## Column Chromatography Of Plant and Leaf Pigments

When fall arrives, students often wonder why leaves turn different colors. Column chromatography provides a means to show that the colored plant pigments seen in the fall were actually present in the leaf year 'round but were masked by the dominant green coloration of chlorophylls.

Materials and apparatus needed:

Neutral, chromatographic-grade alumina
Sand
Glass wool
Chromatography column, glass, 13.3/mm interior diameter and 300/mm long (a 50-ml buret is fine)
Food blender or knife and large mortar and pestle
Wide-mouthed, screw-cap jar
Funnel for column
2:1 solution of benzene–methanol
Quantities of assorted leaves or food vegetables (lettuce, cabbage, celery, spinach, carrots, etc.)
Distilled water
Separatory funnel
Wooden dowel tamping rod

To extract pigments, begin by mixing a sizeable quantity (50/gm) of each plant sample with 200/ml of the 2:1 benzene–methanol solution (Ventilation!) in a food blender or large mortar and pestle after partially dividing with a knife. Mince each sample well. The benzene is used to dissolve the plant pigments; and, because water is immiscible with benzene, methanol is used to ensure homogeneity due to the high water-content of the plant samples. Transfer the minced plant sample and solution to a wide-mouthed, screw-cap jar. Tumble it for 15 to 20 minutes to extract the pigments. Roughly filter the resulting colored solution through a wire or paper filter to remove most of the solid.

Transfer the filtered solution to a separatory funnel and add 100 ml of distilled water. This will remove the methanol. Discard the aqueous layer. A benzene solution of colored pigments suitable for chromatography remains.

To prepare the column, clamp it in a vertical position with the stopcock closed. Using a wooden dowel as a tamping rod, push a wad of glass wool into the bottom of the column. This will keep the sand and the sorbent out of the stopcock. Cover the wool with about a 6.5-cm layer of sand, using a funnel. Pour dry alumina into the column to within 7.5 cm of the top. Tamp it down and add more until it is tamped to within 7.5 cm of the top. After opening the stopcock, add pure benzene to the top of the column until it percolates through the stopcock. Close the stopcock after adding an additional 50–100 ml of benzene and allowing it to run through. Leave 2.5 cm of benzene in the top and stopper. The column should appear as in the figure.

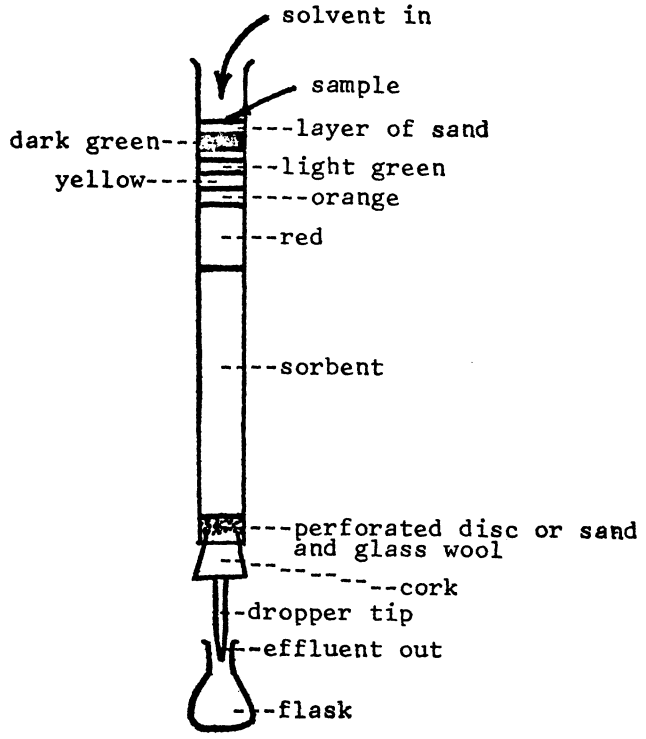
If the benzene extract appears light in color, it may be necessary to concentrate it before applying it to the column. This can be done by carefully evaporating it to 5–10 ml by heating it in a beaker of hot water (Ventilation or hood!). After it has been concentrated, carefully add it to the top of the column by means of a small pipet or a medicine dropper. After it has soaked into the sand, add pure benzene to begin elution. This can be done by hand; or a small dropping-funnel containing the benzene can be supported atop the column—but be sure its flow rate is adjusted to prevent the column from running dry.

After a time, different-colored bands of pigments should be visible on the white column.

If it is desired, the continued addition of benzene will allow the elution and collection of each of the pigments one at a time, and thus their isolation from a biologic mixture.

Questions and further points:

1. Why is this an example of adsorption and not partition chromatography?




**Typical chromatographic column,** showing the separation of pigments obtained from a lettuce extract.

2. How could one adequately determine which pigments dissolve in methanol?

3. Using different laboratory groups, determine the pigment compositions of as many different leaves and plant foods as possible.

4. It is possible to obtain commercially $\beta$-carotene and other plant pigments. Dissolving these in benzene and chromatographing as above would provide interesting qualitative and quantitative comparisons.

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## An Experiment with Color Adaptation

The manner in which protective coloration ensures the survival of a species is a topic dealt with in most biology classes. Usually, examples of organisms that display this evolutionary adaptation to their environment are presented to students through pictures or through the use of preserved specimens and samples of their environments. Both methods fail to involve the student actively and do little to further his understanding of the dynamic nature of the process.

The experiment described here enables students to discover for themselves, in the classroom, the principle of survival through color adaptation. This experiment requires a tame bird of any species, a chessboard, brown pebbles, gray pebbles, and equal quantities of birdseed dyed gray and brown. The birdseed can be dyed by using conventional food coloring and referring to the color charts printed on the package.

The bird is fed dyed birdseed for a week and then is given no food for the six hours preceding the actual experiment. The chessboard is placed in the center of a large table, and the brown and gray pebbles are placed on the dark and light squares, respectively. The students then place 20 grains of seed (10 brown and 10 gray) randomly on the 16 squares of the board. During the experiment the bird is tied with a light string approximately 1 m long that has one end fastened to the corner of the board and one end attached to its leg. The bird is allowed to eat any birdseed it can find on the board during a two-minute trial. After the trial the student should determine and record the number of color-adapted seeds (brown seeds on brown squares or gray seeds on gray squares) and non-color-adapted seeds (brown seeds on gray squares or gray seeds on brown squares) the bird has eaten.

The student should randomly place a total of 10 brown and 10 gray seeds on the board for each trial. The data obtained from a series of 10 trials can then be used to determine the survival value of color adaptation.

Students may wish to extend the procedures to an experiment that uses insect larvae of different colors placed on the appropriate backgrounds.

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## "What If . . ."

As a biology teacher, how often have you been asked a question by your students beginning with those all-encompassing words, "What if"? What if no humans died on earth? What if man found a cure for all diseases? What if man could create life? What if life is found on Mars? What if scientists were able to control all environmental conditions on earth?

Most students are concerned with "what if" questions, but quite often teachers treat these questions lightly. Some teachers probably recognize that much class time could be spent discussing this kind of question, at the expense of "covering" the prescribed course-content. Other teachers may assume that many questions asked by students are irrelevant within the framework of the present-day curriculum. Still others may feel unsure of themselves in dealing with broad, open-ended questions.

An analysis of various "what if" questions would reveal a futuristic quality among them. There is an apparent contrast between (i) the questions asked by teachers, those written by curriculum writers, and the ones found in biology textbooks and curriculum guides and (ii) the questions proposed by students. If one surveys popular biology textbooks and curriculum guides, the absence of future-directed questions becomes quite obvious. I believe that teachers deal almost exclusively with the past; but students, with their "what if" questions, look to the future.

Neil Postman and Charles Weingartner (1969: *Teaching as a Subversive Activity,* Dell Publishing Co., New York) have said that "future-orientation is essential for everybody. Its development in schools is our best insurance against a generation of 'future shock' sufferers" (p. 203). Paul DeHart Hurd recently pointed out: "Students should leave a biology course feeling that their future will not be like the past—recognizing they will never live in the kind of world in which their teachers were educated and knowing they will never experience the world of their parents" (1971: "Biology as a Study of Man and Society," *American Biology Teacher* 33 [7]: 397-400, 408). Alvin Toffler, in *Future Shock* (1970: Random House, Inc., New York), has said: "If our children are to adapt more successfully to rapid change . . . We must sensitize them to the possibilities and probabilities of tomorrow. We must enhance their sense of the future" (p. 423).

What can biology teachers do to guard against a generation of future-shock sufferers? We may begin