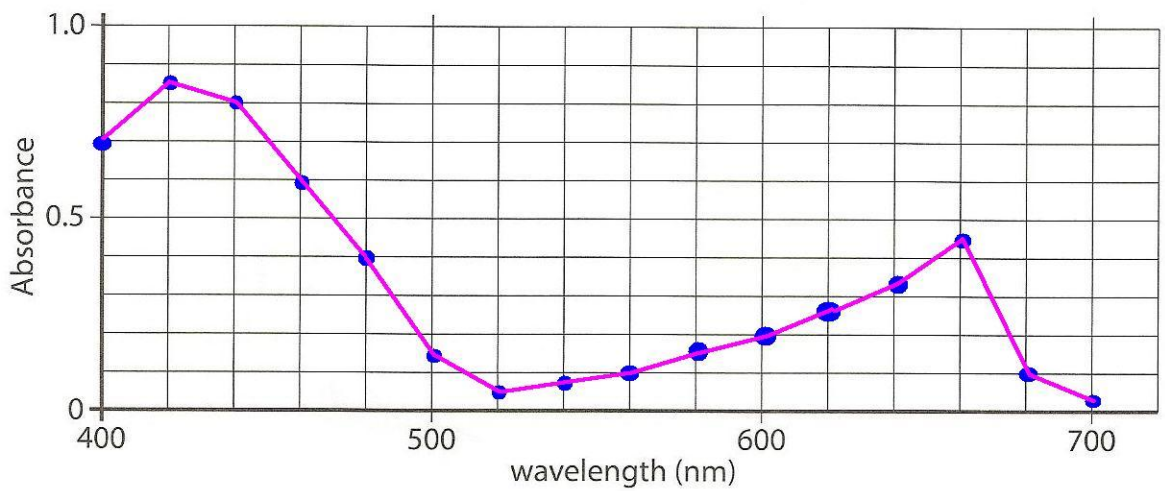
Since all green plants don't have exactly the same type and amount of photosynthetic pigments, the spectra may vary from one species of plant to another.

Table 3: Absorption Spectrum Data for Spinach Chloroplast Extract

Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
400	.700	500	.131	620	.129
420	.840	520	.019	640	.189
440	.764	540	.085	660	.339
460	.568	560	.093	680	.094
480	.318	580	.107	700	.023
		600	.123		

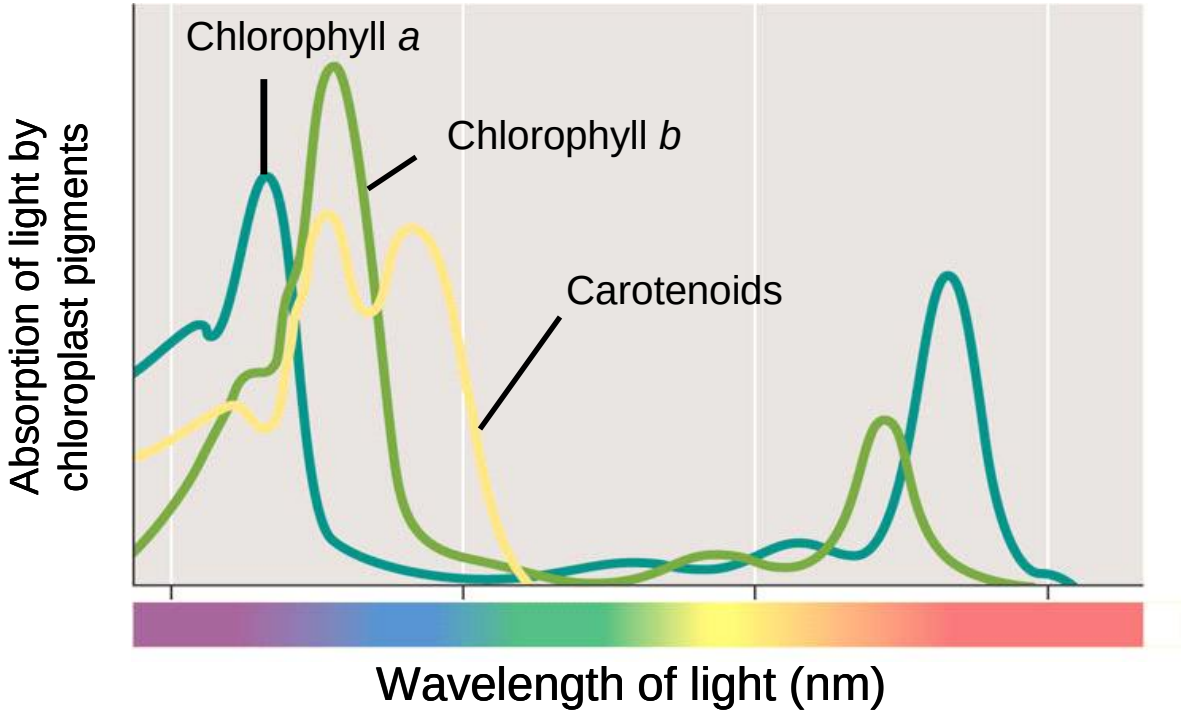
Use the data in Table 3 to construct an absorption spectrum graph, below.

Absorption Spectrum Graph of Spinach Chloroplast Extract



The absorption spectra of three types of pigments in chloroplasts

Provides clues to the relative effectiveness of different wavelengths for driving photosynthesis



Questions

1. What type of energy is used in this reaction?

2. What absorbs that energy?

3. A spectrophotometer measures?

4. Which colors of light absorb the most pigment?

5. Which colors of light will be *most effective* for photosynthesis?

6. This is the absorption spectrum for which pigment?

7. Paper chromatography separators pigments on the bases of which 3 properties?

Questions

1. What type of energy is used in this reaction? **Sunlight (light intensity - wattage)**

2. What absorbs that energy? **Chlorophyll**

3. A spectrophotometer measures? **Absorption of light wavelengths**

4. Which colors of light absorb the most pigment? **Violet/blue and a little red.**

5. Which colors of light will be *most effective* for photosynthesis? **Violet/blue some red.**

6. This is the absorption spectrum for which pigment? **Chlorophyll**

7. Paper chromatography separators pigments on the bases of which 3 properties?

a) **Molecular weight**
b) **Solubility in the solvent**
c) **Affinity for the chromatography paper**