



Kool-Aid Lab

Separation of components of Kool-Aid by liquid chromatography

Introduction

Liquid chromatography (LC) is an analytical technique chemists use to separate mixtures into individual components. Simple liquid chromatography consists of a column that holds *the stationary phase* (Figure 1) which is in equilibrium with a solvent, *the mobile phase*.

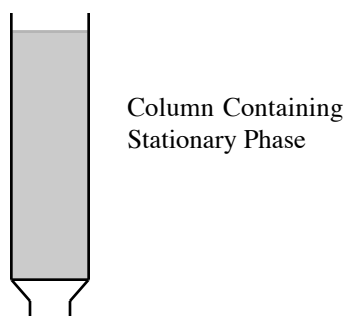


Figure 1.

There are three stages in the LC process: **injection**, **separation**, and **elution**. (Elution is the term used to describe the passing of a solvent through the column to separate each of the components from the mixture.). At the **injection stage**, the mixture to be separated is loaded into the LC column (Figure 2).

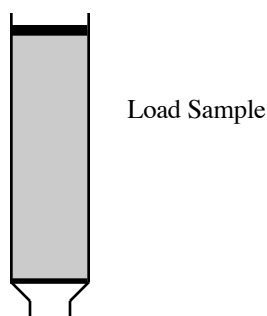


Figure 2.

Separation of each component of the mixture is achieved by passing different solvents (mobile phase) through the stationary phase. The initial band separates into individual bands for each component of the mixture (Figure 3).

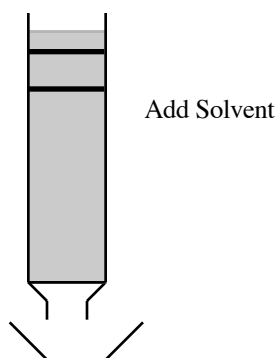


Figure 3.



Elution of each component is achieved by separately passing solvents of differing polarities through the column. The different components in the mixture pass through the column at different rates due to competing attractions of the polar mobile phase and the nonpolar coating on the stationary phase for each component of the mixture. The most polar components will be eluted first, as the most polar solvent is passed through the column because the polar molecules are more strongly attracted to the polar mobile phase molecules than to the nonpolar stationary phase. The less polar components and nonpolar molecules will be more attracted to the stationary phase and travel down the column more slowly. These compounds are eluted by reducing the polarity of the solvents that are passed through the column (Figure 4) so that they will be attracted to the mobile phase and will move down the column.

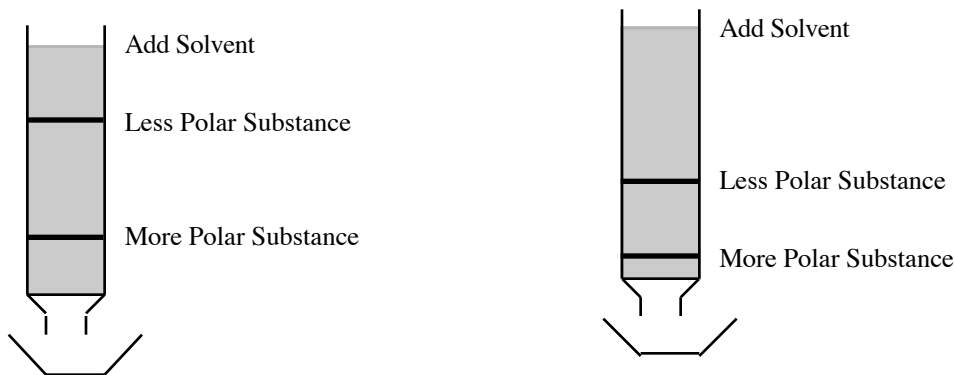


Figure 4.

In this experiment a Sep-Pak® C18 column, which holds the nonpolar stationary phase, will be used to separate the components of grape Kool-Aid®. The grape Kool-Aid® used in this experiment has four major components: **nonpolar additives**; **red dye #40**; **blue dye #1**; and **polar additives**. Each of the components has some affinity for the nonpolar stationary phase in the column. Therefore, when the Kool-Aid® sample is injected onto the column only one initial band is observed. When the column is eluted with a series of solvent mixtures with varying polarity, the components can be separated one at a time.

The four major components of the grape Kool-Aid® will be eluted by separately passing four different solvent mixtures through the Sep-Pak® column. The solvent mixtures to be used are: water, 8.25% isopropyl alcohol solution, 17.5% isopropyl alcohol solution, and 70% isopropyl alcohol solution.

Water (Figure 5) is a very polar molecule. The large difference in electronegativity results in very polar O-H bonds. The molecule is angular so the bond polarities do not cancel and the large bond polarities result in a large net molecular polarity through the center of the molecule.

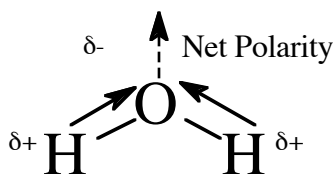


Figure 5

Isopropyl alcohol (Figure 6) is also a polar molecule but not as polar as water. The difference in electronegativity between carbon and oxygen is less, resulting in a less polar bond. Carbon is more electronegative than hydrogen so these these bonds are polar towards the carbon. These carbon-hydrogen bonds have the effect of moving electron density towards the carbon-oxygen bond reducing its polarity and the net polarity of the molecule. The molecule still has a net



polarity towards the oxygen but the net polarity will not be directly through the center of the molecule.

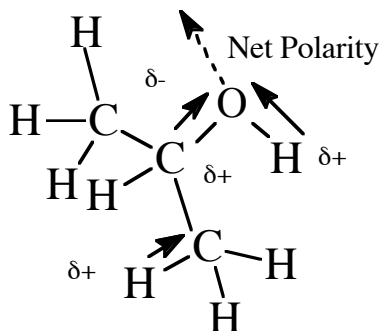


Figure 6

Procedure

Part I. Preparation

1. Acquaint yourself with the use of a syringe with the Sep-Pak® cartridge by following the general instructions below:
 - a. Do not squeeze the Sep-Pak® cartridge during handling.
 - b. Remove the piston from the syringe barrel.
 - c. Push the *longer end* of the Sep-Pak® cartridge snugly onto the syringe tip.
 - d. Hold the assembly with the open barrel up and add 5 mL of water to the syringe barrel.
 - e. Insert the piston and use it to push the water in the barrel slowly through the cartridge into a waste beaker. The flow rate in should not exceed 5 mL per minute.
 - f. Remove the cartridge from the syringe tip.
 - g. Remove the piston and repeat Steps c through e twice more.

2. Label four 18x150 mm test tubes: H₂O, 8.25%, 17.5%, 70%,

Part II. Separation of mixture

1. Remove the cartridge from the syringe. Remove the piston from the syringe and add 10 mL of 70% isopropyl alcohol to the barrel. Attach the cartridge. Insert the piston and slowly push the alcohol through the cartridge into a waste beaker.
2. Remove the cartridge from the syringe. Remove the piston from the syringe. Attach the cartridge. Add 5 mL of distilled water to the barrel. Insert the piston and slowly push the water through the cartridge into a waste beaker.

If you make a mistake or want to start over once the separation has begun, you can restore the cartridge to its original condition by redoing Steps 1 & 2.

3. Remove the cartridge from the syringe. Remove the piston from the syringe. Attach the cartridge. Add 2 mL of grape Kool-Aid® to the barrel. Insert the piston and slowly force the Kool-Aid® into the cartridge. Collect the liquid coming through the column in the waste beaker. Notice that a band of color is concentrated near the top of the cartridge.
4. Remove the cartridge from the syringe. Remove the piston from the syringe. Attach the cartridge. Add 10 mL of water to the barrel. Insert the piston and slowly elute the first component of the Kool-Aid®, collecting the fraction in the test tube labeled “H₂O”. Record the color of this component in the Data Table.
5. Remove the cartridge from the syringe. Remove the piston from the syringe. Attach the cartridge. Add 15 mL of 8.25% isopropyl alcohol solution to the barrel. Insert the piston and slowly elute



the second component of the Kool-Aid®, collecting the fraction in the test tube labeled “8.25%”. Record the color of this component in the Data Table.

6. Remove the cartridge from the syringe. Remove the piston from the syringe. Attach the cartridge. Add 15 mL of 17.5% isopropyl alcohol solution to the barrel. Insert the piston and slowly elute the third component of the Kool-Aid®, collecting the fraction in the test tube labeled “17.5%”. Record the color of this component in the Data Table.
7. Remove the cartridge from the syringe. Remove the piston from the syringe. Attach the cartridge. Add 10 mL of 70% isopropyl alcohol to the barrel. Insert the piston and slowly elute the fourth component of the Kool-Aid®, collecting the fraction in the test tube labeled “70%”. Record the color of this component in the Data Table.

Part III. Conductivity Testing

1. Labpro, Apps- Datamate- setup probes- conductivity- set it at 2000us (the same setting that is on the small black box attached to the sensor). Hit enter or ok to get back to the main menu, set the mode by placing the cursor on the mode, hit enter, select Events with Entry, select enter or ok, then start.
2. Measure the conductivity of each component of the Kool-Aid®. Record the conductivity of each component in the data table. Rinse the probe with distilled water before and between each sample.



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DATA TABLE

	Color of Solution	Conductivity
H₂O		
8.25%		
17.5%		
70%		

Questions

1. Rank the solvents used in this lab (water, 8.25% isopropyl alcohol, 17.5% isopropyl alcohol, 70% isopropyl alcohol) from least polar to most polar. Explain your reason for this order.
2. Using the data collected in this lab, explain why grape Kool-Aid® is purple.
3. Which is more polar, red dye #40 or blue dye #1? Explain.
4. Using the data collected in this lab, were the ionic components of the Kool-Aid® completely separated from the molecular components. Explain.
5. Is there relationship between the polarity of the solvent that eluted each component and the conductivity of each component? If so, describe this relationship.
6. The ingredients of grape Kool-Aid® are: citric acid, calcium phosphate, salt, maltodextrin, modified corn starch, artificial flavor, ascorbic acid (vitamin C), red #40, blue #1.
 - a. Which of these ingredients were eluted with the water? Explain.
 - b. Does the conductivity data support this conclusion? If so, how?
 - c. Which of the remaining ingredients might have been eluted with 70% isopropanol?
 - d. Were any of these ingredients electrolytes? Explain
 - e. Does the conductivity data support this conclusion? If so, how?