**2018 Q1L4**: Physical Separation Techniques 🡪 Column Chromatography

Introduction: **Chromatography** is a popular physical separation technique used in research and industry to separate and ultimately purify components of mixtures.

Column liquid chromatography (LC) is often used in industry to separate mixtures and detect trace components of a mixture. High performance liquid chromatography (HPLC) is an instrument of choice for quantitative (“how much”) analysis! The steps involved in this lab activity can be compared to HPLC. There is a solvent delivery system (for this lab it’ll be a syringe), an injector (again, the syringe), a column (here’s it’s the Sep Pak cartridge) and a detector (our eyes).

Essentially, for this lab we will *mimic* the process of HPLC by attempting to separate the dye molecules in graph kool aid. The molecules in a mixture will either be more attracted to the solvent (water or alcohol solutions) or the Sep Pak chromatography material. We will be able to make qualitative observations about how the Sep Pak and solvents were able to help separate the dyes in the graph kool aid.

Most molecules (compounds) typically fall into one of two categories: (1) polar or (2) nonpolar. **Polar** molecules form when atoms bond together by sharing valence electrons unequally with each other thus generating an **asymmetric distribution** **of charge**. The result is a molecule with partially charged ends, one end being partially more positive (where less negatively charged electrons spend time) and the other being partially more negative (where more - electrons spend time). **Nonpolar** molecules behave just the opposite. They have atoms bonded together that share valence electrons equally and have an overall **symmetric distribution of charge**, so that means NO partially negative or positive ends result.

So what does this mean? Let’s use water as a reference molecule. Water, H2O, is often called **universal polar solvent** and is a very polar molecule. Oxygen and the two hydrogens share **valence electrons.** However, the difference is the oxygen has a **higher electronegativity**, which means it has a stronger attraction for the valence electrons than the two hydrogen atoms do. Therefore, **the O becomes slightly more negative** and **the two H’s slightly more positive** 🡺 thus it is an asymmetric polar molecule! This is commonly shown as you see in the diagram below:



How do we make solutions? We need a solute and solvent to mix (dissolve) uniformly to form a homogenous mixture, or solution! **Solute + solvent 🡺 solutions**

The solute is the substance we are trying to dissolve. The solvent is the substance that does the dissolving. If the solute dissolves fully in a **water solvent** we get a homogenous mixture called an **aqueous (aq)** solution. \*\* The rule: “Like dissolves Like” means polar dissolves polar, and nonpolar dissolves nonpolar, but polar and nonpolar never mix (creates heterogenous mixture)

Procedure:

1. Retrieve:
	* 1. 6 compartment well plate
		2. 1 Syringe
		3. 1 piece of white paper background
		4. Chemical inventory sheet for ONE small HALF-Filled cup with
			+ 1. Kool Aid
				2. distilled water
				3. 70% I-ol solution
				4. 25% I-ol solution and
				5. 5% I-ol solutoin
2. Set your well plate on white background lab sheet.

 **\*\* Steps 3 – 5 MUST be repeated twice!\*\***

1. We need to first pre-treat, clean and prepare the Sep-Pak column for the separation lab. To do this we must carefully draw 10.0-mL of 70% I-ol into the syringe, then gently push and twist place the short end of the Sep-Pak cartridge snuggly onto the syringe.
2. Carefully expel (by pushing the plunger) the alcohol out of the syringe through the Sep-Pak column into the sink (wash down with running water).
3. Remove the cartridge and repeat steps 4 – 5 using 10-mL of distilled water instead of 70% I-ol.

**\*\* At this point the Sep-Pak should appear clean (white) and ready to go! If not then repeat a third time!**

1. Remove the Sep-Pak cartridge from the syringe and draw **10-mL of grape kool** aid from its cup into the syringe.
2. Place the short end of the Sep-Pak cartridge back onto the syringe and slowly and steadily force the Kool Aid solution through the Sep-Pak into an empty clean well of your well plates on its white paper background.
3. *Data Line #1*: What color was the solution that elutes (exits) from the syringe?
4. *Data Line #2*: Describe the appearance of the Sep-Pak packing material?

1. Remove the Sep-Pak cartridge from the syringe. Remove any grape Kool Aid left in the syringe.
2. Next, draw **10 mL 5%** into the syringe then place the short end of the cartridge back on the syringe and slowly force the 5% I-ol through the column into a second clean well of your well plate on the white paper background.
3. *Data Line #3*: What color is the solution that elutes from the syringe?
4. *Data line #4*: Describe the appearance of the Sep-Pak packing material?
5. Remove the cartridge from the syringe and draw 10 mL of 25% I-ol solution into the syringe and place the short end of the cartridge back onto the syringe.
6. Slowly force the 25% I-ol through the Sep-Pak into your final clean well of your well plates on the white paper background.
7. *Data Line #5*: What color is the solution in the final well?
8. *Data Line #6*: Describe the final appearance of the Sep-Pak packing material?
9. **CLEAN UP AND DISPOSAL**: CLEAN THE SEP PAK CARTRIDGES BY REPEATING STEPS 3 – 5 (they should appear cloudy white when clean).
10. CLEAN AND DRY THE well plate and RETURN.
11. Rinse out the syringes with water AND RETURN.
12. Carefully return the remaining liquids in the original five cups.

Data Table:

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| --- | --- |
| Data Line #1 observations |  |
| Data Line #2 observations |  |
| Data Line #3 observations |  |
| Data Line #4 observations |  |
| Data Line #5 observations |  |
| Data Line #6 observations |  |

Post Lab Analysis Questions:

1. Why is Kool Aid is purple?

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1. Water is often referred to as the **universal polar solvent** and is one of the most polar molecules we will see in this course. The polarity of Isopropyl alcohol solutions depends on its concentration of alcohol dissolved in a certain amount of water. Essentially, the different I-ol solutions are created by mixing the alcohol and water in *different proportions*.

Create a list of the % alcohol solutions used in this lab by % from most to least polar and EXPLAIN your reasoning.

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1. After the sep pak was pre-treated, passing the Kool Aid through the Sep-Pak produced a \_\_\_\_\_\_\_\_\_\_\_\_\_\_ liquid. Explain in terms of attractive forces between the dyes in kool aid and the sep-pak material, how this happened?

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1. We also passed 5% I-ol through the Sep-Pak, the liquid seen exiting the Sep Pak was \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_. Explain in terms of attractive forces between the dyes in kool aid and the sep-pak material, how this happened?

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1. When we passed the 25% I-ol through the Sep-Pak, a \_\_\_\_\_\_\_\_\_\_\_\_\_\_ liquid exits the column. Explain in terms of attractive forces between the dyes in kool aid and the sep-pak material, how this happened?

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6. Below is a sketch of the separation of the molecules found in ink using the process of **paper chromatograph** with a select solvent (see a real-world similar example of this taped to the side white board).

The original ink mixture was placed as a dot on the start line of the chromatography paper and then just the very bottom of the paper was carefully placed in the solvent that would be drawn up and through the paper and the original ink droplet.

After a certain amount of time, the separation of the original ink droplet into “A” and “B” was seen as a green (A) spot and a red (A) spot. The solvent used was pure H2O. You can see the solvent traveled to the opposite end of the paper.



Based on the diagram above complete the following statements:

The green dye *component A* was [more or less] attracted to the [paper or solvent] because it traveled the [most or least].

The red dye *component B* was [more or less] attracted to the [paper or solvent] because it traveled the [most or least].