

Chromatography...Oh Yeah!

What's In Kool-Aid? – An Introduction to Extraction and Chromatography

OVERVIEW:

The purpose of the experiment is to separate and analyze the components of prepared Kool-Aid using a solid phase extraction (SPE) column.

OBJECTIVES:

Before doing this lab you should understand:

- The concept of a mixture.
- The concept of a solution.
- The difference between a homogenous mixture (i.e. a solution) and a heterogeneous mixture.

After doing this lab you should be able to:

- Explain the concept of extraction and how it relates to environmental analysis.
- Explain the difference between chromatography and filtration.
- Separate the components of a given solution based on polarity.

INTRODUCTION:

Extraction is a technique commonly used in many areas of chemistry and biology, particularly in environmental analysis. It is one of many techniques used to remove certain chemicals from a mixture. This isolation is done to purify the desired chemical or to separate it so that it can be identified or studied. Extraction is often the first step done when a chemist wishes to identify a chemical, determine its structure, and possibly synthesize it.

For example, artificial vanilla exists because the natural chemical was isolated from a plant, and its structure was determined. Once the structure was known, a procedure for producing it less expensively in the laboratory was developed. Pollutants in soil or water are often dissolved in the environmental sample and must be extracted out to determine how much is there and if there is enough there to cause harm to humans or wildlife.

For many of these applications, **filtration** is not effective because the desired components are **dissolved** in the sample material. **Filtration** is effective for removing particles or other un-dissolved components of a **heterogeneous mixture**, but means other than physical separation are needed to separate out desired **dissolved** components.

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Solid phase extraction (SPE) will be used for this procedure. Very small silica beads covered with a material called C-18 (18 carbon atoms bonded together) are packed in the column.

When a mixture is passed through the column, some components are more powerfully attracted to the C-18 material than others. These components "stick" to the column and are held while the other components pass through. By selecting proper solvents, the materials adhered to the column can be removed and isolated for further study.

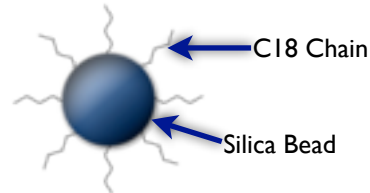
MATERIALS:

C-18 SPE column
8 test tubes
10 mL syringe
Colored pencils (optional)

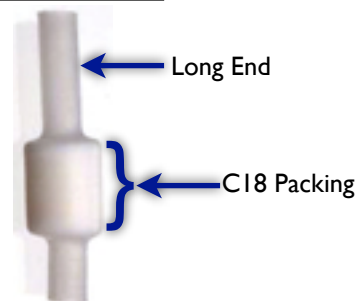
SAFETY:

- Always wear safety glasses in the lab - isopropanol solutions are flammable and could splash!
- Use equipment only as directed.
- Never eat or drink in the lab.

C-18 Silica SPE Bead Structure



SPE Column



SOLUTIONS:

Kool-aid solution
Isopropanol (5%, 10%, 20%, 70%)
DI water

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- f. Conditioning a column –
- g. Solvent –
5. Using the line below, rate the relative polarity of the solvents used in the Kool-Aid lab: 100% isopropanol, water, 5% isopropanol, 10% isopropanol, 20% isopropanol.

Write the solvent names above the line, in the order and place they should be in based on their relative polarity.



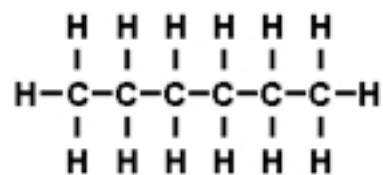
6. Identify the following about hexane (circle choices):

C18 Column

Bonds: polar or non-polar or both

Circle any polar bonds on the molecule.

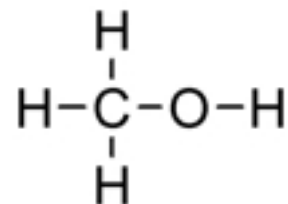
Whole molecule: polar or non-polar



7. Identify the following about isopropanol (circle choices):

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Isopropanol



Bonds: polar or non-polar or both

Circle any polar bonds on the molecule.

Whole molecule: polar or non-polar

8. Assign the roles for your group members and record them here:
- Data Recorder** – Records all data and observations in the data table page of your lab handout.
 - Name: _____
 - Solution Adder** – Adds the solutions to the top of the column with a syringe.
 - Name: _____
 - Solution Manager** – Prepares and gives solution tubes to Solution Adder; Keeps track of what solution to add next by following the procedure.
 - Name: _____
 - Column Manager** – Keeps the column in proper position and over the correct tube number; Pushes the solutions through the column with the plunger after the Solution Adder has added them.
 - Name: _____

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PROCEDURE:

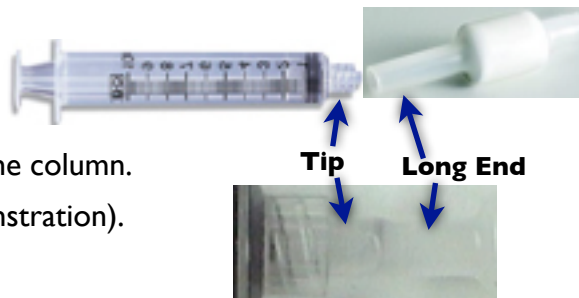
★ Label test tubes in the following order:

| Tube Order | Tube | Solution Added |
|------------|----------|-----------------------|
| 1 | 70% | 70% isopropyl alcohol |
| 2 | Water | Water |
| 3 | Kool-Aid | Kool-Aid |
| 4 | Water | Water |
| 5 | 5% | 5% isopropyl alcohol |
| 6 | 10% | 10% isopropyl alcohol |
| 7 | 20% | 20% isopropyl alcohol |
| 8 | 70% | 70% isopropyl alcohol |

★ Go through the following steps to add the solutions in the table (**IN THIS ORDER**) to the column and collect the effluent from each step in a **different test tube**.

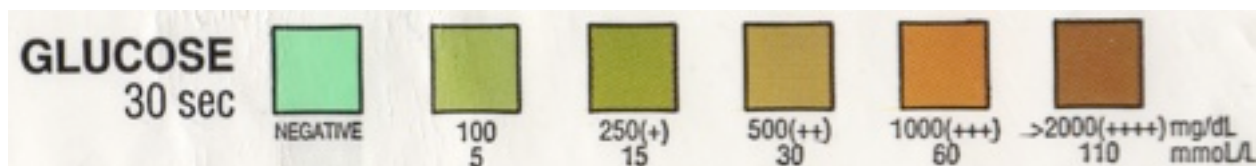
STEPS FOR EACH SOLUTION/TUBE:

1. Place the **first tube** underneath the column.
2. Fill the 10mL syringe.
3. Attach the tip of the syringe into **long end** of the column.
4. Slowly push through with the syringe (See demonstration).
5. Remove the syringe.
6. **Record** the **colors** of the **effluent** and the **column** on the data table for each step.








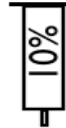


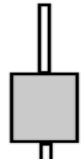
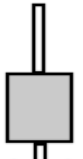
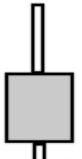
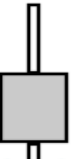
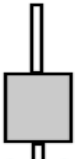
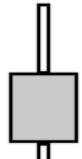
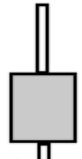
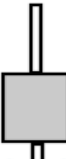








TEST FOR SUGAR:

1. Decide as a group which tubes would be the most likely to contain **sugar**:
2. Dip the test strip in your test tube and **wait 30 seconds** for the color to develop.
3. **Compare strip color to the chart below** for relative sugar concentrations.

















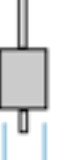










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RECORD YOUR DATA BELOW

| | | | | | | | | |
|-----------------|---|---|---|---|--|---|---|---|
| Solution Added: | 70% | H ₂ O | Kool-Aid | H ₂ O | 5% | 10% | 20% | 70% |
| |  |  |  |  |  |  |  |  |
| |  |  |  |  |  |  |  |  |
| |  |  |  |  |  |  |  |  |
| Glucose: | _____ | _____ | _____ | _____ | _____ | _____ | _____ | _____ |

Hypotheses for what's happening:

Example

| | | | | | | | | |
|----------------------------------|---|---|---|---|---|--|---|---|
| Solution Added: | 70% | H ₂ O | Kool-Aid | H ₂ O | 5% | 10% | 20% | 70% |
| |  |  |  |  |  |  |  |  |
| |  |  |  |  |  |  |  |  |
| |  |  |  |  |  |  |  |  |
| Glucose: | _____ | _____ | + | _____ |  | _____ | + | _____ |
| Hypotheses for what's happening: | | | | | First non-polar solvent pulled red dye from the Kool-Aid on the column | | | |

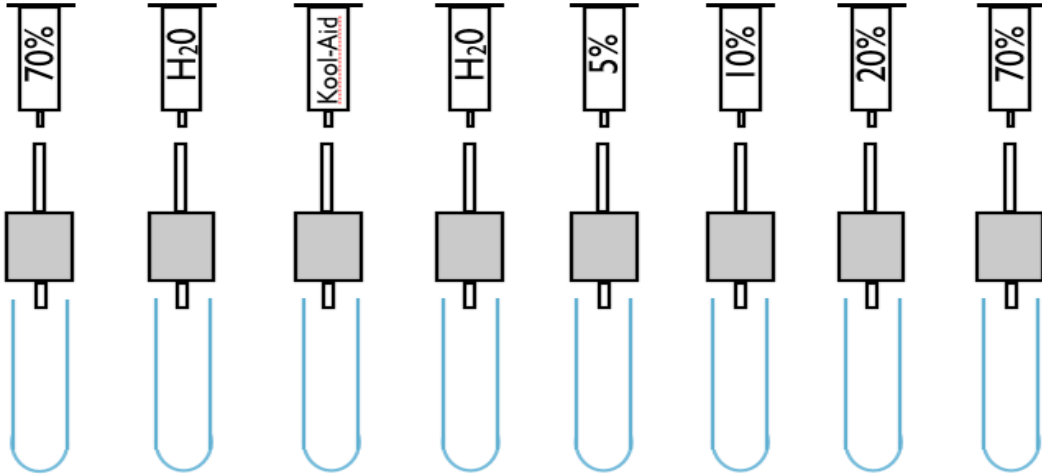
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CLASS COMPARISON DATA

Use this sheet to record the other groups' data. **Illustrate** the changes they observed in the color of the **column and effluent** AND where the **glucose** strip test was positive.

Kool-Aid: Cherry

Solution Added:

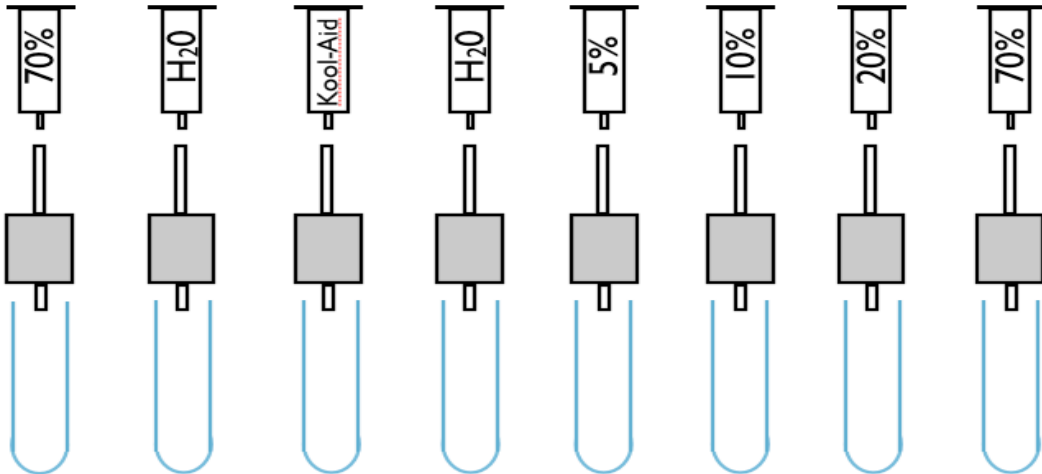


Glucose: _____

Hypotheses for what's happening:

Kool-Aid: Grape

Solution Added:

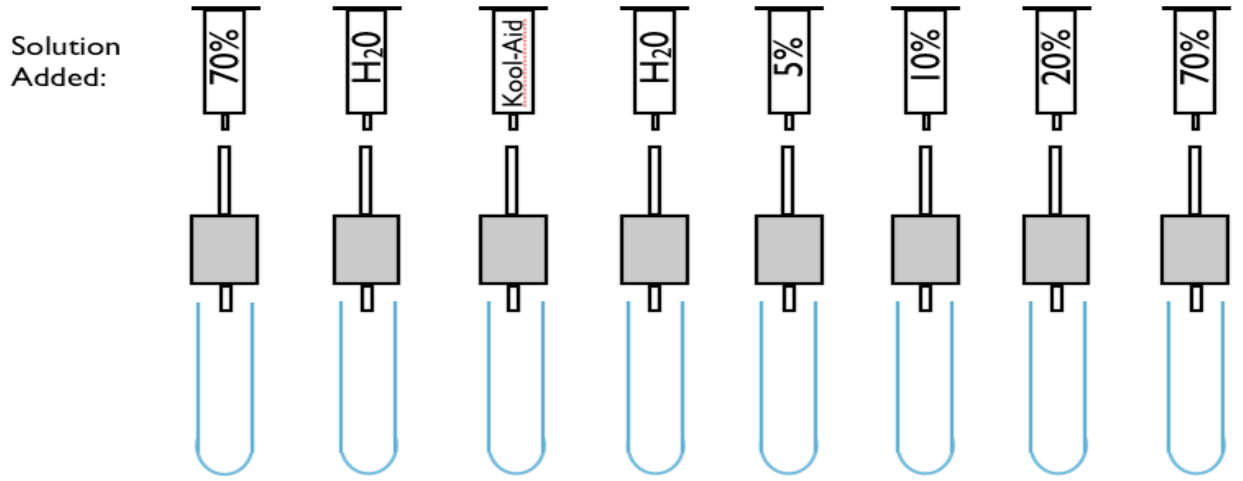


Glucose: _____

Hypotheses for what's happening:

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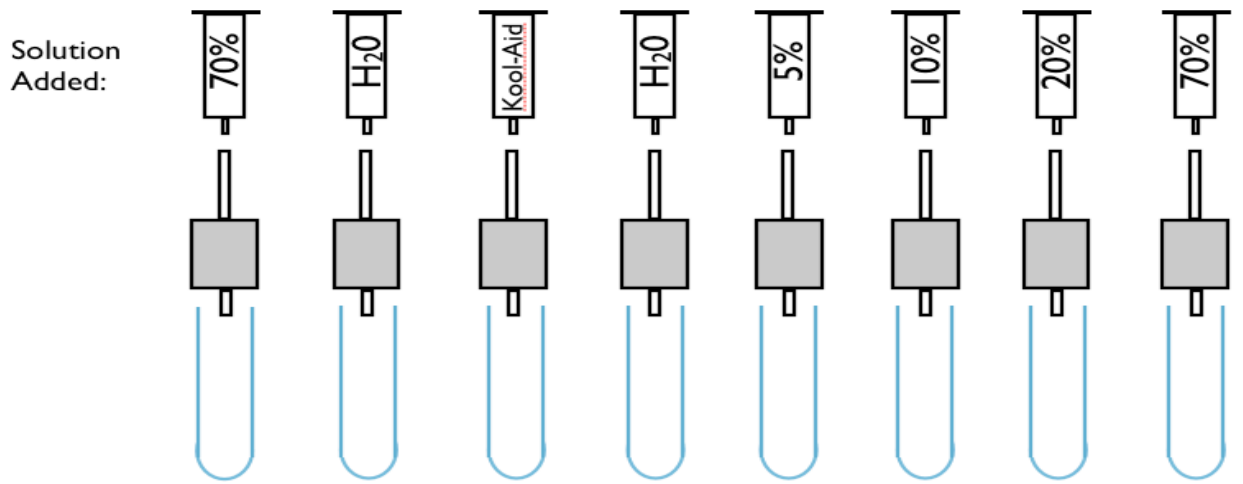
Kool-Aid: Orange



Glucose: _____

Hypotheses for what's happening:

Kool-Aid: Lemon-Lime



Glucose: _____

Hypotheses for what's happening:

Essay for Kool-Aid Lab**Due:**

Describe (in words, or pictures, or both) what would happen if we mixed all three flavors of Kool-Aid solutions (Cherry, Orange, Grape) and ran them through our column extraction system.

- ✓ Be very specific in regards to the changing color of the column and effluent when the different polarity solvents are added.

- ✓ You should think about the different dyes involved and glucose and their relative polarities in relation to the order that they come off of the column.

