Student Manual

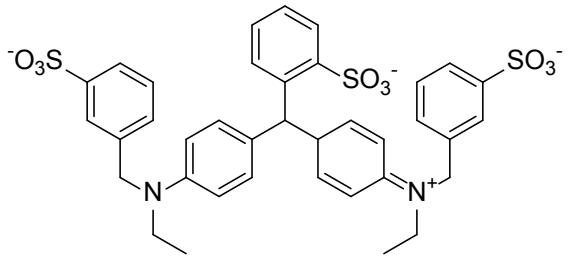
Background

How do you pick the foods you eat? The appearance of the food is a major deciding factor. For example, color can tell you whether a fruit is ripe or whether meat has spoiled. But the effect of food color goes much further than just helping to choose which food is fresh. Color affects our perception of how food tastes. For example, in taste tests, consumers may think that purple colored grape soda tastes better than uncolored grape soda, even though the two sodas are identical except for their color.

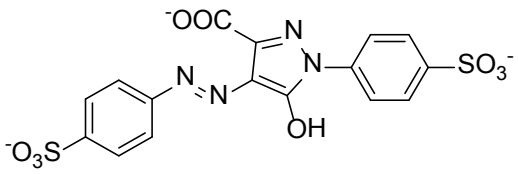
Use of artificial colors in food and drink has been around for thousands of years. What is new, however, is that governments now control the substances that can be used to color foods. Before food colors were regulated, many foods were colored with substances that were not safe to consume, such as bread whitened with chalk or cheese made yellow by lead tetroxide. Candy was one of the worst offenders. Because children gravitated to brightly colored sweets (something that has not changed through the years), vivid colors were produced with heavy metal salts, such as copper sulfate (blue), lead chromate (yellow), and mercury sulfide (red).

In the U.S., the Food and Drug Administration oversees the use of artificial coloring agents in food. They currently approve seven food color additives, all of which are petroleum derivatives. In addition, there are a number of natural food colors in use, such as caramel, beet juice, and carmine.

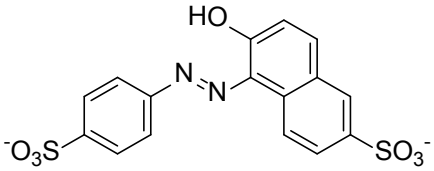
In this experiment, you will extract the food coloring from various candies and then analyze the dyes using agarose gel electrophoresis. In addition to your samples, you will run the four most commonly used artificial food dyes on your gel. The reference dyes are Blue 1, Red 40, Yellow 5, and Yellow 6. The four dyes are similar (see Figure 3), and all are negatively charged at pH 8.



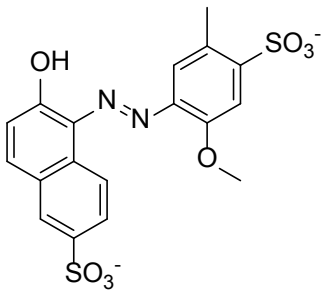
Blue 1



Yellow 5



Yellow 6



Red 40

Fig. 3. Reference dyes used in this experiment.

Molecules can be separated by size using a process known as **gel electrophoresis**. The term electrophoresis means *to carry with electricity*. The mixture of molecules is placed in a small well formed in an agarose gel, which has a texture similar to that of gelatin. A buffer is then poured to cover the gel. When an electric current is applied to the gel, the molecules move in the gel depending on their charge. The negatively charged molecules move toward the positive electrode (red) and the positively charged molecules move toward the negative electrode (black). Any molecules with no charge will not leave the well.

The gel acts as a strainer with tiny pores that allows the smallest molecules to move through it very quickly. The larger the molecules, however, the more slowly they move through the gel. So the electrical charge causes molecules to move depending on their charge and the gel slows down bigger molecules relative to smaller molecules. This allows us to separate them.

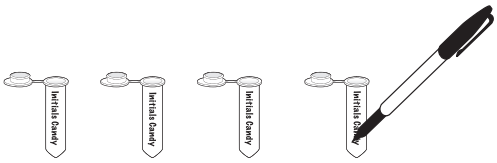
Dye Extraction From Candies

Student Workstation	Quantity
Dye extraction solution	2 ml
2 ml microcentrifuge tubes	4
Microcentrifuge tube rack	1
Marking pen	1
Plastic cups or small beakers	4
Eyedropper	1
Colored candies	4 varieties, 1–4 candies per variety*

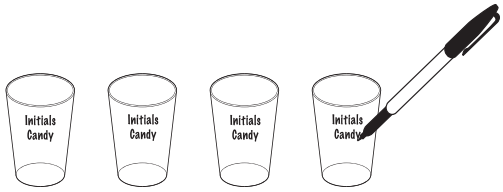
*Candy example: 3 green Skittles, 3 orange jelly beans, 4 Red Hots, 1 brown gumball

Protocol

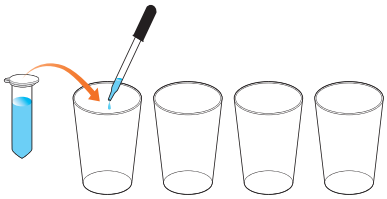
1. Label the four microcentrifuge tubes with your initials and the names and colors of the candies you are using.



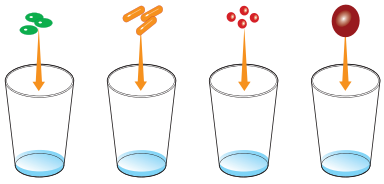
2. Label four cups with your initials and the names and colors of the candies you are using.



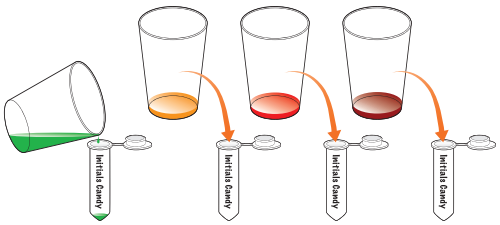
3. Using an eyedropper or pipet, add 0.5 ml of dye extraction solution to each cup. Use the volume marks on the 2 ml microcentrifuge tube to measure the correct volume.



4. Place your candy into the appropriately labeled cup and swirl the candy in the dye extraction solution. If using a candy such as M&M'S or Skittles, just dissolve the color coating off until you get to the white layer of the candy. For all other candies, try to get as dark a solution of dye as possible.



5. Remove your candy from the cup. Pour the solution containing the dissolved colored candy coating into the appropriately labeled microcentrifuge tube.

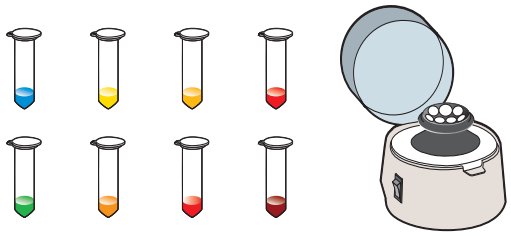


Agarose Gel Electrophoresis

Student Workstation	Quantity
Agarose gel electrophoresis system	1
Agarose gel	1
Power supply (may be shared by multiple workstations)	1
Electrophoresis buffer, 1x TAE	275 ml
Blue 1 reference dye	15 µl
Yellow 5 reference dye	15 µl
Yellow 6 reference dye	15 µl
Red 40 reference dye	15 µl
Dyes extracted from candies from Lesson 1	4 labeled tubes
2–20 µl adjustable-volume micropipet	
or 10 µl fixed-volume micropipet and 8 tips	1
Marking pen	1

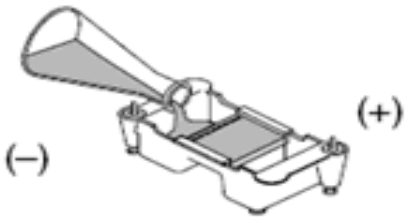
Protocol

1. Prepare your extracted candy dye samples. If a centrifuge is available, pulse spin the microcentrifuge tubes in the centrifuge to bring all the liquid to the bottom of the tube and to settle any insoluble particles. Spin down your dye standard samples as well, if necessary.



2. Obtain a prepoured agarose gel from your teacher or, if your teacher instructs you to do so, prepare your own gel.

3. Place the casting tray with the solidified gel in it into the platform in the gel box. The wells should be at the (–) cathode end of the box, where the black lead is connected. If the comb is still in the tray, remove it very carefully from the gel by pulling it straight up.

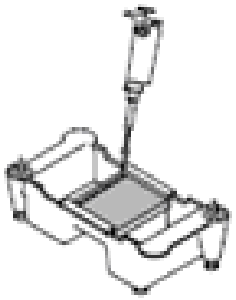


4. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel, using approximately 275 ml of buffer.

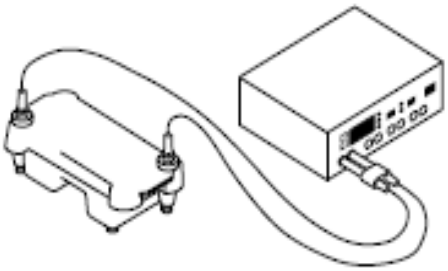
Protocol (cont.)

5. Using a separate tip for each sample, load 10 µl of your reference dyes and candy dye extracts into the gel, with one sample per well. The first sample is loaded in the well at the left hand corner of the gel.

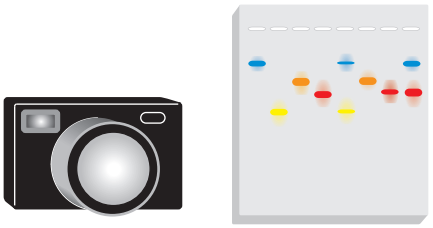
    Lane 1: Blue 1 reference dye
    Lane 2: Yellow 5 reference dye
    Lane 3: Yellow 6 reference dye
    Lane 4: Red 40 reference dye
    Lane 5: Candy 1 dye extract
    Lane 6: Candy 2 dye extract
    Lane 7: Candy 3 dye extract
    Lane 8: Candy 4 dye extract



6. Place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid will align with the red and black jacks on the base. Plug the electrodes into the power supply.
7. Turn on the power supply. Set it for 100 V and electrophorese your samples for 15 min.
8. When the electrophoresis is complete, turn off the power and remove the top of the gel box. Carefully remove the gel and tray from the gel box. Be careful — the gel is very slippery!



9. Take a photograph of the gel for your records immediately.

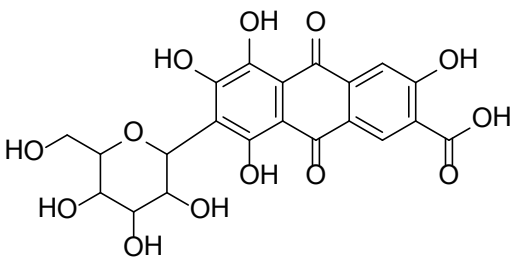


Focus Questions

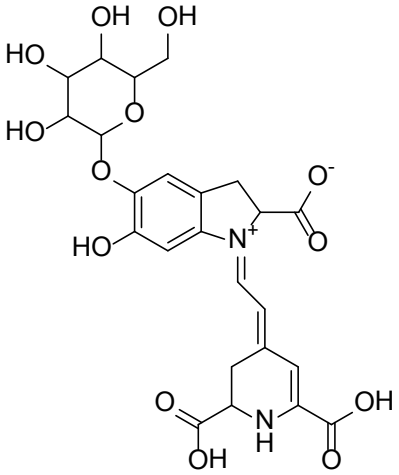
1. When you analyzed the results of your gel, did any of your experimental samples contain dyes that did not match the four reference dyes? For example, did any of your samples produce:
   a. Dye bands that are a different size than any of the reference bands?
   b. Dyes that are a different color than any of the reference bands?
   c. More than one color band?
   d. Dyes that you observed moving in the "wrong" direction (toward the cathode)?

   What might these dyes be?

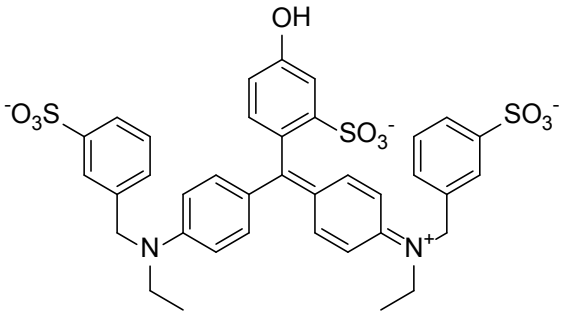
2. Look at the structures of the dyes pictured here. Which of these dyes would migrate similarly to the dyes you examined in this lab? Why?



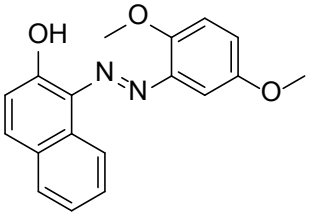
Carminic acid



Betanin (beetroot red)



Fast green FCF



Citrus red 2

3. Many popular dry dog foods and dog treats have FD&C dyes among their ingredients. For example, Beneful dry food contains Yellow 5, Red 40, Yellow 6, and Blue 2, and Snausages Breakfast Bites contain Red 40 lake, Yellow 6 lake, and Yellow 5 lake. (Lake dyes are the insoluble forms of the FD&C dyes.)

   Why do dog food manufacturers put artificial food colors in dog food?

4. Keep a log of the coloring agents in the foods you consume for a 1-week period. What are the most common dyes on your list? Are there any natural dyes on your list?

   List the artificial dyes in a table, and look up natural food dyes that would produce the same color.

FD&C Dye	Color	Natural Alternative	Source of Natural Dye

   Are there any reasons why artificial food colors might be preferable to natural food colors?

5. What two factors control the distance the colored dye solutions migrate?

6. What force helps move the dyes through the gel?

7. What component of the electrophoresis system causes the molecules to separate by size? Explain

8. Agarose electrophoresis is commonly used to separate molecules of DNA. Explain how you expect DNA molecules with molecular weights of 600, 1000, 2000, and 5000 daltons to separate.