Follow-up Activities

- Ask students to research other separation technologies and their many applications in research and industry, and report their findings to the class. Examples include high-pressure liquid chromatography (HPLC), ion-exchange chromatography and capillary electrophoresis.
- Using paper chromatography, have students compare pigments from a plant with multicolored leaves to pigments from a plant that has pale leaves. If possible, have them elute a specific band for analysis in a spectrophotometer. The following Web site of The Association for Biology Laboratory Education provides a protocol for the thin layer chromatographic separation as well as the spectrophotometric analysis: academic.bowdoin.edu/bio/grants/spec/speclinks.shtml.
- For a given plant, compare thin layer chromatograms with paper chromatograms of the same pigments. Are the R_f values constant for each pigment? Have students discuss the advantages and the disadvantages of each method.
- Introduce students to other tools used by forensic scientists to investigate crime scenes. If possible, take a field trip to a crime laboratory or invite a forensic scientist in to speak to the class about his or her education and career.

Suggested Internet Resources

Periodically, Internet Resources are updated on our Web site at www.LibraryVideo.com

- **thechalkboard.com/Corporations/Dow/Programs/NSTA_Lessons/T-shirt.html**
  This NSTA site contains an interesting high school chemistry lesson plan that allows students to use the process of ink chromatography to make tie dye t-shirts.
- **pubs.acs.org/journals/chromatography/index.html**
  "Chromatography– Creating a Central Science" is an online supplement produced by the American Chemical Society that contains an overview of the history of chromatography in all its variations.
- **antoine.frostburg.edu/chem/senese/101/matter/chromatography.shtml**
  "General Chemistry Online!" explains some of the many ways chromatography is used to separate mixtures.
- **science.csustan.edu/tutorial/color/index.htm**
  This Web site developed by the University of California at Stanislaus explains how to analyze ink samples via chromatography and gives basic information about the science of colors.

(Continued)

- **www.pharm.uky.edu/ASRG/HPLC/hplcmytry.html**
  This User's Guide to HPLC gives the history of the development of high-pressure liquid chromatography and explains the theory behind the technology as well as listing many applications.

Suggested Print Resources

- Snyder, Carl. *The Extraordinary Chemistry of Ordinary Things.* Wiley Publishers, New York, NY; 1997.
- Bodner, George. *Chemistry: An Experimental Science.* Wiley Publishers, New York, NY; 1995.
- Lee, Henry. *Cracking Cases: The Science of Solving Crimes.* Prometheus Books, Amherst, NY; 2002.

TEACHER'S GUIDE CONSULTANTS

Dr. Robert Orr
Professor of Chemistry
Delaware Valley College of Science & Agriculture

Paula J. Bense, M.Ed.
Schlessinger Media
Curriculum Specialist

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SCIENCE LAB Investigations!

CHROMATOGRAPHY

Grades 9–12

Instruction in both scientific ideas and processes is necessary for students to have a well-balanced science education. By practicing the skills of science while solving everyday problems, students will learn to raise good questions and find accurate answers about the objects, forces and organisms in their world.

Inquiry-based teaching and learning goes well beyond the traditional scientific method to focus not only on engaging students in the "doing" of science, but also in thinking of science as an active process that allows them to focus on their own questions as they develop the ability to plan and execute a scientific investigation. Students' ability to use a variety of technologies should be an integral component of all scientific investigations. Mathematics should also play a role in all aspects of scientific inquiry. Students find that these skills empower them to engage in problem solving in all areas of their lives.

At various points throughout the video, there are opportunities for the educator to actively involve students in the topic by expanding upon the onscreen discussion or by recreating the investigations.

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Background

Everything around us — the earth, the oceans and the air — is a vast mixture of materials. Even living organisms are made up of thousands of different substances: proteins, sugars, fats, salts and all sorts of other compounds. By separating and purifying those components, scientists are able to learn a great deal about the world around us. One of the most powerful analytical techniques for separating compounds is a process called chromatography. This technique is used to isolate proteins, DNA and other molecules vital for life. It can be used to separate and purify components of food and is a critical tool for solving crimes. A Russian scientist, Mikhail Tswett, realized the power of this technique in the early 1900s. He used it to separate pigments found in plants, and named the technique after the Greek words for "color" and "writing."

Chromatographic separation has two parts that come in contact with one another — a stationary phase that stays still and a mobile phase that moves. In paper chromatography, the paper is the stationary phase and a liquid solvent is the mobile phase. Due to the fact that the molecules of each compound have distinct properties such as size and polarity, different components of the mixture travel at different rates through the mobile phase. The tendency of compounds to stick to the stationary phase or move with the mobile phase is called affinity, and how much the components separate is based on how strong the affinity is for each phase.

A common laboratory experience is to extract the colored compounds from a leaf and separate them via paper chromatography. When this is done in an ideal solvent, a number of pigments can be identified by calculating the R_f values for each colored band. This is done by measuring the distance the band traveled from the spot where the leaf solute was first placed and dividing that number by the distance the solvent traveled from the origin. This ratio should remain constant for each unique pigment as long as the chromatogram is run under a set of given conditions. Since many of the nonpolar mobile phases used to separate plant pigments are toxic and flammable, working with solvents is best done under a fume hood and with gloves and goggles.

Ink consists of many pigments that may be either soluble or insoluble in water. Ink that is insoluble in water is referred to as indelible ink. The separation of water-soluble ink (a polar mixture) is accomplished using water because it is a polar solvent. Indelible ink is a mixture that is significantly less polar than water and must be separated with a relatively nonpolar solvent. Using paper chromatography, students analyze a number of brands of ink and discern which brand was used to make a mark on a piece of paper. They further their investigation by performing the separation using a number of different solvents to discover the mobile phase that gives the best separation. The separation patterns produced by different brands of pen and different solvents are governed by several factors: the composition of the ink, the solubility of each component dye in the mobile phase and the extent to which the ink clings to the stationary phase. ***(Continued)***

Chromatography can also be a powerful tool used to separate and purify colorless compounds as well as pigments. In some instances, the substance being separated can be detected with ultraviolet light; others can be sprayed with a reagent to detect the bands once the chromatogram is completed. In fact, applications for this technique include the analysis of pesticides in drinking water, the detection of drugs in a person's urine and the presence of antibodies of a disease in blood samples.

Vocabulary

chromatography — The separation of a mixture into its components by taking advantage of the components' different affinities for a stationary phase and a mobile phase.

mixture — A combination of two or more substances in which the substances retain their chemical identity.

stationary phase — The immobile phase involved in the chromatographic process. In paper chromatography, the stationary phase is the paper.

mobile phase — The moving phase involved in the chromatographic process. In paper chromatography, the mobile phase is the solvent.

affinity — A measure of the strength of binding of one molecule to another.

solvent — The liquid in which the components of a mixture are added prior to separation.

polar — Possessing a partial positive or negative charge; polar molecules are easily dissolved in water.

nonpolar — Possessing a neutral charge; nonpolar molecules are not easily dissolved in water.

chromatography paper — High quality cellulose paper used as the stationary phase in chromatography, created to be very uniform.

solvent front — The distance moved by the mobile phase.

capillary action — The spontaneous movement of a liquid into thin tubes or fibers; determined by adhesive forces, cohesive forces and surface tension.

R_f (retention factor) — The distance moved by a compound from its original spot divided by the distance moved by the mobile phase (the solvent). R_f values remain constant for a component under similar experimental conditions and allow scientists to compare results from different chromatograms.

beta carotene — An orange plant pigment that is carried along near the solvent front.

xanthophyll — A yellowish pigment that is found further from the solvent front because it is less soluble in the solvent and has been slowed down by hydrogen bonding to the cellulose. ***(Continued)***

chlorophyll — The green primary photosynthetic pigments in plants, which bind more tightly to chromatography paper than other pigments and therefore travel less distance relative to the solvent front.

thin layer chromatography — A chromatographic separation technique that involves a stationary phase of plastic or glass that is coated with a layer of aluminum or silica gel. Thin layer chromatography is often used to isolate naturally occurring compounds and has an advantage over paper chromatography because stronger solvents can be used as the mobile phase.

column chromatography — Any form of chromatography that uses a column or tube to hold the stationary phase. The components of the mixture separate because they travel through the tube at different rates, determined by the affinity of each to the stationary material.

gas chromatograph — An analytical instrument that rapidly heats and vaporizes a sample and then a stream of gas carries it along a column that contains a stationary phase. The sample becomes distributed between the mobile gas phase and the stationary phase, and the higher a substance's affinity for the stationary phase, the more slowly it comes off the column. The substances are detected as peaks on a chart recorder.

Discussion Topics

1. Why do some stains migrate in water and others do not?
2. What makes the solvent move along the paper?
3. Why do some spots move with ethanol as the solvent, but not with water?
4. In chromatography, what is the difference between the stationary phase and the mobile phase?
5. What is meant by the phrase "like dissolves like?"
6. How does affinity affect the way a component travels up the chromatography paper?
7. What is the difference between paper chromatography and thin layer chromatography?
8. Why is it important to cover the solvent in the developing chamber and allow it to sit for a time?
9. Why is it important to use a pencil when drawing the origin line on the chromatography paper?
10. What will happen if the chromatogram is left in the developing chamber too long?
11. Why should you work with developing solvents under a fume hood?
12. Would you expect the R_f value of a pigment to be the same if a different solvent were used? Explain.