



College of the Canyons: Introduction to Biotechnology

HPLC Pilot Lab: Post Lab Write Up

1. What chemical characteristic is the separation of the different dyes based on?
Polarity, specifically ratio of polar to non-polar functional groups

2. The ratio of –OH (hydroxyl) groups to carbon atoms provides the basis for determining polarity of the molecules used in this lab.

a. What chemical property does the –OH group exhibit that accounts for this polarity?
Polar bonds as a function of unequal electron sharing (also function of electronegativity).

b. What other atoms would also provide a strong polarity in relationship to carbon atoms? O, N, S, P, Cl All highly electronegative elements that will share electrons (albeit unequally) with carbon. Fluorine too is in this group, but it is rare in biological systems.

c. Which Grape Kool-Aid dye was more polar? Justify your answer by examining and citing the molecular structure.

Red dye, 2 lines of evidence: 1) Red dye eluted in lower alcohol (more polar soln) than blue dye 2) Structure...more polar groups in red dye

3. Red dye eluted in a more polar water concentration than the blue dye. This idea is essentially the opposite of point number 1 FYI!

3. Why is it HPLC a useful technique in science?

HPLC involves separation/purification. The substances may be purified further (by size, density, other properties) or used for additional experiments. For example, DNA purified via HPLC may be used in genetic engineering experiments. Separation is important therefore as it is often the first step in identification, analysis, purification, etc.

4. Rank the following in increasing order of polarity, methanol, water, isopropanol, and ethanol. Isopropanol alcohol, ethanol, methanol, water (least to most polar)

5. Name two other physical or chemical characteristics could be used to separate molecules in chromatography (the textbook is a good reference)?

Shape, size, hydrophobicity, ion groups, highly specific interactions (i.e. antibody/antigen), and other "lock and key interactions".

6. Define the terms elute and eluant.

Elute: To dissolve in a chromatographic system. The dyes were eluted with alcohols.

Eluant: The substance doing the dissolving. The alcohols were the eluants in our HPLC experiment.

7. Occasionally, some very polar functional groups exist in molecules, but the entire molecule has little to no polarity. Provide or explain **two** examples of such molecules and show, diagram, etc. how they may exist with polar groups, yet have no net polarity.

The “net” charge is the idea. You could have many negative and positive groups on a large molecule, but the net charge of the molecule would be zero if the charged groups that are opposite each other are in equal in number.

Charge to mass: The charge may be present, but if it is say a single or a few functional groups that are on an enormous molecule (say an antibody with its 100,000 plus amino acids) the end result is that the charge may be too insignificant to detect, separate, etc.

Dipoles. This is one of the more esoteric ideas. Some charges (especially polar covalent partial charges) are arranged in such a way that the charges essentially cancel each other out when their position in the molecule is considered. CO₂ is one such example. According to the molecular formula, it looks like it should be quite polar (lots of oxygen), when in reality, it is a non-polar gas. The reason is when we look at the dipole. Each oxygen is negative, and the carbon positive,,but the two oxygens are on opposite sides of the carbon, essentially canceling each other out! If you need help visualizing this idea, ask the instructor. Also..understanding dipoles and the over-all molecular structure is beyond the scope of this class, so do not worry if this idea is tricky or hard. Even chemistry folks struggle with dipoles and their implications on net charge!