THE EXTRACTION AND IDENTIFICATION OF ARTIFICIAL FOOD COLORS

Teacher Notes

This is a laboratory experiment designed for students working singly or in groups of two.

This procedure is based on a procedure used by the FDA to determine the identity of colors in food products. The FDA uses thin layer chromatography, not paper chromatography.

Food materials must be in their original packages with labels intact so students can determine which colors are present in that particular foodstuff. It is recommended that candies be purchased, when possible, in small snack bags rather than large bags. Save the large bags that the candies are packaged in so that all the ingredients and nutritional information is available.

The initial color is removed from the food materials using water or slightly acidic water. Care should be exercised to remove the color from candy coated foods and not the candy coating, which contains emulsifiers. Student tend to soak the food materials too long. Inform the students that they should gently swirl the candies in the water or 1% acetic acid solution and that they should decant the liquid from the food as soon as they can see the white layer under the color.

The means of extraction is by use of a liquid ion exchange resin, Amberlite LA-2 in n-butyl alcohol.. This will remain as a separate organic layer in the separatory funnel. (Amberlite LA-2 ion exchange resin is available from Rohm and Haas Company, Philadelphia, PA.)

Demonstrate how to shake and vent a separatory funnel.

After initial mixing, the layers should separate within one to two minutes, with most of the color in the upper organic layer. If it does not separate, then an emulsion has formed and saturated ammonium sulfate solution must be added to salt out the emulsion. It will be necessary to swirl, or to gently shake, the solution after the addition of the saturated ammonium sulfate solution. Depending on the amount of emulsifier that dissolved in the original extraction liquid, it may take several additions of the ammonium sulfate to break the emulsion.

The addition of ammonium sulfate will make the solution acidic and it will turn the blue litmus paper red, thus, washing of the extraction liquid with water is limited to 3 total washings.

After the final washing, hexane is added to the mixture in the separatory funnel to displace any remaining water from the solution.

The food color is eluted from the ion exchange resin by addition of concentrated ammonia. Generally, 1 mL of ammonia will be sufficient, if the majority of the acid has been removed. Almost all the color will be concentrated in the ammonia layer after shaking.

The easiest way to prodvided the F D & C colors to a class of students is to place approximately 5 mL of each of the standard F D & C colors in small, labeled 25 or 50 mL beakers at some central location in the laboratory (I usually place them under a hood) with a capillary tube (opend both ends) in each beaker. Students use these solutions to spot their chromatography paper. (There should not be a long line-up at these beakers as students tend to complete their extraction procedures at different times. Also, if students are working in groups of two, one member of the group can spot a sheet of chromatography paper while the other member of the group is working on the separation of the food color.)
Demonstrate how to spot the chromatography paper using the capillary tubes in the F D & C colors.

Even if the student’s final color solutions appear dark, they may need to spot their chromatography paper several times with this solution. Instruct the students that they must allow the spot of color to dry before placing another drop of solution on top of the spot. After separation, the extracted colors may still be light in intensity.

The extracted colors may not move the same distance as the pure F D & C standards in the separation. There will be sufficient movement that the colors can be compared between the F D & C standards and the colors extracted from the foods used.

Students should use both the visual colors and the $R_f$ values to identify the colors in their “unknowns”.

A long range UV lamp may be used to help see some of the separated food colors.

**Laboratory Preparation Notes:**

**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...)
- Colored candy-coated materials (M&M’s, Skittles, 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.

**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

**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, HC$_2$H$_3$O$_2$
- concentrated ammonia, NH$_3$
- saturated ammonium sulfate solution, ($\text{NH}_3$)$_2\text{SO}_4$ (in a dropping bottle)  
  Note: there should be a small amount of ammonium sulfate undissolved on the bottom of the bottle.
- hexane, C$_6$H$_{14}$
- concentrated acetic acid (in a dropping bottle)
- blue litmus paper
ANSWERS TO QUESTIONS

1. Why is it important to keep the size of the color/cation spots small when spotting the chromatography paper?

   If spot is too large, there will not be an adequate separation of a mixture. Also, the large spot may extend below the solvent level, coloring the solvent.

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)

   The rates of migration should be similar each time. This, along with the visual color, could constitute a chemical “fingerprint.”

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?

   Temperature and evaporation of solvent, resulting in different rates of flow, would be the main reason. Also, impurities in the solution can affect the separations.

4. Why is it useful to record the initial color of the food color mixture?

   The original color should give some clues to the colors in the food.

5. What changes in the procedure would be necessary if the original food used was only lightly colored?

   Use a larger amount of food material to get more color.

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?

   Retest to confirm. Once confirmed, contact the company and/or the FDA