

THE EXTRACTION AND IDENTIFICATION OF ARTIFICIAL FOOD COLORS

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OBJECTIVE

In this experiment, artificial food colors will be extracted from foods by use of a liquid ion exchange resin (LA-2 Amberlite) and paper chromatography will be used to separate and identify the synthetic food colors.

INTRODUCTION

Artificial food colors are added to many foods to increase their visual appeal. Most individuals are familiar with their use in candies, other sweets, and soft drinks, but may not be aware of their widespread use in foods such as cheese, butter, and various prepared foods. A short term effect of food additives, and particularly food colors, is hyperactivity in some children, believed to be a form of an allergic response. In the long term some of these foods colors are believed to be carcinogenic and some colors, mostly reds, have been banned from food use. It is not the purpose of this experiment to explore the controversy surrounding food colors, but rather to see how they can be extracted from foods and identified.

SAFETY PRECAUTIONS AND DISPOSAL

Goggles or safety glasses must be worn at all times in the laboratory.

Hexane is flammable. Keep it away from heat or open flames. Vapors may irritate the respiratory system.

Acetic acid vapors irritate the respiratory system. The vapors and liquid irritate and burn the eyes. Concentrated solutions burn the skin. In the event of contact, flood affected areas with cold water.

Ammonia vapors irritate all parts of the respiratory system. The vapors and solution can cause severe eye burns. The solution burns the skin. Work in a well ventilated area. Keep the container closed when not in use. In the event of contact flood affected areas with water.

When shaking a separatory funnel, hold the stopper in place and release the internal pressure venting the funnel every few shakes. The separatory funnel is vented by holding it tip upward, at a slight angle, pointing it away from yourself and everyone else, and opening the stopcock.

DISPOSAL

Dispose of all materials in the proper waste containers which are provided in the laboratory.

EXPERIMENTAL PROCEDURE

A. Extraction of Synthetic Colors From Food

1. Foods that can be used:

Artificially colored beverages (such as grape or orange soda), highly colored gelatin desserts (Jello), highly colored hard candy, gelatin candy (Jelly beans, Dots, etc...), or colored candy-coated materials (M&M's, candy-coated gum, etc...).

Note: Do not use white or black colored materials. White colored materials do not contain artificial colors and black colors are often achieved by using carbon.

2. Apparatus needed:

10 mL graduated cylinder
100 mL graduated cylinder
separatory funnel (250 or 500 mL)
400 or 600 mL beaker
20 or 50 mL beaker
rule, graduated in mm

3. Reagents needed: (all solutions prepared by mass)

10% solution of LA-2 Amberlite resin (Rohm and Haas Co.) in n-butyl alcohol.
1% acetic acid solution, $\text{HC}_2\text{H}_3\text{O}_2$
concentrated ammonia, NH_3
saturated ammonium sulfate solution, $(\text{NH}_3)_2\text{SO}_4$ (in a dropping bottle)
hexane, C_6H_{14}
concentrated acetic acid (in a dropping bottle)
blue litmus paper

4. Procedure:

Record all observations and measurements in your laboratory notebook in ink.

Use any one of the following:

About 25 mL of artificially-colored beverage.

A solution of 2 grams gelatin dessert in 20 mL hot water.

A solution made by soaking 2 or 3 pieces hard candy or gelatin-type candy in 20 mL of 1% acetic acid. (Do not mix different brands of candy.)

A solution of the candy-coating made by soaking 2 pieces of gum or 5 pieces of M & M's in 10 mL of 1% acetic acid solution. (Note: soak candy long enough to dissolve the color off of the candy coating, trying to dissolve as little of the white candy coating as possible. Discard any solid candy pieces remaining.)

The color solution should have a fairly intense color.

Pour the color solution, as prepared above, into a separatory funnel supported on a ring support. Add 10 mL of 10% LA-2 Amberlite resin solution to the separatory funnel, stopper it, and shake gently until the color is transferred to the upper layer (resin in butyl alcohol). Place the separatory funnel on the ring support and allow the layers to separate. (See Figure 1) If the solution does not separate into two distinct layers within about 1 to 2 minutes, then an **emulsion** has formed. The emulsion can usually be separated by adding 5 to 10 drops of saturated ammonium sulfate solution, $(\text{NH}_4)_2\text{SO}_4$, to the solution, dropwise, with gentle swirling. If separation does not occur, additional ammonium sulfate solution should be added in 5 drop increments. Draw off and discard the lower layer.

NOTE: If an emulsion formed in the above procedure, there is

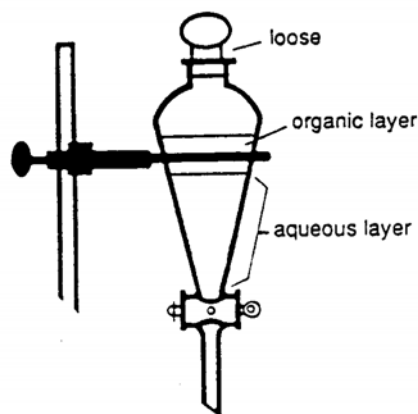


Figure 1. The separatory funnel.

a high probability that you will have an emulsion form in the following steps where the resin solution is washed with water. In each case, the emulsion can be separated using the saturated ammonium sulfate solution. The ammonium sulfate solution is acidic and will cause the blue litmus paper to turn pink.

Wash the solution remaining in the separatory funnel by adding 10 mL of distilled water, shaking, letting the layers separate, and then draining off the water layer (the lower layer) into a clean beaker. Test the water with blue litmus paper. If the litmus paper turns red, add 10 mL of fresh distilled water to the separatory funnel and repeat the washing procedure, above. If the water from the second washing is still acid, repeat the washing with another 10 mL of fresh distilled water. Draw off and discard the water layer. (Note: Do not wash the solution more than a total of three times or too much of the food color may be lost.)

Add 20 mL of hexane to the colored resin layer remaining in the separatory funnel, shake, allow the separatory funnel to stand for 1 to 2 minutes, and discard any water that separates. The hexane is used to remove traces of water from the solution so that the final food color will not be too diluted.

Extract the color from the resin layer by adding 1 mL of concentrated ammonia solution, NH_3 , and shaking gently. (Ammonia causes the food color to be released from the resin.) All the color should now be concentrated in the lower layer; if it is not, shake again. If the color is still not concentrated in the lower layer, add another 1 mL of concentrated NH_3 and shake again. Collect the color solution in a small clean beaker. (If Blue No. 2 is thought to be present, add concentrated acetic acid to the color solution dropwise, testing with litmus, until it is neutral - only a few drops will be required. This will prevent this color from fading.) Save the color solution for Part B of this experiment and do not discard it until the chromatographic separation is complete.

B. Chromatographic Separation of the Unknown Color Mixture

1. Reagents needed:

- 1% sodium chloride solution, NaCl (by mass)
- 0.1 % solutions of F D & C certified colors

2. Apparatus needed:

- Chromatography paper (Whatman No. 1 or equivalent), 10 cm x 20 cm
- Capillary tubes (open both ends)
- 600 mL beaker
- Plastic wrap (Saran Wrap or similar) or watch glass to cover beaker
- Stapler with staples
- Food color standard solutions: 0.1% solutions of current, permitted, F D & C colors

3. Procedure:

Obtain a sheet of chromatography paper. Draw a light **pencil** line across the paper about 1.0 cm from the bottom of the sheet. Using a capillary tube, pointed glass rod, or capillary-tipped dropper, apply a small spot of the unknown color mixture (obtained in Part A of this experiment) on the pencil line near the center of the paper. (The color spot should not exceed about 0.4 cm in diameter.) Mark the paper, in **pencil** at the top, above the spot with an **X** to signify the unknown color mixture (see Figure 2). If the spot seems to be too small or too light in color, you can make it darker by applying a second spot of color directly on top of the **dry** first spot. (It is necessary to allow the spot to dry between applications of color in order to keep it small in size.) If the spot is still seems to be too light in color, additional spots can be applied on top of the dry color spot.

Spot the paper with each of the available F D & C colors allowing about 2.0 cm minimum distance between each different spot. Note the identity of each color at the top of the paper above each spot.

Add 1% NaCl solution to a clean 600 mL beaker to a depth of about 0.5 cm (this will require approximately 25 to 30 mL of solution). Bend the spotted chromatography paper into a cylindrical shape, **butt** the ends together (do not overlap the ends) and staple them. Place the chromatography paper into the beaker making sure that the spots of dye are not below the solvent level and that the paper is not touching the sides of the beaker. Cover the beaker with a piece of plastic wrap or with a watch glass. (See Figure 3)

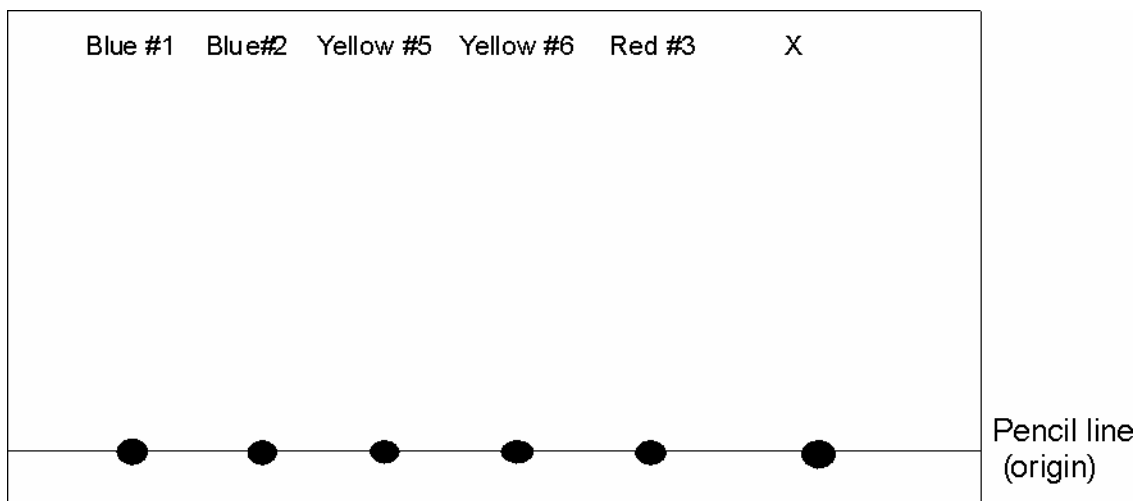


Figure 2. Chromatography paper showing pencil line, spots of color, and identification information

Allow the solvent to move up the paper to within 1 cm of the top (this will take anywhere from 15 to 35 minutes). Remove the paper from the beaker, open it flat, and, using a pencil, mark the solvent front (the level reached by the solvent). Lay the chromatography paper on a paper towel to dry.

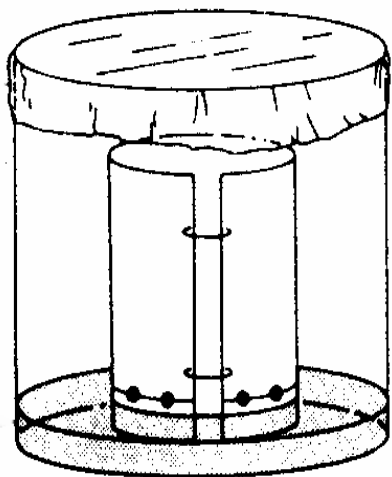


Figure 3. Chromatography paper in the 600-mL beaker developing chamber.

Outline each spot on the chromatography paper in pencil. Measure and record the average distance from the origin to the solvent front. Measure and record the distance each spot moved from the origin. (See Figure 4) Determine the R_f values for each spot in the unknown mixture and for each of the F D & C colors that you used. The R_f values for the components in the unknown mixture will be the same for the same component in the F D & C known colors. By matching both the color and the R_f value of each of the spots, you can determine the identity of the colors you extracted from the food.

Save the chromatogram to hand in with your laboratory report.

CLEAN UP

Clean up the separatory funnel with soapy water. Use a brush to help remove traces of organic materials from its interior walls. Rinse well with water before returning the separatory funnel to the central supply area.

Clean up all beakers and glassware with soapy water. Use a brush to help remove traces of organic materials from the glass surfaces. Rinse well with water.

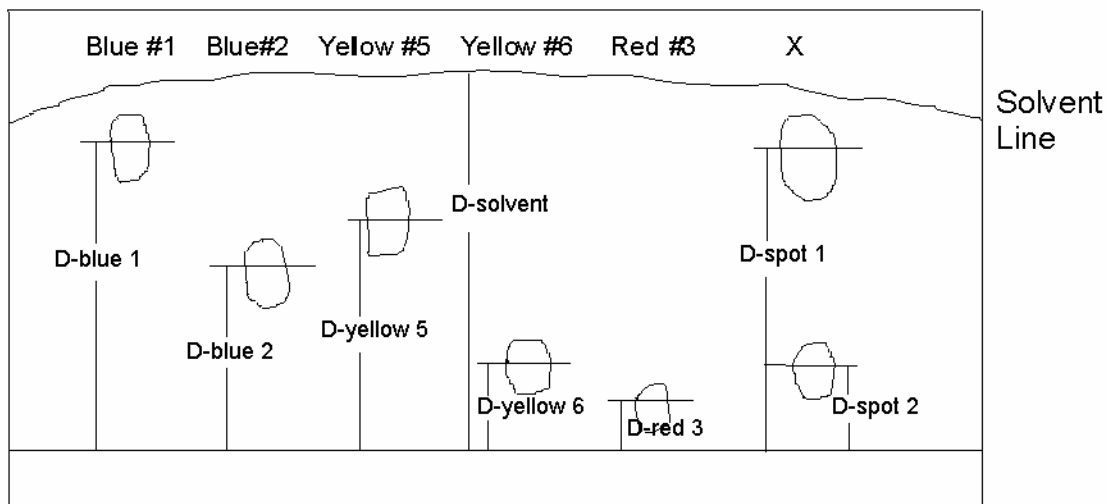


Figure 4. Chromatography paper showing how spots may have moved with solvent line and measurements of distances spots and solvent moved from original pencil line.

Calculations

The R_f value for a spot is calculated using the formula:

$$R_f = \frac{\text{distance spot moved in cm}}{\text{average distance to solvent line in cm}}$$

The R_f value will be a decimal fraction

References

“Identifying Synthetic Colors in Food”, **Chemistry**, **42** (11), 27-8, Dec., 1969

Graichen, Charles, “Quantitative Determination of F D & C Colors in Foods”, Division of Colors and Cosmetic Tech., Food and Drug Administration, Washington, D.C.

THE EXTRACTION AND IDENTIFICATION OF ARTIFICIAL FOOD COLORS

Name _____ Course/Section _____

Partner's Name (If applicable) _____ Date _____

DATA AND RESULTS

Foodstuff used (include brand name): _____

Color(s) of original food: _____

Color of NH₃ extract (at the end of procedure, Part A): _____

Average distance traveled by solvent from the pencil line: _____ cm

Distance traveled, from the pencil line, by spots of known F D & C colors:

<u>F D & C color</u>	<u>Distance traveled</u>	<u>R_f value of spot</u>
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____

Distance traveled, from the pencil line, by spot(s) (i.e., colors) in unknown:

<u>Color of the spot</u>	<u>Distance traveled</u>	<u>R_f value of spot</u>
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____
_____	_____ cm	_____

Identity of colors in unknown: _____

Show a sample calculation for an R_f value for one of the colors above:

QUESTIONS

1. Why is it important to keep the size of the color/cation spots small when spotting the chromatography paper?
2. Since the colors move at different rates up the chromatography, do you think that this rate of migration could be used as a chemical “fingerprint” for color identification? (explain your answer)
3. Of the possible factors affecting the chromatographic separation of mixtures, which single factor would most probably be responsible if you obtained different R_f values on the same sample when it was tested at different times?
4. Why is it useful to record the initial color of the food color mixture?
5. What changes in the procedure would be necessary if the original food used was only lightly colored?
6. If you were to find a color in a food that did not appear to be one of the F D & C approved colors, what action would you take?