Liquid chromatography lab

Liquid Chromatography – Laboratory #18 Introduction: We are using liquid chromatography to separate the colored substances in grape-flavored drinks. We separate the component dyes, and then we separate the flavorings and citric acids. Background: Chromatography is a process that is used to separate a substance into its component parts. The separation occurs between the stationary and moving phase of the lab. The moving phase consists of a fluid and the stationary phase consists of a solid. The mixture we are trying to split up is integrated into the mobile phase.

When the mobile phase interacts with the solid phase, the components of the mixture are attracted to the solid phase in varying degrees. Components with higher levels of attraction for the mobile phase result in a faster speed of transport throughout the solid phase. Components with higher levels of attraction for the solid phase result in a slower speed of transport throughout the solid phase. These differing levels of attraction end up in separation of the mixture into component bands, which exit the system as distinct liquids. [pic] Liquid chromatography labs are composed of six main parts: . A separation column filled with a fine-grain solid. b. A solvent (mobile phase) that moves through the separation column. c. An injection system to transport the solvent to the separation column. d. A pump to force the solvent through the separation column. e. A detector to determine when the components exit the separation column. f. A recorder [pic] Although in most chromatography labs the solid phase is polar and the mobile phase is nonpolar, we are using Reverse Phase Liquid Chromatography, where the mobile phase is polar and the solid phase is nonpolar.

When the mobile phase interacts with the solid phase, the polar parts of the mobile phase are attracted to each other, so they wash through the column quickly. The less polar components of the mobile phase are attracted to the nonpolar solid in the column, so they wash through the column slowly. This results in a separation of the components, whose degree is measured as the resolution. [pic] Pre-lab Questions 1. What is the process of chromatography used for? 2. In the chromatography, components of a mixture distribute themselves between the stationary phase and the mobile phase.

Explain how the components can be separate with these two phases. 3. In the liquid chromatography column used in this experiment, the solid has a C18 hydrocarbon bonded to it. Would a C18 hydrocarbon be polar or nonpolar? Explain. 4. Below are the typical data for this experiment. 1 mL of a Kool-Aid solution was loaded on a Sep-Pak C18 Column. The red and blue dyes were eluted from the column with a constant flow of 18% isopropyl alcohol. The eluted solution was collected in a 10 mL graduated cylinder. The volumes of eluant were recoreded at the beginning and end of each color band. pic] Information: The first step in calculating the selectivity and resolution of the system is determining the volumes of eluant corresponding to the band widths and band centers for each eluted dye. a. Bandwidth W is the volume in mL of eluant containing each dye as it emerges from the column. Calculate the bandwidth W for each dye for each of the three runs and then determine the average bandwidth W average for each dye. b. Center of band, called Average Retention Volume V Rave corresponds to the center of each band.

The average retention volume is calculated by taking the average starting volume for each band and adding one half the corresponding average band width. V Rave = V start + (?) W ave Calculate the average retention volume volume V Rave for the red and blue dyes. c. For each dye, a capacity factor k’ can be calculated. This term is a relative measure of the attraction of the dye for the stationary phase as compared to its attraction for the mobile phase. The equation fo capacity factor is : k’ = (V Rave – V M)/V M here V Rave is the average retention volume for each dye and V M is mobile phase or eluant volume in the cartridge. V M can be estimated to be one half the cartridge volume, with the stationary phase occupying the other half. For the Sep-Pak cartridges, this V M value is . 49 mL. Calculate k’ for each dye. d. A selectivity or separation factor, alpha, can now be calculated. This is the ratio of the k’ values for each dye, with the larger value in the numerator. For good separation, a mobile phase is usually chosen that gives an alpha value between 2 and 10. Calculate alpha for this separation: alpha = (k’ Blue)/(k’ Red) e.

The resolution R, a measure of how well the two dyes are separated by the column and eluant, is determined by the equation R = 2(V Rave Blue – V Rave Red)/(W Blue + W Red) where the numerator is the volume between the band centers and the denominatory represents the average band width. The greater the selectivity, the larger the numerator and therefore the greater the resolution. The resolution can also increase as the efficiency of the column increases, since this results in a lower average band width. Calculate R for this separation. Materials: Isopropyl Alcohol, 70% 50 mL

Isopropyl Alcohol, 28%, 10 mL Isopropyl Alcohol, 18% 50 mL Isopropyl Alcohol, 5% 10 mL Graduated Cylinder, 10mL Graduated Cylinder, 25 mL Distilled Water, 300 mL Grape Koolaid Solution, 20 mL Sep-Pak C18 Cartridge 10 mL Syringe w/ male Luer tip Beaker, 100 mL, 3 Beaker, 50 mL, Safety Precautions: Isopropyl alcohol is inflammable and a fire hazard. Do not conduct this laboratory in the presence of flames. This alcohol is slightly toxic by ingestion and inhalation. Chemical-resistant goggles, gloves, and aprons are required. Wash and rinse hands thoroughly with soap and water after conducting the lab.

Procedure Part 1: Isocratic Separation (Constant rate of flow and solvent concentration) Pretreatment of the Sep-Pak C18 Cartridge 1. Cut off the exit tube/shorter end of the cartridge at the point where it meets the body of the cartridge. 2. Load the syringe with 10mL of 70% isopropyl alcohol. 3. Connect the tip of the syringe to the long end of the Sep-Pak cartridge. 4. Pump the isopropyl alcohol through the syringe cartridge at a rate of 5-10 mL/minute. 5. Collect the alcohol in a 10 mL graduated cylinder to monitor flow rate. 6. Repeat previous steps with distilled water. Sample Injection . Use 10 mL syringe to slowly inject 1 mL of Kool-Aid solution onto the column. 2. Discard the effluent that washes out. 3. Remove the cartridge from the syringe. 4. Rinse the syringe with 10 mL of distilled water 3 times to erase Kool-Aid residue. Sample Elution 1. Fill the syringe with 18% isopropyl alcohol eluant and attach the syringe to the Sep-Pak Cartridge. 2. Pump the alcohol through the cartridge with a flow rate of 5-10 mL/min. 3. Collect effluent in 10 mL graduated cylinder. 4. Record volume of effluent collected as first and last of colored drops of each of the dyes exit.

If separation is imperfect, record data for beginning/end of intermediate purple bands. Center of the purple band acts as the end of the first band and beginning of the last. Column Regeneration Repeat measurements two more times. Between injections, wash the column with 10 mL of distilled water at the same flow rate of 5-10 mL/min. If colored residue remains, repeat preatreatment. Part 2: Step Gradient Separation Now, we change composition of the eluting liquid. We first use a polar solvent, and then we reduce the polarity of the solid phase by adding isopropyl alcohol.

Through this, we wash out citric acid and flavoring oils in addition. Pretreatment of the Sep-Pak C18 Cartridge Follow the pretreatment in Part 1. Sample Injection and Component Elution 1. Inject 1 mL of Kool-Aid solution into the column. 2. Elute polar components of the mixture (citric acid and sugar) by passing 5 mL of distilled water through the column. 3. Collect effluent in the first small beaker. 4. Elute the red dye by passing 10 mL of 5% isopropyl alcohol through the column. 5. Collect effluent in the second small beaker. 6. Use 10 mL of the 28% isopropyl alcohol to elute blue dye. 7. Collect effluent in the third small beaker. . Use 10 mL of 70% isopropyl alcohol to elute nonpolar flavor oils and additives. 9. Collect effluent in the fourth small beaker. 10. Record the color of each effluent. Evaporate the solvents and examine the components. 1. Allow the solutions to evaporate and leave them overnight in the fume hood until next lab period. Label solutions properly. 2. Observe and describe contents of each of the beakers. Measure using color, odor, and appearance. Data Table Part 1: Isocratic Separation | | Red Dye | Blue Dye | | Run #1 | Run #2 | Run #3 | Run #1 | Run #2 | Run #3 | | Start of Band (mL) | | | | | | | End of Band (mL) | | | | | | |

W (mL) | | | | | | | Vrave (mL) | | | | | | | K’ | | | | | | Part 2: Step Gradient Separation Beaker | Eluant | Observations | | 1 | H2O | | | | | | 2 | 5% isopropyl alcohol | | | | | | 3 | 28% isopropyl alcohol | | | | | | 4 | 70% isopropyl alcohol | | | | | Calculations Determine the following values and show calculations. Refer to question six in the Pre-Lab Questions. Enter results in the Part 1 data table. 1. Bandwidth W for each dye. 2. Average Retention Volume V Rave for each dye. 3. Capacity Factor k’ for each dye. 4. Selectivity alpha for the two dyes with this isocratic separation. 5. Resolution R for the two dyes with this isocratic separation.

Post-Lab Questions 1. What is meant by polarity of molecules? What causes differences in polarity? 2. In discussing solubility, the rule “ like dissolves like” is frequently used. What does this mean? 3. Draw the structural formula of isopropyl alcohol. Explain how it differs in polarity from water. 4. For good separation of the dyes, the resolution should be greater than one. What was the value you calculated? Did the two dyes overlap as they emerged from the column, or was the separation a good one? 5. In the step gradient separation, four separate fractions were collected. How were these related to the polarities of the column and of the eluting solvent?