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iBIO STEM Kit: Chromatography Part 2

iBIO STEM Kit welcomes you to a SCIENTIFIC JOURNEY!

Today, we will continue investigating **methods for separating mixtures**. The purpose of this investigation is to challenge you to separate the materials using their properties. In Part 1, you not only physically separated a mixture, but you also used paper chromatography to separate food dyes from solution. Now, we will use 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.

Follow the QR code at the top of the page for additional resources on this activity. There are many resources for you to use on our 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:
5 medicine cups
3 – 9 oz cups
Grape Kool-Aid
One 10ml syringe
Mystic Sand

Supplied from Welcome Kit:
Isopropyl alcohol
Sharpie

Supplied from home:
Water and a 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.

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Chromatography-Chemistry

Part A: 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

Materials for Column Chromatography:

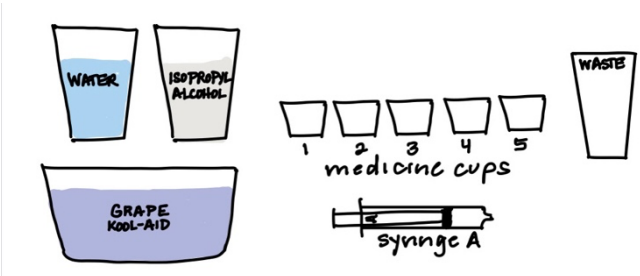
- 5 medicine cups
- 9 oz cup with 2 cm of Isopropyl alcohol
- 9 oz cup with 2 cm of water
- Grape Kool-Aid
- Hydrophobic Sand
- 9 oz waste cup
- One 10ml syringe
- Sharpie

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 an 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 to the right.



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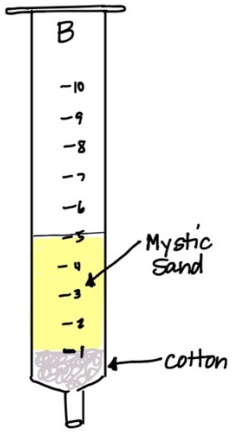
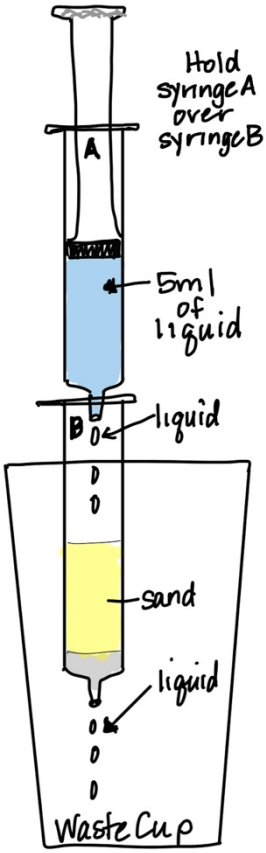
Materials to set up your column:

- 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!

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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.**

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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!