iBIO STEMfamily: Chromatography

iBIO STEMfamily Camp welcomes you to a SCIENTIFIC JOURNEY!

Today, we will be investigating **methods for separating mixtures**. The purpose of this investigation is to challenge you to separate the materials using their properties. After physically separating a mixture, we will use paper chromatography and column chromatography to separate food dyes from solution. We also challenge you to explore this chemistry problem as a scientist would. What does this mean?

Scientific exploration is different than just playing around because it asks you to think about HOW you investigate. This means you need to do your investigation by observing what happens when you change a variable you have carefully chosen. This helps you to understand WHY something happens. Scientific exploration also means that you record WHAT you see or measure and that you record WHY you think it happens. The Young Scientist Journal that you are holding will help to guide your investigation and give you a place to record your observations, measurements and conclusions.

We have video guidance for your investigation at this website: **www.ibio.org/STEMfamily**

There are many resources for you to use at this website. This type of investigation is associated with some very exciting careers! We hope that you will explore these resources while you are doing your investigation!

Let's Get Started!

FIRST, you will need to prepare your workspace. This can be a very wet and messy investigation, so make sure that you are using a space that will not be easily damaged. A kitchen table will work nicely. To make your clean up easier, you should protect your surface by laying out some used newspaper or opening up a paper grocery bag.

SECOND, you want to lay out your materials. Use the list below to identify which materials are used in each part and organize them in your workspace. There are some additional materials that you will need to supply from your home.

Materials for Part A:

- A MIXTURE in a plastic bag
- A ceramic magnet
- 9 oz cups
- Plastic spoon
- Piece of wool felt

Supplied from home:

- Water
- A piece of paper

Materials for Part B:

- 2 medicine cups
- Grape Kool-Aid & Lemon-Lime Kool-Aid
- Plastic spoon
- 2 chromatography paper strips
- 2 cotton swabs
- 1 tsp of salt
- 9oz cups
- Two small binder clips
- Two craft sticks
- Ruler

Supplied from home:

Water, pencil

Kit Materials for Part C:

- 5 medicine cups
- 9oz Cups
- Isopropyl alcohol
- Two 10ml syringes

Supplied from home:

- Water
- Large bowl or pitcher

LAST, you need to be prepared for experimenting safely. Always be careful when working with chemicals (even if they do not seem hazardous) to prevent injury. DO NOT eat or drink the Kool-Aid.

Chromatography-Chemistry

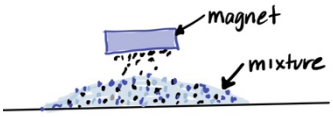
Part A: How can we use the properties of materials to separate a mixture?

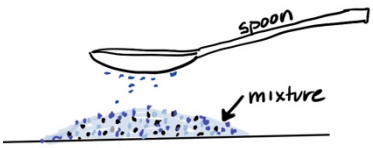
What is a mixture?
A mixture is made when two or more materials are combined, but they are not combined chemically. That means that when you look at the mixture, you can still see each item as a separate thing. Each of the materials could be easily separated from one another. The materials in a mixture still have their own properties.

Here's what you will need to separate your mixture:

- A MIXTURE in a plastic bag containing these materials:
  - •Salt
  - •Sand
  - •Iron filings
  - •Black pepper
- A ceramic magnet
- 9 oz cup that is half-filled with water
- Extra 9 oz cups
- Plastic spoon
- Piece of wool felt

From home:
A piece of paper that you can use as a surface to unmix your mixture!





You have a plastic bag that contains a mixture of salt, sand, iron filings and black pepper. If you look carefully at the mixture, you should be able to identify each material. You could actually pick them apart and move them into separate piles, but that would take a long time. An easier way to separate a mixture is by using the properties of each material to do the separation for you. Let's try it out!

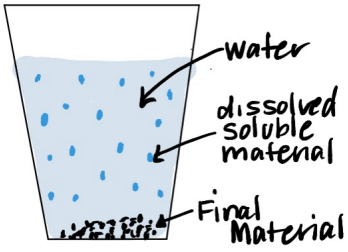
<u>Important ideas for separating a mixture:</u>

1. **Magnet:**
   Magnets create an invisible charged field around themselves that can attract or pull on some metals. If a material in a mixture is attracted to a magnet, the magnet will pull that material to the magnet. This force can be used to move it out of the mixture.

   One of the materials in the mixture can be moved by a magnet! Take your magnet and run it over the mixture, and one of the materials will easily lift out of your mixture! Remove that material and put it into a pile on your paper. Which material is it? **Find its name on Table 1 and record your observations there.**

2. **Static Electricity:**
   Static electricity can be **created** by rubbing one object against another object. This is because the rubbing releases negative charges, called electrons, which can build up on one object to produce **a static** charge. This charge is attractive to the opposite charge.

   One of the materials is lightweight and will be lifted out of the mixture by static electricity. This is easily done by rubbing your piece of wool felt on the plastic spoon. Once the static electricity is made, hovering the spoon over the mixture will cause the component to jump out of the solution. You may have to do this many times to remove all of this material from the mixture. Make a pile of it on your paper. Which material is it? **Find its name on Table 1 and record your observations there.**



3. **Solubility**:
   Solubility is the ability to dissolve in a solvent, like water. When a material dissolves, it will no longer be visible because its molecules are hidden between the water molecules. It forms a solution. Materials that cannot dissolve will remain visible because they are not a part of the solution.

   One of your materials is soluble in water and can be removed when it is in solution. Put the remaining mixture into the cup of water and stir it with the plastic spoon. The material that is soluble will dissolve into the water. Pour your new solution into the empty cup and put it aside. Which material dissolved? **Find its name on Table 1 and record your observations there.**

4. **Final Material**:
   The final material will be left in the cup. It is not magnetic. It does not respond to static electricity and it is not soluble. Which material is it? **Find its name on Table 1 and record your observations there.**

Clean up your materials before you start Part B. You will be reusing your 9oz cups and your plastic spoon.

Table 1: Observations of the Separation of Mixture

Component of Mixture	What method did you use to move this component out of the mixture?	What was it like to use this method to remove this material?	Describe the appearance of this material. Can you still see it?
Salt			
Iron filing			
Sand			
Black pepper			

Part B: How can we use paper chromatography to separate a solution?

What is a solution?

A **solution** is a type of mixture that consists of two or more substances dissolved in a liquid form. This means that atoms of the solute are evenly dispersed throughout the solvent. Liquid drinks are common solutions. Coffee is a solution that contains water, sugars, and proteins. Flavored drinks are solutions made of water, sugar, flavorings and food coloring.

What is paper chromatography?

Colored drink mixtures are interesting to separate. In this investigation, we will be using paper and gravity to separate the food colorings (dyes) in two different kinds of Kool-Aid. This separation technique is called **paper chromatography.** The dyes we put on a strip of paper from each type of Kool-Aid will be carried up the paper by a salt solution. Each color dye is a different size. The big dyes will be heavy and will not move very quickly against gravity. The smaller dyes will be lighter and will move more quickly up the paper. This will separate them so that we can see each color in the Kool-Aid solution!

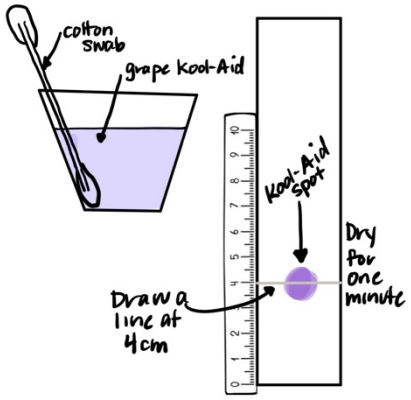
Note: This can get messy, so make sure you have covered your workspace!

Here's what you will need to make your solution:

- 2 medicine cups
- Water
- Grape Kool-Aid
- Lemon-Lime Kool-Aid
- Plastic spoon (from part 1)

Here's what you will need to set up your paper strip:

- 2 chromatography paper strips
- Ruler
- Pencil
- Lemon-Lime Kool-Aid solution
- Grape Kool-Aid solution
- 2 cotton swabs



<u>Setting up your solutions:</u>

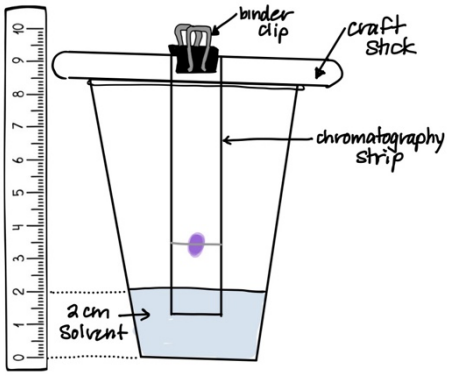
1. Your medicine cups are marked so that you can measure with them. Put 20 ml of water in each of your medicine cups.
2. Open the grape Kool-Aid and pour all of the powdered mixture into one of the medicine cups. Use the spoon to stir the mixture until it is dissolved. Rinse the spoon-you will be using it again.
3. Open the lemon-lime Kool-Aid and pour all of the powdered mixture into one of the medicine cups. Use the spoon to stir the mixture until it is dissolved. Rinse the spoon--you will be using it again.

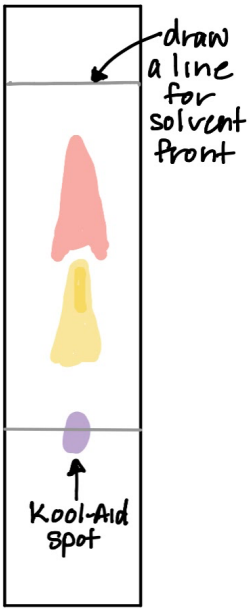
<u>Setting up your paper strip:</u>

4. Take your first paper strip and lay it out in front of you.
5. With your ruler, measure 4 centimeters from the bottom. Make a line with your pencil across the width of the paper. At the top of the paper, write "Grape". It should look like the picture.
6. Take the Grape Kool-Aid Solution and a cotton swab. Stir the solution with the cotton swab so that the cotton swab is soaked.
7. Dab the cotton swab on the edge of the medicine cup or on a piece of paper towel to make sure that the cotton swab is not too wet. Lightly touch the swab in the center of your pencil line as shown in the picture below. The solution will soak into the paper quickly. Let it dry for one minute.
8. Repeat this three more times until the mark is very dark. Put the paper and the solution aside for now.
   You will be using the grape solution in Part C so DO NOT throw it away.
9. Now, take your second paper strip and repeat steps 4-8 with the Lemon-Lime Kool-Aid

Here's what you will need to set up your chromatography cup:

- 1 tsp of salt
- 1 9 oz cup of water
- Plastic spoon
- 2 empty 9 oz cups
- Two small binder clips
- Two craft sticks
- Your Grape paper strip
- Your Lemon-lime paper strip





Setting up your chromatography cup:

10. Pour your salt into the 9 oz cup of water and stir until it is dissolved. This is your solvent. It will dissolve the dyes on the paper.
11. Set out your two 9 oz cups. Pour 2 cm of the salt solution into each cup, by holding your ruler next to the cups and pouring the solution in.
12. To set up your paper strip hanger, you need a craft stick and a small binder clip. Wrap the top of the paper strip over the craft stick as shown in the diagram.
13. Use the binder clip to secure the paper on the craft stick.
14. Place the whole hanger across the cup so that the paper strip is suspended in the liquid as shown in the diagram. You will see the salt solution begin to rise up the paper. You will need to let it sit for 5-10 minutes.
15. Now set up the Lemon-Lime paper strip hanger using directions 12-14. It will need to sit for 10 minutes as well.
16. Soon you will see the food coloring in the Kool-Aid begin to move up the paper as well. The solvent is dissolving the dyes and carrying them up the paper. You will need to let it sit for 10 minutes to see the complete separation of the dyes in the paper.

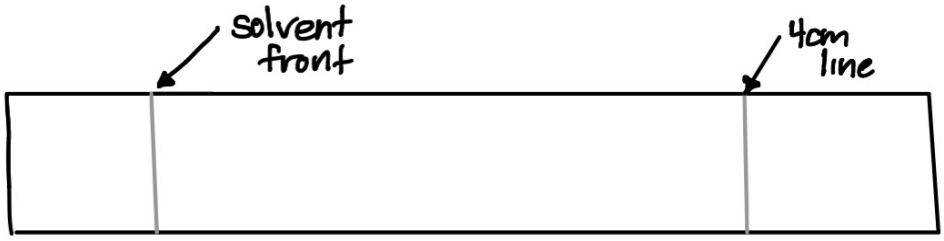
When the solvent is close to the top:

17. After 10 minutes, remove the paper strip holders and remove the paper strips from the craft stick.
18. With your pencil, draw a line across the top edge of the wet area of the paper strip. This is called the **solvent front**. It is how far the salt water was able to move up the paper.
19. Allow your paper strip to dry and then tape your paper strips in Table 2.
20. What do you see? What does it tell us about the different Kool-Aid mixtures?

Table 2: Analyzing the Paper Strips

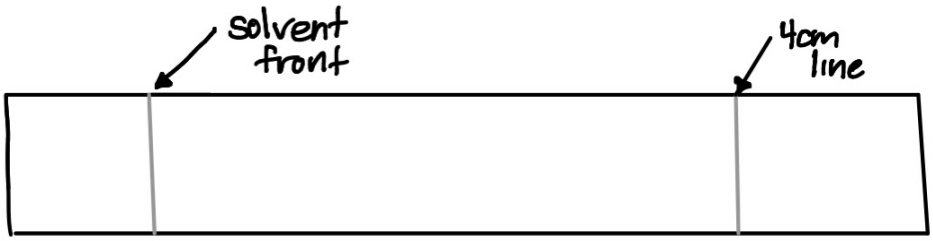
Each color dye is a different size. The big dyes are heavy and will not move very quickly against gravity. The smaller dyes are lighter. They move more quickly up the paper and will be closest to the solvent front.

Tape your GRAPE KOOL-AID Paper strip on top of the drawing below. There should be two colors. Label one color dye 1 and the second color dye 2.



What color is Dye 1: _____	What color is Dye 2: _____
This is the (**larger dye** or **smaller dye**) (circle answer)	This is the **(larger dye** or **smaller dye**) (circle answer)
How far did the dye move? Circle the answer. All of the way! Not quite all of the way. Halfway Not quite halfway	**How far did the dye move? Circle the answer.** All of the way! Not quite all of the way. Halfway Not quite halfway

Tape your LEMON-LIME KOOL-AID Paper strip on top of the drawing below:



What color is Dye 1: _____	What color is Dye 2: _____
This is the (**larger dye** or **smaller dye**) (circle answer)	This is the **(larger dye** or **smaller dye**) (circle answer)
How far did the dye move? Circle the answer. All of the way! Not quite all of the way. Halfway Not quite halfway	**How far did the dye move? Circle the answer.** All of the way! Not quite all of the way. Halfway Not quite halfway

Part C: How can we use column chromatography to separate a solution?

Adapted from: Science Buddies, Column Chromatography

What is column chromatography?

Column chromatography in chemistry is a chromatography method used to separate chemical compounds from a solution. Column chromatography can be done using gravity to move the solvent, like paper chromatography, but they often use pressure to push the solvent through the column. We will be using a syringe as our column and use both gravity and pressure to separate the dyes.

Some dyes love to dissolve in water (hydrophilic) and some dyes do not love water (hydrophobic). We will be using these two properties to help us to separate two different dyes found in Grape Kool-Aid. We will be using water to attract the hydrophilic dye. We will be using isopropyl alcohol and a specially-treated sand (Mystic Sand) to separate the hydrophobic dye.

This experiment is a more complex separation technique and it may be easier to work with another person so that you have two sets of hands! Remember that if this looks confusing, you can follow along with the video!

Here's what you will need to do your column chromatography:

Making the Kool-Aid Solution

- Four cups of Water
- Pitcher or large bowl for mixing
- Grape Kool-Aid solution from Part B

Materials for Column Chromatography:

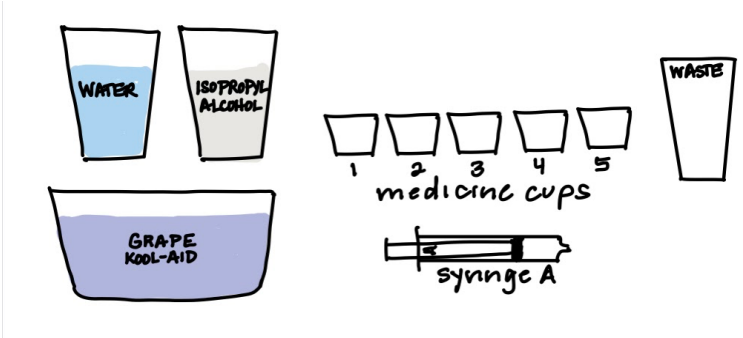
- 5 medicine cups
- 9 oz cup with 2 cm of Isopropyl alcohol
- 9 oz cup with 2 cm of water
- Grape Kool-Aid
- 9 oz waste cup
- One 10ml syringe
- Sharpie

Make the Kool-Aid Solution and setting up for testing:

1. We will need to have a more dilute solution of Kool-Aid for column chromatography. That means that we need to add water.
2. In your pitcher (or large bowl), add four cups of water. Add the Grape Kool-Aid Solution (in the medicine cup) that you made in Part B to the four cups of water. Stir to mix.

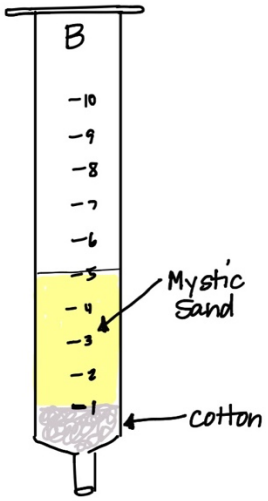
Setting up materials:

3. Now set up your materials. This investigation will be easier if your materials are organized.
4. Put your grape Kool-Aid, cup of isopropyl alcohol and cup of water off to one side.
5. Place the waste cup off to the other side.
6. Line up your five medicine cups in front of you. With your sharpie, write a A on the syringe at the top of the syringe. Put the syringe next to the medicine cups in the workspace. Your workspace should look like the picture below.



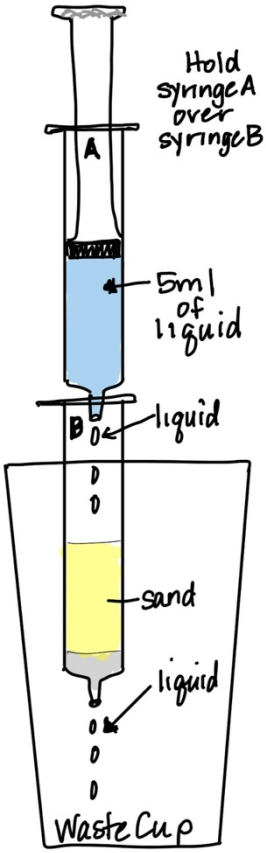
Materials to set up your column:

- The second 10ml syringe
- Cotton ball
- Pencil
- 2 Tbsp Mystic Sand



Make your column:

7. Take your second syringe. Label it “B” This will be your column. Remove the plunger and put it aside.
8. Take a small piece of cotton from the cotton ball and push it to the bottom of syringe “B”. Use the eraser end of the pencil to push it to flatten it to the bottom. It should fill up to the 1 ml mark on the syringe as you can see in the diagram.
9. Now, pour Mystic Sand (about 4ml) into syringe “B” above the cotton. This should be fill the syringe to the 5ml mark. The cotton will prevent sand from falling out of the syringe. Tap the bottom of the syringe on the table so that the sand packs together. If you need to add more sand so that it is filled to 5ml, add and tap the sand again.
10. Now syringe “B” is ready to use. Place it, standing up, in the waste cup.



Prepare your syringe for the Kool-Aid:

11. Use your empty syringe “A” to suck up 5 mL of 70% isopropyl alcohol from its cup.
12. Hold the sand-packed syringe “B” over the waste cup. Then, (in your other hand) hold the alcohol-filled syringe “A” over the sand and *slowly* push out the alcohol into the sand in syringe “B”. The diagram shows how the syringes should be arranged. Be careful to not disturb the sand too much while adding the alcohol.
13. Let the alcohol drip out of syringe “B” and into the waste cup below. To speed up the process, you can put the plunger back into syringe “B” and slowly push out the remaining alcohol. Be careful not to disturb the sand.
14. When all of the alcohol has moved through and has emptied into the waste cup, move to the next step.
15. Take the empty syringe and use it to suck up 5 mL of distilled water.
16. Carefully remove the plunger from syringe “B”.
17. Repeat steps 11 -14 with distilled water.
18. Now your column is ready for the Kool-Aid!

Separating the dyes in the Kool-Aid: STEP 1

19. Take the **EMPTY** syringe "A" and use it to slowly suck up 5 mL of **GRAPE KOOL-AID**.
20. Carefully remove the plunger from the sand-filled syringe "B". Hold the sand-filled syringe "B" over your **1st** medicine cup. Then (in your other hand) hold the **grape Kool-Aid** syringe "A" over the sand-filled syringe and *slowly* push the **grape Kool-Aid** into the sand. Try not to disturb the sand too much.
21. Let the grape Kool-Aid drip out of syringe "B" and into the medicine cup below. To speed up the process, you can put the plunger back into syringe "B" and slowly push out the remaining Kool-Aid. Be careful not to disturb the sand.
22. **The solution that is coming out of the syringe is our HYDROPHILIC dye. It is in the water that was used to make the Kool-Aid. The hydrophobic dye is sticking to the Mystic Sand.**
23. What is the color of the liquid that comes out of the syringe? What is the color of the sand in the syringe? Write your observations in **Data Table 3.**

Adding water for better separation of the hydrophilic dye: STEP 2

24. Take the EMPTY syringe "A" and use it to slowly suck up 5 mL of WATER.
25. Carefully remove the plunger from the sand-filled syringe "B". Hold the sand-filled syringe "B" over your 2nd medicine cup. Then (in your other hand) hold the WATER syringe "A" over the sand-filled syringe and *slowly* push the WATER into the sand. Try not to disturb the sand too much.
26. Let the liquid drip out of the sand filled syringe "B" and into the medicine cup below. To speed up the process, you can put the plunger back into the sand-filled syringe "B" and slowly push out the remaining liquid. Be careful not to disturb the sand.
27. What is the color of the liquid that comes out of the syringe? What is the color of the sand in the syringe? Has it changed? Write your observations in **Data Table 3.**

Removing the hydrophobic dye from the sand: STEP 3

28. Take the EMPTY syringe "A" and use it to slowly suck up 5 mL of ISOPROPYL ALCOHOL.
29. Carefully remove the plunger from the sand-filled syringe "B". Hold the sand-filled syringe "B" over your 3rd medicine cup. Then (in your other hand) hold the ALCOHOL syringe "A" over the sand-filled syringe "B" and *slowly* push the ALCOHOL into the sand. Try not to disturb the sand too much.
30. Let the liquid drip out of the syringe "B" and into the medicine cup below. BUT WATCH CAREFULLY. The first few drops will be the same color again. But as you watch, you will see the drops change to the second color. AS SOON AS YOU SEE THE DROPS CHANGE COLOR, move to the 4th medicine cup to collect the second color. This is the hydrophobic dye.
31. NOW, to speed up the process, you can put the plunger back into the sand-filled syringe "B" and slowly push out the remaining liquid. Be careful not to disturb the sand.
32. **Remember that the hydrophobic dye was sticking to the Mystic Sand. The isopropyl alcohol is able to remove the hydrophobic dye from the sand.**
33. What is the color of the liquid in the 3rd and 4th medicine cups? What happens to the color of the sand in the syringe? Write your observations in **Data Table 3.**

FINAL STEP

34. Take the EMPTY syringe "A" and use it to slowly suck up 5 mL of ISOPROPYL ALCOHOL.
35. Carefully remove the plunger from the sand-filled syringe "B". Hold the sand-filled syringe "B" over your 5th medicine cup. Then (in your other hand) hold the ALCOHOL syringe "A" over the sand-filled syringe "B" and *slowly* push the ALCOHOL into the sand. Try not to disturb the sand too much.
36. Let the water drip out of the syringe "B" and into the medicine cup below. To speed up the process, you can put the plunger back into the syringe "B" and slowly push out the remaining liquid. Be careful not to disturb the sand.
37. What is the color of the liquid in the 5th medicine cup? What happens to the color of the sand in the syringe? Write your observations in **Data Table 3.**

Table 3: Column Chromatography Observations					
	1st medicine cup	2nd medicine cup	3rd medicine cup	4th medicine cup	5th medicine cup
Into syringe	Kool-Aid	Water			
Out of syringe	Hydrophilic dye	The rest of the hydrophilic dye			
COLOR OBSERVED of the LIQUID					
	1st Sand Observation	2nd Sand Observation	3rd Sand Observation	4th Sand Observation	Final Sand Observation
COLOR OBSERVED of the SAND					

Conclusion- Purple Kool-Aid is really made of red and blue dyes!

When purple Kool-Aid powder is mixed with water and is pushed through a sand filter, red dye comes out into the first two cups. This is because the red dye is not attracted to the sand and passes through it. The blue dye that was in the original purple Kool-Aid stuck to the sand rather than passing through with the red dye. When isopropyl alcohol is added, it dissolves the blue dye off of the sand and then passes through to the 4th and 5th cups!