**Paper chromatography** is a chemical process that separates the chemicals present in a mixture or compound. The separation takes place by absorption and capillary action.

Paper is used to hold the substances by absorption. Capillary action moves the substances up the paper. Each substance will move up the paper strip at its own rate of speed and will appear as colored streaks on the paper. The pattern of separated chemicals on the paper is called the *chromatogram*.

This process can be used to separate various chemicals that are contained within a single substance or structure, such as the many different *pigments* in a plant’s leaf cells.

The cells of living things contain many different forms of chemicals including many varieties of proteins. Pigments are proteins that can absorb energy from the sun. People have a pigment in their skin called *melanin* that helps protect them from the sun’s rays.

Plants have a variety of light-trapping pigments to help them gather the energy they need to perform their food-making process, known as *photosynthesis*.

Paper chromatography can be used to find out which pigments might be in the structure of a particular type of plant leaf, since not all species possess identical types of pigments.

**PROCEDURE**

- Cut a narrow strip of filter paper and trim one end to a point as shown in the diagram above.

- Hook the flat end of the paper to the paper clip hanging from the cork.

- Place the paper strip on a clean paper towel. **DO NOT** touch the pointed end of the paper strip.
• Draw a faint pencil line just above the pointed end of the paper.
• Dot a small amount of pigment extract at one point on the line as shown in the diagram above.
• Allow the dot to dry then apply more pigment to build up the dot. Let each application of the pigment dry before adding the next.
• When the pigment spot is dark and dry add 5 ml of chromatography solvent to the test tube.
• Carefully suspend the paper strip into the test tube containing the solvent. Make certain that only the pointed tip of paper located below the dot of pigment touches the solvent.
• DO NOT let the solvent touch the dot of pigment.
• Allow time for the solvent to move up the paper strip. When the entire paper is wet with solvent remove the paper strip and allow it to dry on a paper towel.
• Measure the distance from the bottom of the spot to the top of each color streak.
• Measure the entire distance traveled by the solvent.
• Record your observations on Chart #1.

The **Rf value** of a pigment is the speed with which the pigment moves over the paper when compared to the speed of the solvent.

\[
R_f = \frac{\text{Distance moved by one individual pigment}}{\text{Distance moved by the solvent}}
\]

The possible pigments that can be extracted from this particular type of leaf are (1) carotenes (orange), (2) xanthophylls (yellow), (3) chlorophyll b (yellow/green), (4) chlorophyll a (blue/green) and (5) anthocyanin (red).

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<th>COLOR OF PIGMENT</th>
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