

Introduction: this is a Sep-Pak "column" chromatography Lab using grape "Purple" Kool Aid. The C18 Sep-Paks I ordered were like \$11 each from Flinn. I have a materials list at the bottom of this page. This lab guide includes the student lab report guide (rubric), background notes, post lab review notes, procedure, lab quiz (paper & virtual versions), Teacher procedure notes, and student example of lab report.

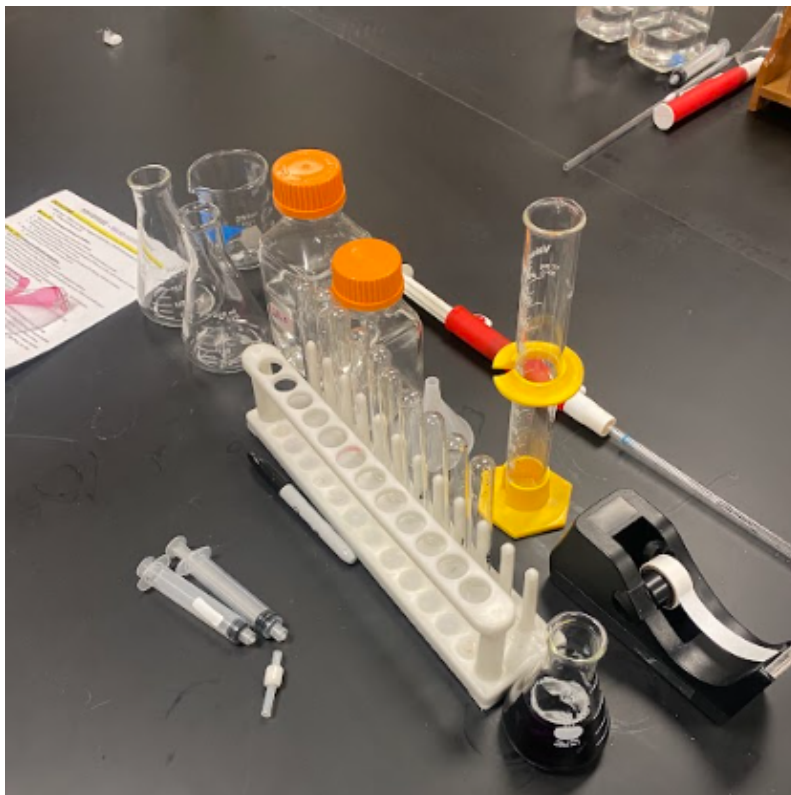
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*** [virtual version of lab quiz click here](#) - edulastic (peat assessment platform)

*** [virtual version of lab quiz click here](#) - Google Form Platform

Materials @ Each Station

- C18 Sep Pak
- 10 ml Syringe
- Kool-Aid Stock
- 70% Iso. Alcohol Stock
- dH₂O Stock
- transfer Pipet or serological pipet
- Sharpie
- Test tube rack
- 10 test tubes
- Waste beaker
- 2 – empty reagent bottles or flask
- Labeling tape
- 100 ml Graduated cylinder



Kool-Aid Column Chromatography

Pages 46- 48 1st edition Textbook / Pages 54-56 2nd Edition Textbook

(4 pts) Lab Objectives:

- Copy the 3 lab objectives.

(4 pts) Safety:

- List 4 safety highlights.
- Make a bulleted list (like this one)

(30 pts) Background

- Copy the background notes into your lab notebook.
- Try to copy as identical as possible.
- Use color!!!!

(12 pts) Pre-Lab Questions – refer to background and class notes.

1. What is the formula you will use to calculate how to prepare your 5% and 25% solutions?
2. The Sep-Paks we are using to separate the pigments within the Kool-Aid mix contain solid resin beads. Explain the properties of these beads and the solid state of chromatography.
3. Once the Kool-Aid is in the Sep-Pak we will push 2 different solvents through. A 5% alcohol solution and a 25% alcohol solution. The remaining % of the solution is water (polar). What is the purpose of the two different alcohol solutions? Do you expect them to yield different results?

(6 pts) Key Vocabulary – refer to background notes

- C₁₈ Sep-Pak Cartridge
- Solid phase
- Liquid Phase
- Elution
- Fraction

(20 pts) Calculations:

- @ Show the work for calculating the 5% and 25% solutions.
- @ The stock solution is 70% Isopropyl Alcohol

100ml of 5% Alcohol Solution	100ml of 25%Alcohol Solution
*Unlimited 70% Alcohol Stock Solution	* Unlimited 70% Alcohol Stock Solution
Formula: $(C_1)(V_1) = (C_2)(V_2)$ 70% alcohol 5% alcohol	Formula: $(C_1)(V_1) = (C_2)(V_2)$ 70% alcohol 25% alcohol
Calculate: $(\quad) (\quad) = (\quad) (\quad)$	Calculate: $(\quad) (\quad) = (\quad) (\quad)$
In a 100ml graduated cylinder how much 70% alcohol will you start with?	In a 100ml graduated cylinder how much 70% alcohol will you start with?
Then you will dilute the 70% alcohol with dH ₂ O in the same graduated cylinder until the bottom of the meniscus touches which graduation mark?	Then you will dilute the 70% alcohol with dH ₂ O in the same graduated cylinder until the bottom of the meniscus touches which graduation mark?

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(12 pts) Data Table:

👍 In the data table below write the name of the color and color in the box with the closest matching colored pencil or marker you have access to.

Kool-Aid Chromatography Results		
Added to Syringe	Color of Fraction (color in the test tube)	Color of Sep-Pak
Kool-Aid		
5% alcohol		
25% alcohol		

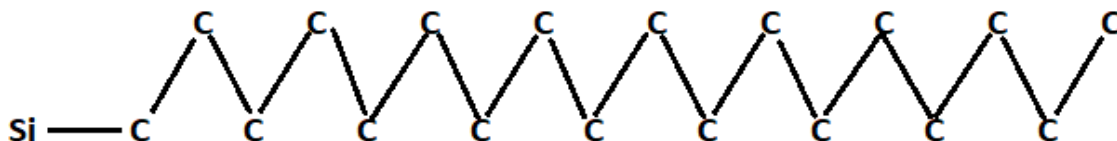
(12 pts) Post Lab Questions:

1. What was eluted into test tube 1? Refer to the post lab review notes if needed.
2. After pushing through the purple Kool-Aid, what color was the Sep-Pak & Why was it this color?
3. Which color dye or pigment was most similar in polarity to the 5% alcohol solution?
4. After pushing through the 5% alcohol, what was the color of the Sep-Pak & why was it this color?
5. Which color dye or pigment was most similar in polarity to the 25% alcohol solution?
6. What color is the inside of the Sep-Pak at the conclusion of the lab? Why?

Kool-Aid Chromatography Quiz.

- After you finish and submit your lab notebook report, your next task is to take the lab quiz.
- The lab quiz is not part of your lab report grade.

Chromatography Lab Background Notes



C₁₈ Cartridge: Sep-Pak

- ✓ Contains C₁₈ solid resin beads.
- ✓ It is made of a 18 carbon hydrocarbon chain!!!
- ✓ There are no polar (charged) sides.

C₁₈-Sep-Pak

There are resin beads inside the Sep-Pak that are....

- Very Nonpolar
- Saturated
- Lipid/fat

S-Rule

compounds that are more similar in polarity to the stationary phase will have longer retention times. The stationary phase is when it is bound to resin inside the Sep-Pak

Polarity Rules

Polar Likes Polar
(attracted)

Nonpolar likes
Nonpolar
(attracted)

Polar HATES
Nonpolar
(repel/DON'T Mix)

Water is Polar
Dyes are NonPolar

SET-UP

Syringe

Add Kool-Aid to syringe

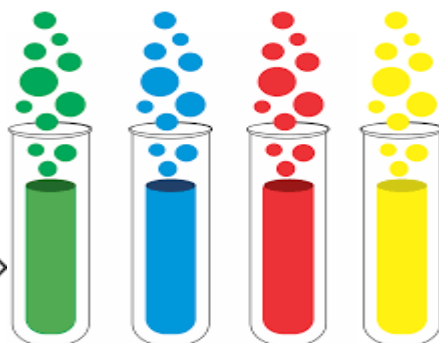
Sep-Pak

Elution:

- ☐ Process of expelling
- ☐ Extruding material by washing with solvent
- ☐ When the solvent is closer to the polarity of compounds than that of the resin beads, the compounds will flush out with that solvent.

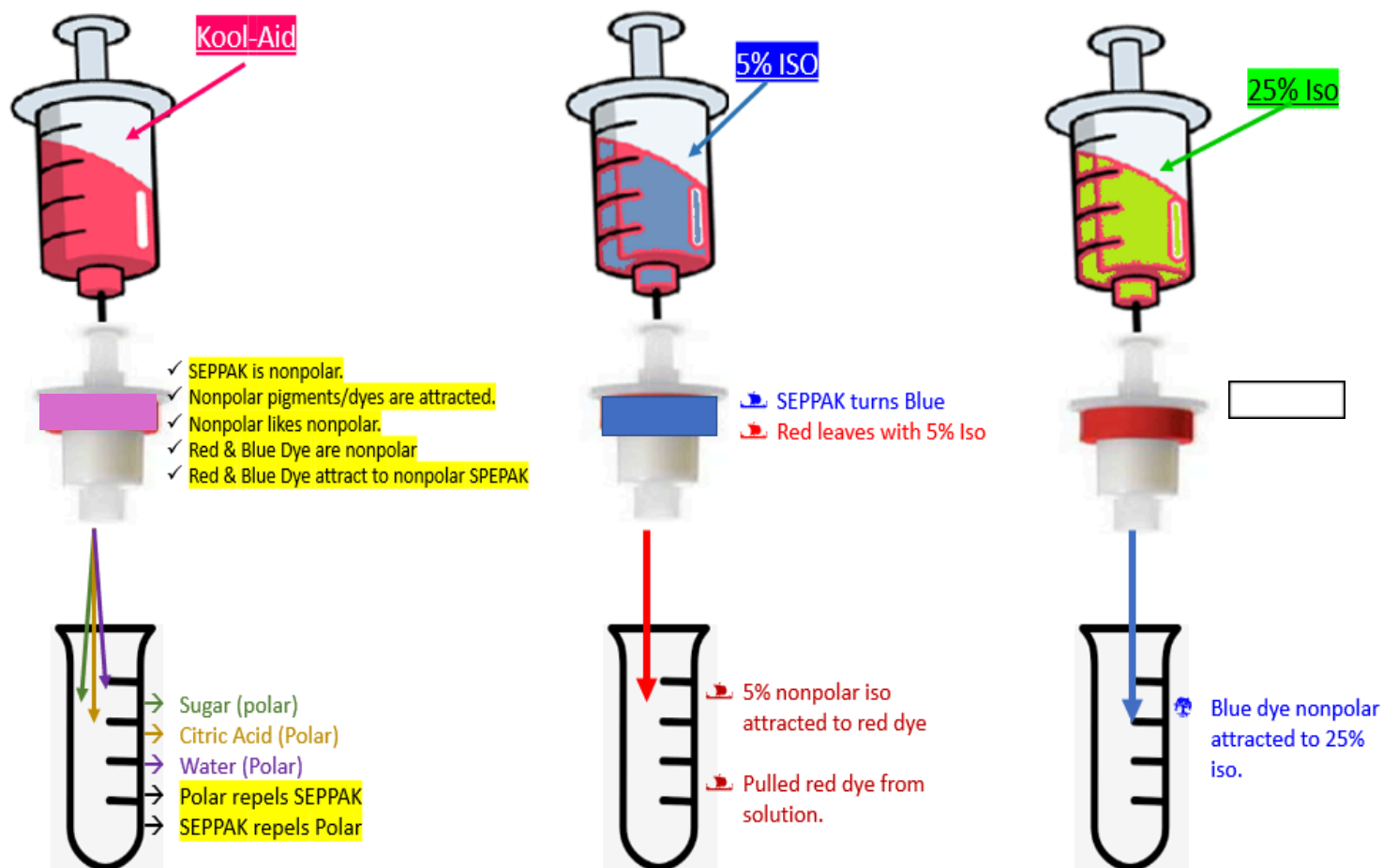
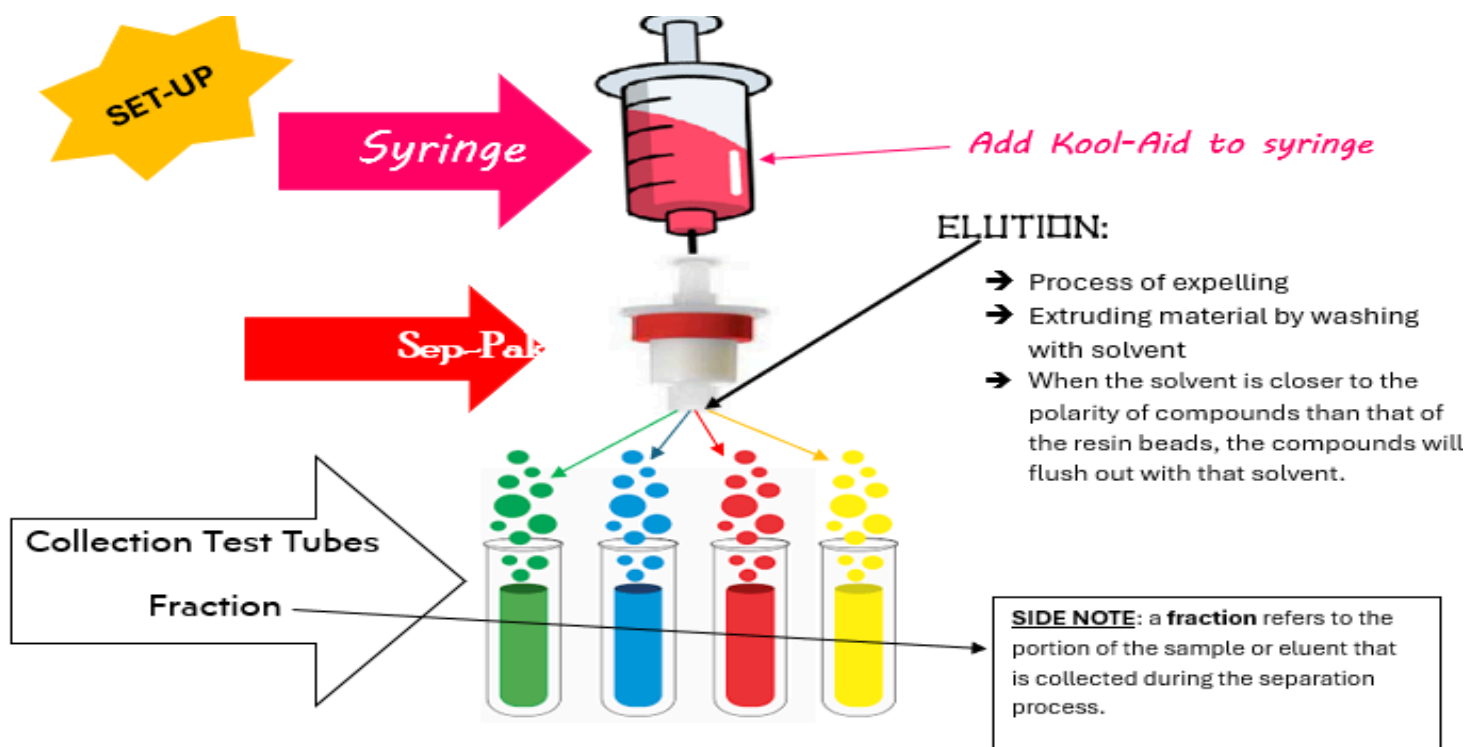
Collection Test Tubes

Fraction



SIDE NOTE: a **fraction** refers to the portion of the sample or eluent that is collected during the separation process.

Kool-Aid Lab Post Lab Review



DATA & RESULTS – KOOL-AID CHROMATOGRAPHY

Added to Syringe	Color Fraction	Color Sep Pak
Kool-Aid	Clear	Purple
5% Iso	Red	Blue
25% Iso	Blue	Clear

Procedure – Kool-Aid Chromatography

Calculations: make 5% & 25% alcohol solutions from the 70% isopropyl stock alcohol solution.

1. Label two - 100mL or more reagent bottles for a 5% & 25% alcohol solution.
 (follow labeling protocol)

Make 5% isopropyl alcohol solution.

2. Refer to your calculations table in your lab notebook.
3. Add the calculated volume of 70% stock solution to a 100ml graduated cylinder.
4. Dilute the stock solution by filling the graduated cylinder up to the 100ml mark with dH₂O.
 Remember to measure at the bottom of the meniscus.
5. Pour solution from graduated cylinder into reagent bottle labeled for 5% iso. alcohol solution.
 Use a funnel and pour with confidence to prevent spills.

Make 25% isopropyl alcohol solution.

1. Refer to your calculations table in your lab notebook.
2. Add the calculated volume of 70% stock solution to a 100ml graduated cylinder.
3. Dilute the stock solution by filling the graduated cylinder up to the 100ml mark with dH₂O.
 Remember to measure at the bottom of the meniscus.
4. Pour solution from graduated cylinder into reagent bottle labeled for 25% iso. alcohol solution.
 Use a funnel and pour with confidence to prevent spills.

Kool-Aid Column Chromatography.

- ☐ Take some time familiarizing yourself with filling the syringe to 10ml and connecting it to the sep-pak.
- ☐ It is vital that the sep-pak goes on the right way every time.

Prepping the Sep Pak.

1. Label test tubes 1 through 10. These test tubes will collect fractions from Sep-Pak
2. Use syringe push 10ml of 70% alcohol through Sep-Pak & collect fraction in waste beaker.
 - push the solution through at a rate that will cause steady drops but not a steady stream.
3. Add 10 ml of dH₂O to the syringe. Push through Sep-Pak. Collect fraction in waste beaker.
4. Add 5ml of Grape (purple) Kool-Aid drink to the syringe. Push through Sep-Pak, but this time collect fraction in test tube #1 to analyze later.

Chromatography Time:

- ☐ From here on out, fill out your data table as you use the Sep-Pak and collect fraction.
 1. Add 10ml of 5% iso. alcohol to syringe
 2. Push 5% iso. alcohol through Sep-Pak, collecting fraction in test tube #2.
 3. Repeat the previous step with 5% iso. alcohol until the fraction is clear.
 - Move to a new test tube with each fraction collection #2, #3, etc... stop once fraction is clear.
 4. Add 10ml of 25% iso. alcohol to the syringe & push through Sep-Pak collecting fraction in next available test tube. (likely Test tube #5, but could be different)
 5. Repeat the previous step with 25% iso. alcohol until the fraction is clear.
 - Move to a new test tube with each fraction collection #6, #7, etc... stop once fraction is clear.
 6. Finally push through 10ml of the 70% iso. alcohol stock solution and collect flow-through in next available test tube.

Kool-Aid Chromatography Lab Quiz

1. Match the explanation with the term together below by drawing a line.

- | | |
|---------------------|---|
| ↓ Polar | ➤ Highly Polar |
| ↓ Nonpolar | ➤ low polarity |
| ↓ Isopropyl alcohol | ➤ The molecule has very distinctive partial positive and partial negative charged sides. |
| ↓ Distilled water | ➤ The presence of a hydroxyl group creates a partial negative and positive charged area on a little corner of the molecule. |

2. Grape (purple) Kool-Aid is delicious and purple. If we run the Kool-Aid through column chromatography, the color is separated due to polarity of the dyes, alcohol, and the resin in the Sep-Pak. What two colored dyes are used to make grape Kool-Aid purple.

*Hint*** food dyes approved by the FDA are primary colors.*

- a. Red
- b. Orange
- c. Yellow
- d. Green

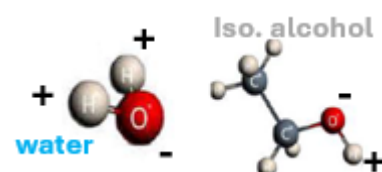
- e. Blue
- f. Indigo
- g. Violet

3. The pigments in the Kool-Aid are all nonpolar but are different degrees of nonpolar. Nonpolar substances have an affinity (attraction/liking) for nonpolar things. Polar substances have an affinity for polar things. Water is polar. Alcohol is nonpolar.

- a. Circle the most polar solution.
- b. Cross out the least polar solution.

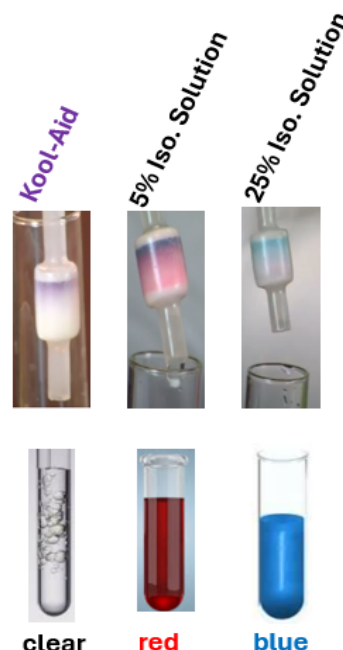
- s dH_2O
- s 5% isopropyl solution

- s 25% isopropyl solution
- s 99% isopropyl (aka 2-propanol)



4. When you flushed the Sep-Pak with 5% and 25% alcohol solutions. The color of the fraction and inside of the Sep-Pak changed. Based on the analysis of this data. Circle the colored dye below that is most polar and cross out the colored dye below that is the least polar based on the Kool-Aid chromatography data.

- a. Red
- b. Orange
- c. Yellow
- d. Green
- e. Blue
- f. Indigo
- g. Violet



clear red blue

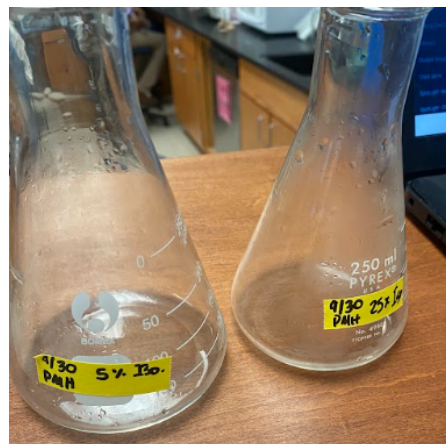
Teacher Procedure Notes – How I Run students through the lab.

- I am typically a big believer in students need to be able to read through procedures and go through the lab as independently as possible; however, the first half of this lab I use more as a “hands on” lecture about labeling and making solutions with the concentration formula. I turn them loose after I go over how to properly use the Sep Paks.

How to Label:

As part of my standards & end of pathway assessment, students need to be able to label. I have students label two reagent bottles for their 5% and 25% isopropyl alcohol solutions. There are 4 things that every label should have, see bullet list below. (We make this list together in a large group setting, basically I let them guess for a few minutes before completing the list.

- ✓ Name/ID of solution
- ✓ Concentration of solution
- ✓ Date solution was made
- ✓ Initials of who made solution



Have students go ahead and label the 5% and 25% Erlenmeyer flask before proceeding to calculations.

Calculations:

Students should have a section copied into their lab notebooks for this step. Alternatively, print the data table on page 2 of this document to hand out to them.

- Write the concentration formula on the board: $C_1V_1 = C_2V_2$

- Discuss what C/V mean.

- Make the following two points clear.

- We have 70% stock solution

- We need 100ml of 5% iso. alcohol

- Set up blank spots under it:

$$\square () () = () ()$$

- Fill in formula and solve

- Show work

- Keep units in ()

- Image illustrates rough ratio of how much alcohol and water are in graduated cylinder.

Handwritten notes and calculations for making 5% and 25% isopropyl alcohol solutions.

Label

- 1) Name ID
- 2) Concentration
- 3) DATE
- 4) initials

5% Iso Alcohol Solution

70% iso. alcohol is stock solution
We need 100ml of 5% iso solution

$$(70\%)(V_1) = (5\%)(100\text{ml})$$
$$V_1 = 7.14\text{ml}$$

→ dilute 7.14ml of 70% isopropyl alcohol with 92.86ml of H₂O

25% Iso Alcohol Solution

70% Iso Alcohol is Stock Solution
We need 100ml of 25% iso alcohol

$$(70\%)(V_1) = (25\%)(100\text{ml})$$
$$V_1 = 35.7\text{ml}$$

→ dilute 35.7ml of 70% iso alcohol w/ 64.3ml of H₂O

Diagram shows two graduated cylinders. The first cylinder contains 7.14ml of 70% isopropyl alcohol and 92.86ml of H₂O, resulting in a 5% solution. The second cylinder contains 35.7ml of 70% isopropyl alcohol and 64.3ml of H₂O, resulting in a 25% solution.

Name: _____

_____(4 pts) Lab Objectives

- ☐ 3 Lab Objectives

_____(4 pts) Safety

- ☐ 4 safety bullet list

_____(30 pts) Background

- ☐ Complete Notes
☐ Neat & Orderly
☐ Color

_____(12 pts) Pre-Lab

- ☐ $C_1V_1=C_2V_2$
☐ Solid state, nonpolar beads
☐ Different levels of nonpolar

_____(6 pts) Key Vocab

- ☐ Sep Pak
☐ Solid Phase
☐ Liquid Phase
☐ Elution
☐ Fraction

_____(20 pts) Calculations

- ☐ Table Set up
☐ Complete
☐ Accurate
☐ Units

_____(12 pts) Data Table

- ☐ Table Set Up
☐ Complete/Accurate
☐ Color

_____(12 pts) Post Lab

- ☐ Other KoolAid Stuff
☐ Purple, why...
☐ Red
☐ Blue, why...
☐ Blue
☐ Clear/White, Why

_____ Total Points

_____ Penalty/Bonus

Final Score: _____

Name: _____

_____(4 pts) Lab Objectives

- ☐ 3 Lab Objectives

_____(4 pts) Safety

- ☐ 4 safety bullet list

_____(30 pts) Background

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☐ Solid Phase
☐ Liquid Phase
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☐ Fraction

_____(20 pts) Calculations

- ☐ Table Set up
☐ Complete
☐ Accurate
☐ Units

_____(12 pts) Data Table

- ☐ Table Set Up
☐ Complete/Accurate
☐ Color

_____(12 pts) Post Lab

- ☐ Other KoolAid Stuff
☐ Purple, why...
☐ Red
☐ Blue, why...
☐ Blue
☐ Clear/White, Why

_____ Total Points

_____ Penalty/Bonus

Final Score: _____

Name: _____

_____(4 pts) Lab Objectives

- ☐ 3 Lab Objectives

_____(4 pts) Safety

- ☐ 4 safety bullet list

_____(30 pts) Background

- ☐ Complete Notes
☐ Neat & Orderly
☐ Color

_____(12 pts) Pre-Lab

- ☐ $C_1V_1=C_2V_2$
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☐ Different levels of nonpolar

_____(6 pts) Key Vocab

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☐ Solid Phase
☐ Liquid Phase
☐ Elution
☐ Fraction

_____(20 pts) Calculations

- ☐ Table Set up
☐ Complete
☐ Accurate
☐ Units

_____(12 pts) Data Table

- ☐ Table Set Up
☐ Complete/Accurate
☐ Color

_____(12 pts) Post Lab

- ☐ Other KoolAid Stuff
☐ Purple, why...
☐ Red
☐ Blue, why...
☐ Blue
☐ Clear/White, Why

_____ Total Points

_____ Penalty/Bonus

Final Score: _____

Exp. No.	Experiment/Subject Kool-Aid Column Chromatography	Date 9/25/24	09
Name [REDACTED]	Lab Partner [REDACTED]	Locker/ Desk No. N/A	Course & Section No. BT

Overview:

- Teaches you how to make volume per volume Percent solutions.
- make solutions ~~containing~~ containing different percentages of alcohol.
- These solutions will be used to separate the colors of Kool-Aid on a chromatography Column by using differences in the polarity of the pigments

Safety:

- Take precautions since Alcohol is flammable
- All lab safety protocols should be followed
- PPE must be worn
- Be familiar with the MSDs

Background:



$\text{Si}-\text{C}_{18}$

C18 Cartridge: SEP-PAK

- Contains C18 solid resin beads
- It is made up of a 18 carbon hydrocarbon chain
- There are no polar (charged) sides

C18 SEP-PAK

There are resin beads inside the sep-pak that are

- very Non polar
- Saturated
- Lipid/fat

S-Rule

Compounds that are more similar in polarity to the stationary phase will have longer retention times. The stationary phase is when it is bound to resin inside the sep-pak

POLARITY RULES

Polar likes Polar (attracted)

Non Polar likes Non Polar (attracted)

Polar hates Non Polar (Repel/Don't mix)

Student Example

Experiment/Subject	Activity 2, c Kool-Aid	Date	9/30	10
Lab Partner	[Redacted]	Locker/Desk No.	N/A	Course & Section No. BT

calculations:

$$C_1 V_1 = C_2 V_2 \text{ 5\% Iso Alcohol}$$

$$(70\%) (V_1) = (5\%) (100 \text{ ml})$$

$$\frac{70\% \cdot V_1}{70\%} = \frac{500\% \text{ ml}}{70\%}$$

$$10x = 500$$

$$10x = 500$$

$$7.14 \text{ ml}$$

100 ml of 5% Alcohol Solution

• Unlimited to 70% Alcohol stock Solution

Formula: $(C_1)(V_1) = (C_2)(V_2)$

70% Alcohol 5% Alcohol

In a 100ml graduated cylinder how much 70% Alcohol will you start with?

You will dilute the 70% Alcohol with d₂O in the same graduated cylinder until the bottom touches with the graduated mark

$$C_1 V_1 = C_2 V_2 \text{ 25\% Iso Alcohol}$$

$$\frac{(70\%) (V_1)}{70\%} = \frac{(25\%) (100 \text{ ml})}{70\%}$$

$$\frac{2500\% \cdot \text{ml}}{70\%}$$

$$V_1 = 35.7 \text{ ml}$$

100 ml of 25% Alcohol Solution

• Unlimited to 70% Alcohol stock Solution

Formula $(C_1)(V_1) = (C_2)(V_2)$

70% Alcohol 25% Alcohol

In a 100ml graduated cylinder how much 70% Alcohol will you start with?

You will dilute the 70% Alcohol with d₂O in the same graduated cylinder until the bottom touches with the graduated mark

Pre-lab question - 1. $(C_1)(V_1) = (C_2)(V_2)$

2. The beads are very non polar saturated, lipid/fat.
3. The difference between the two is one has more alcohol than the other.

Key Vocabulary: C18 Sep-pack Cartridge - C18 SepPak, S-rue, Polarity

- Elution - Process of expelling, Solid phase - SepPak
- Fraction - The portion of the liquid phase

Data Table:

Add to Syringe	Color of Fraction	Color of Sep-Pak
Kool-Aid	Purple	Purple
5% Alcohol	Pink	Purple
25% alcohol	Purple + Pink	Blue

Post lab questions:

1. Alcohol
2. Pink Because the Alcohol set into the solution
3. Pink
4. Purple + Pink
5. Blue
6. Clear b/c it faded

Signature	Date	Witness/TA	Date
[Redacted]	9/30	[Redacted]	9/30