Column Chromatography

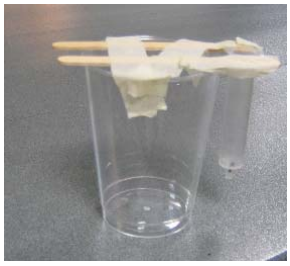
Deconstructing Gatorade

Separate the components of Gatorade using a home-made affinity column.

Materials
activated charcoal – pre-rinse with water to reduce the amount of dust
5ml syringe barrel for your column
2 paper cups
2 popsicle sticks
masking tape
paper towel or coffee filter
hole puncher
3ml graduated plastic pipets
containers to collect fractions – clear or white watercolor palettes work well
20% isopropanol
40% isopropanol
water
Gatorade or other colored drink (purple-colored drinks work well)
(optional) glucose test strips

To do and notice

1. In a paper cup, mix together half a spoonful of carbon with 10ml of the Gatorade. Let it sit for at least 5 min, swirling every so often.



2. Assemble a stand for your column by taping two popsicle sticks parallel to each other across the top of the second cup. Space them so that there is just enough room for the column to sit between them.

3. Use the hole puncher to cut a circular piece of paper towel or coffee filter. Insert the circle into the column so that it covers the hole at the bottom. Set the column between the popsicle sticks and tape to secure.

4. Place your collection container under the column, and pour the carbon/Gatorade mixture into the column. It may be easiest to pour the liquid first and then scrape the carbon into the column with a wooden stirrer. This first fraction is called the "flowthrough".

5. Place the column above the next collection well. Rinse the column by adding 10 ml of water. This fraction is called the "wash".

6. Now that the column is washed, you are ready to begin collecting your samples. Shift the column so that it's above an empty well and add 5 ml of 20% isopropanol. When things are pulled off the column, you are "eluting" them, so this fraction is called "Elution #1".

5. Shift the column over an empty well once more and elute again by adding 5 ml of 40% isopropanol. This fraction is called "Elution #2".

6. Look at the color of each fraction. How do they compare with the color of the Gatorade you started with? Can you guess what some of the ingredients of Gatorade are?

5. (optional) Use glucose test strips to measure the glucose content in each fraction. Which well has the most sugar?

What's going on?

This activity models the basic principle of affinity chromatography. This technique is used to purify the majority of chemicals as well as a wide range of things from biopharmaceuticals to petroleum products. Gatorade is made up mostly of water, sugar, artificial colors, and flavorings. These compounds all bind to the carbon in the activated charcoal with different strengths or "affinities". Affinity chromatography takes advantage of these different binding strengths to separate out individual components of a mixture. In your activity, the flowthrough fraction may have been dark and cloudy due to residual carbon dust in the charcoal, but there should have been very little color present. This is because the chemical dyes that make up the artificial flavoring have a greater affinity for the carbon than they do for water. The wash fraction should have been relatively clear since the dyes prefer to stay bound to the carbon. Isopropanol has an even greater affinity for the carbon than the dyes to. When it is introduced to the column, isopropanol molecules compete for binding sites on the carbon and knock some of the dye molecules off. You may have noticed that Elution #1 has a bluish tint. This means that some of the blue dye molecules being knocked off of the column. The red dye molecules are smaller than the blue ones and take a little more alcohol to get knocked off, so they come out mostly in Elution #2, which explains the pinkish color. The sugar comes out in the first fraction because it has a much higher affinity for water than for the carbon.

Going Further

The different affinities of each compound in this activity depend primarily on their polarity, which describes how charge is distributed across a given molecule. Since the activated charcoal is very porous, the size of the molecules also affects their affinity with the carbon. You may have heard the phrase "like dissolves like". This refers to the fact that polar compounds, which have an uneven charge distribution, like to mix with other polar compounds, and non-polar compounds like to mix with non-polar compounds. Water is a very polar compound, whereas carbon is very non-polar. Sugar is a very polar compound, so never binds to the carbon and remains in the water that flows through the carbon. The colored dyes and isopropanol are slightly less polar than water, so the dyes dissolve very well in an alcohol solution. The different affinities of the blue and red dyes to the carbon are due to a combination of their polarity and size. The red dye is smaller and can move further into carbon pores. In addition to size and polarity, properties such as ionic strength and antibody specificity are used to separate mixtures by affinity chromatography.