

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

CHROMATOGRAPHY IS USED BY SCIENTISTS TO SEPARATE ONE SUBSTANCE FROM ANOTHER.

IN THIS EXPERIMENT YOU WILL SEPARATE THE DYES THAT GIVE GRAPE KOOL-AID ITS COLOR. YOU WILL USE A TYPE OF CHROMATOGRAPHY CARTRIDGE FASTENED ONTO A SYRINGE.

SEPARATION IS POSSIBLE BECAUSE ONE OF THE KOOL-AID DYES IS MORE STRONGLY ATTRACTED TO A POWDERY MATERIAL IN THE CARTRIDGE THAN THE OTHER DYE IS.

THE KIND OF CARTRIDGE YOU ARE USING TODAY IS USED IN LABS ALL OVER THE WORLD. MANY EVERYDAY PRODUCTS ARE SEPARATED OR PURIFIED BY THIS METHOD. THIS CARTRIDGE IS USED BY:

FOOD AND BEVERAGE COMPANIES

PHARMACEUTICAL COMPANIES

OIL COMPANIES

COSMETIC COMPANIES

WATER PURIFICATION PLANTS

MEDICAL LABORATORIES

ENVIRONMENTAL PROTECTION ORGANIZATIONS

RESEARCH LABORATORIES

INTRODUCTION TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: SEPARATION OF THE DYES IN GRAPE KOOL-AID

OBJECTIVES:

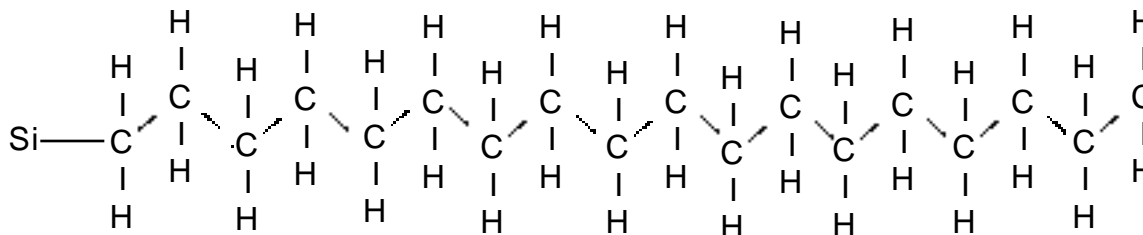
1. To understand the usefulness of separating mixtures in order to analyze them.
2. To separate the different dyes that make grape Kool-Aid purple.
3. To compare the use of methyl alcohol and isopropyl alcohol as solvents.
4. To compare some properties of molecules you use in the separations.

I. BACKGROUND

A fundamental method of study, whether in science, literature, or any other field, is to isolate the parts that make up the whole, and then to start analyzing each of the parts. In this way, a complicated problem can be solved a little at a time.

In analytical science, the technique called High Performance Liquid Chromatography (HPLC) uses precision pumps to measure, mix, and propel a liquid through a tube packed full of a powdery solid adsorbent. Separation of a mixture added to the tube occurs because some molecules in the mixture are more attracted to the moving solvent while other molecules in the mixture are more attracted to the solid motionless adsorbing agent. The molecule that is most attracted (most easily dissolved) in the solvent, and least attracted (adsorbed) by the solid, will flow out of the tube first. Electronic sensors detect the molecules as they exit the tube.

You will gain experience with HPLC in this laboratory exercise. Your hands, eyes, and brain will perform the work of the expensive pumps and other automated equipment, but you will use the same sorts of solvents and adsorbent used by scientific experts performing sophisticated analyses. It is estimated that over 90% of the scientists using HPLC use the same carbon-18 adsorbent that you will be using in this experiment. The chemical formula of the "C-18" adsorbent is shown below. The powder is glass, covered with C-18 chains attached at the silicon atom.



C 18 SEP-PAK CARTRIDGE

Some down-to-earth examples:

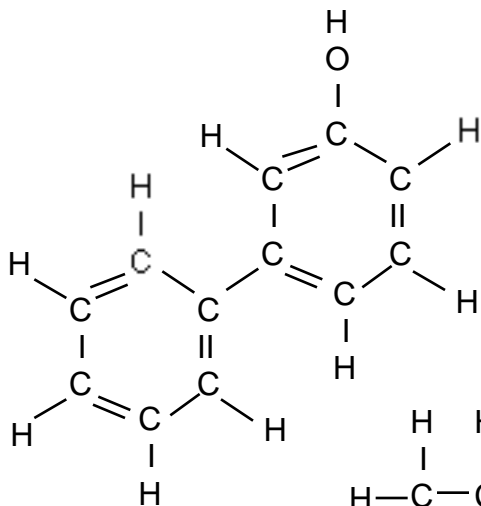
If two molecules are attracted to each other, they tend to stick to one another and to mix. On a large scale, interactions like this make transparent tape stick to paper and food stains stick to clothing. If molecules repel one another, the substances do not stick and they will not mix.

Name _____

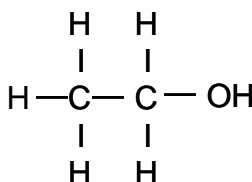
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a. Write the ratio of hydroxyl group (-OH) to carbon atom (C) in the space beside each molecule. Generally speaking, the higher the number of hydroxyl groups per carbon atom, the more polar the molecule.

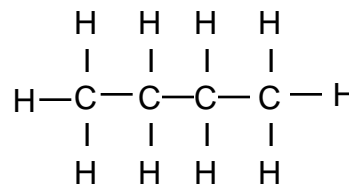
WRITE THE RATIO OF -OH: C IN THE SPACE BELOW EACH MOLECULE



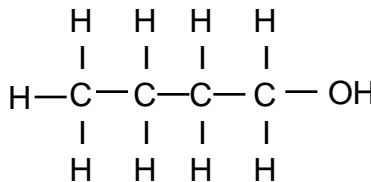
PHENYL PHENOL _____
(LYSOL)



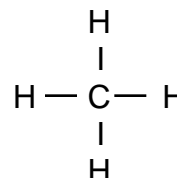
ETHANOL _____



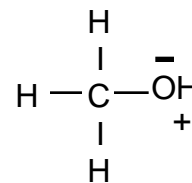
BUTANE _____



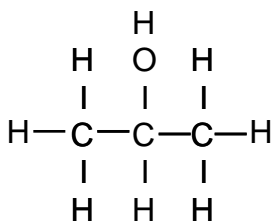
BUTANOL _____



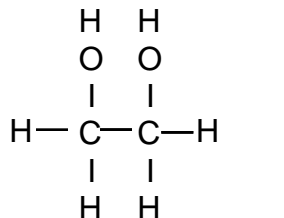
METHANE _____



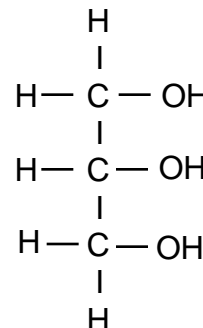
METHANOL _____



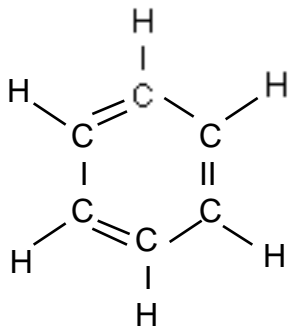
ISOPROPANOL _____
(RUBBING ALCOHOL)



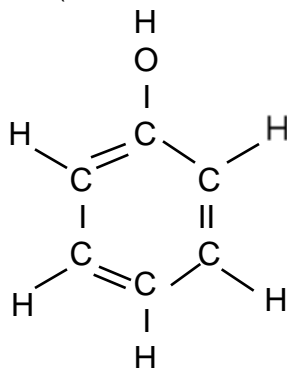
ANTIFREEZE _____
(ETHYLENE GLYCOL)



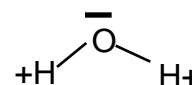
GLYCEROL _____
(USED IN LOTIONS,
COUGH DROPS, AS A
FOOD PRESERVATIVE)



BENZENE _____



PHENOL _____
(USED TO PRODUCE
PLASTICS)



WATER _____

b. Across the bottom of this page, make a list in which you rank the various alcohols and hydrocarbons according to their relative polarity (from most polar to most non-polar). If two or more molecules are equally polar or non-polar, list them together.

High Performance Liquid Chromatography

Kool-Aid Chromatography

You will be separating Grape Kool-Aid into its components via HPLC. This involves a solid phase and a liquid phase. As the concentration of alcohol changes in the liquid phase the pigments either interact more with the liquid or the solid phase. You will determine the effectiveness of these % methanol solutions in separating the components of grape Kool-Aid into different fractions and compare to the same extraction methods using various % propanol solutions. Make **detailed sketches** of the **filters** as you wash various materials through the filters and make **detailed observations** of the material that you collect after it **flows through** the filters.

1. Attach Cartridge (Large end toward the syringe).
2. Pre-wet with 10 ml of 100% methanol. Discard the alcohol as it comes out of the filter.
1. Flush with 10 ml of water. Discard the water that comes through.
2. Add 1 ml Kool-Aid. Collect and make observations.
3. Pump 10 ml water into test tube. Collect and make observations.
4. Pump 10 ml of 5% methanol through. Collect and make observations.
5. Pump 10 ml of 20% methanol through. Collect and make observations. (pump 5 ml more if necessary to remove color)
6. Pump 5 ml 60% methanol through. Collect and make observations.
7. Pump 10 ml 100% methanol through. Discard
8. Combine the two colored samples. Record all observations
9. Repeat with Propanol. Record all observations.

Discuss with your partner the nature of Kool-Aid and what was learned by using HPLC to explore the properties of the chemical mixture.

Questions to ponder and answer in your report;

1. Which did a more complete job of separating the colored in Kool-Aid?
2. Which pigments are more non-polar? Which are polar?
3. Which solvent is more polar? Non-polar?
4. How can the property of a molecule be used to help analyze it?