Procedures

1. Demonstrate the use of CO_2 in plants for photosynthesis.

    A. Pour distilled water into the graduated cylinder to the 50 mL level.

    B. Add one phenol red tablet to the graduated cylinder and stir until dissolved.

    C. Place a straw into the graduated cylinder and blow slowly and steadily, until the phenol red changes to a yellow color. *BE CAREFUL NOT TO BLOW TOO HARD OR THE SOLUTION MAY SPLASH INTO THE FACE.*

    D. Place a small piece of spinach leaf into the test tube.

    E. Pour the phenol red solution from the graduated cylinder into the test tube to the top of the tube.

    F. Carefully put the tube cork into the test tube, leaving no air bubbles.

    G. Place the test tube in front of a light source (light or window) for 30 minutes. Record results of any observed color change.

2. Chromatography

    Remember, acetone is flammable so be certain to keep it away from open flames.

    A. Wrap a rubber band around the jar lengthwise so that the mouth of the jar has a stretch of rubber band around it. The chromatography paper strip will be attached to this rubber band so be certain that it is centered across the opening of the jar.

    B. Attach two paper clips to the rubber band so that they hang loosely in the opening of the jar.

    C. Using the forceps to minimize handling, remove one chromatography strip and while holding it at the terminal end (straight edge), attach the chromatography strip to the paper clip. Note: Only handle the chromatography paper by the extreme edges (as opposed to the flat surfaces) or at the terminal portion of the end that will not go into the acetone/water as the oils

D. The strip should not touch the bottom of the inside of the glass jar. If it does, it may be necessary to fold over the top of the strip. The strip should hang no more than one cm from the bottom of the jar because the solvent (acetone) needs to be poured into the jar. Only the very tip of the chromatography strip should touch the solvent. Once the strip is positioned, remove it from the paper clip and place it on a paper towel. Use this strip to make any adjustments to the second strip. The remaining strip may be cut to size, or the top may be folded over so that it is the same size as the test strip. Place both strips on a clean paper towel and set it aside.

E. Place a small piece of tape on each of the two bowls. Using a marker or pen, label the first bowl "acetone" and the second bowl "water."

F. Remove two, large spinach leaves. Use the scissors to cut up the leaves, or use clean hands to tear the spinach into small bits and place the pieces into the mortar. Be sure to use only the leaves of the spinach and not the stems.

G. Cover the leaf tissue with 10 mL of acetone and add 1/2 teaspoon of fine sand. Using the pestle and sand, grind the spinach leaves until the entire mixture becomes a slurry of a dark liquid (about five to seven minutes). Add additional acetone (no more than 5 mL) and grind for another minute. Let the solution stand for a few minutes (about five) to be sure the pigments are extracted. A very dark pigment solution should be formed.

H. Carefully pour the liquid into the bowl labeled "acetone."

I. Repeat steps E-G, this time using water to cover the plant leaves and pouring the liquid into the bowl labeled "water."

J. Using a *pencil* (ink cannot be substituted as it will migrate along with the solvent) and the metric ruler, draw a horizontal line across each strip about 1 to 1 1/2 centimeters from the tip (bottom). This line is the origin line. Label one strip "A" for acetone and the second strip "W" for water to identify the solvent used to extract the pigment.

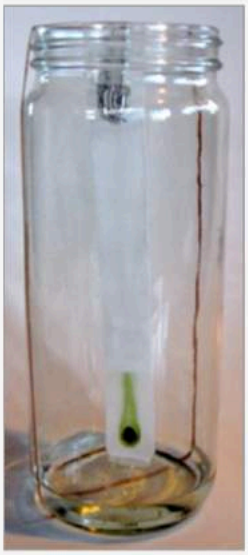
K. Using a micropipette, draw up a small volume of the spinach/acetone slurry and add a small drop to the center of the pencil line (origin line) on the chromatography paper labeled with the "A." Attempt to keep the spot as small as possible to prevent the pigment from spreading across the paper.

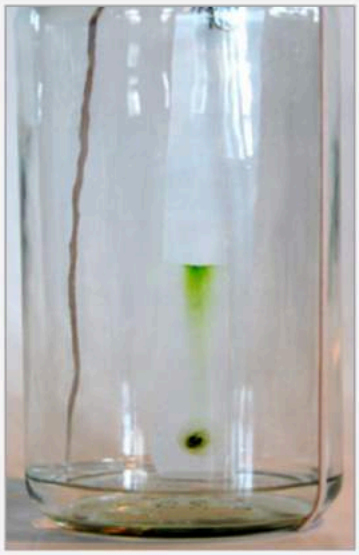
L. Repeat step J a second time, using the second chromatography strip (labeled with the "W") and the spinach/water slurry.

M. Allow the spots to dry (use of a hairdryer on a low setting may facilitate the drying process). Repeat steps J and K several more times until a deep green spot is achieved.

N. Pour acetone into the jar to a height of about 1 to 1 1/2 cm.

O. Place the jar in a location where it will not be subject to shaking or vibration. If the jar is bumped, or disturbed in any way, the solvent will quickly migrate up the paper and the pigments will not be removed.





P. Attach the prepared chromatography strips to the paper clips. The strips should hang so that only the extreme end of the chromatography strip touches the solvent (acetone). DO NOT ALLOW THE ORIGIN LINE TO TOUCH THE SOLVENT.

T. Measure and record the distance from the origin line to the solvent front (end distance of acetone), as well as the distance for each pigment from the starting point (origin line) to the top of the respective pigment line.

U. Calculate the R_f value for each pigment by dividing the distance from the origin line (slurry spot) to the top of the pigment line, by the distance from the origin line (slurry spot) to the solvent front.

V. Record the values obtained.

3. Factors That Affect the Rate of Photosynthesis

A. Prepare a 0.8% bicarbonate solution. Take one plastic cup and label it 0.8%. Using the 100 mL graduated cylinder, pour a total of 150 mL of distilled water into the labeled cup. Add ¼ teaspoon of baking soda and stir until dissolved.

Click on image to enlarge.

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B. Take a second plastic cup and label it 0.2%. Using the 100 mL graduated cylinder, measure out 50 mL of the 0.8 mL solution and pour it into the cup labeled 0.2%. Using the 100 mL graduated cylinder, add an additional 100 mL of distilled water to the cup.

C. Add a few drops of dish soap to each cup and stir.

D. Using a hole punch, cut 30 leaf disks from fresh spinach leaves, trying to avoid any major veins.

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E. Remove the plunger from a 10 mL syringe. Place 10 disks into the body of the syringe and then gently re-insert the plunger. Take care not to damage the leaf disks when inserting the plunger.

Click on image to enlarge.

F. Insert the syringe into the 0.2% bicarbonate solution and draw up about 8 mL. The disks should be floating at this time.

Click on image to enlarge.

G. Hold the syringe upward (tip up) and slowly depress the plunger to remove any excess air.

H. Cover the tip of the syringe with the thumb and with the opposite hand, pull back on the plunger to create a partial vacuum and move air out of the disks. Hold for 10 seconds.

Click on image to enlarge.

I. Simultaneously release the thumb and the plunger. Tap the side of the tube to dislodge any bubbles. The disks should start to sink.

J. Repeat steps H and I until all of the disks sink to the bottom of the syringe (Note: Be patient as this may take several attempts).

K. Place the syringe upright under a light source 10 cm from the syringe and start a stopwatch or record the time.

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L. After the end of one minute, invert the syringe to agitate the disks and then immediately return the syringe to its position under the light source. Record the number of disks that are floating in the syringe.

M. Repeat step L after each one minute interval, until all of the disks are floating.

Click on image to enlarge.

N. Empty the contents of the syringe, including the solution and the disks, down the drain of a sink. Flush the syringe with tap water.

O. Repeat the process two more times, beginning with step F. The first time, modify the experiment by using the 0.8% solution. For the last trial, modify the experiment by using the initial 0.2% solution, but place the syringe 15 cm away from the light source.

P. Calculate the rate of photosynthesis (as indicated by 50% of the disks floated for each trial) by graphing the number of disks that floated as a function of time and extrapolating the point of time where 50% of the disks were floating.