PURDUE UNIVERSITY INSTRUMENT VAN PROJECT

DEMYSTIFICATION OF LIQUID CHROMATOGRAPHY CHROMATOGRAPHY OF GRAPE DRINK (Revised: 1-24-93)

How many components are in grape-flavored Kool-Aid®? By using a Sep-Pak® C18 cartridge, four components in grape-flavored Kool-Aid® can be separated: polar components (sugar and citric acid), red food coloring, blue food coloring, and nonpolar components (flavoring oils).

The Sep-Pak® is a small, reusable cartridge which is used in industry and research to separate liquid mixtures into their component parts.

ACTIVITY GUIDELINES

This activity is written as a demonstration, but it can easily be adapted and used as an individual student experiment.

TIME

Getting Ready: 15 minutes Doing the Activity: 20 minutes plus evaporation time ("Doing the Activity" Step 9)

MATERIALS

Sep-Pak® C18 cartridge package of grape Kool-Aid® (or other grape drink mix) water rubbing alcohol (isopropyl alcohol, 70% by volume, with no color or added scent) 2-quart container one of the following volumetric measures: *25-mL graduated cylinder *1-cup measuring cup 5 small vials or test tubes with labels container to collect liquid waste one of the following liquid dispensers: *one 10- to 50-mL syringe with male Luer® tip and four 50- to 100-mL plastic *six 50- to 100-mL plastic squeeze bottles with dropper tips on the lid

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VARIATION

Other types of drink mixes for comparison tests.

SAFETY AND DISPOSAL

No special safety precautions are necessary. All solutions can be discarded by flushing down the drain. The Sep-Pak® should be saved for reuse.

GETTING READY

1.Prepare the grape Kool-Aid® according to directions on the package, <u>but</u> OMIT the sugar. If you are using bottles, fill one bottle with grape drink.

2.Prepare 40 mL of a 35% isopropyl alcohol solution (20 mL of rubbing alcohol and 20 mL of water). Put the solution into a bottle and label it "35% isopropyl alcohol."

3.Prepare 40 mL of a 17.5% isopropyl alcohol solution (10 mL of rubbing alcohol and 30 mL of water). Put the solution into a bottle and label it "17.5% isopropyl alcohol."

4.Prepare 40 mL of an 8.25% isopropyl alcohol solution (5 mL of rubbing alcohol and 35 mL of water). Put the solution into a bottle and label it "8.25% isopropyl alcohol."

5. Fill one of the remaining solvent or squeeze bottles with water and label it.

6.Fill a sixth bottle with undiluted rubbing alcohol and label it "70% isopropyl alcohol."

7.Label five small vials or test tubes as indicated: "Polar Additives", "Red Food Coloring", "Red and Blue Food Coloring", "Blue Food Coloring", "Nonpolar Additives".

8.Acquaint yourself with the following general instructions for using a syringe or bottle with the Sep-Pak® cartridge:

Notes:Do not squeeze the Sep-Pak® cartridge during handling.

• If a syringe is used,

a.Remove the piston from the syringe barrel.

b.Push the longer end of the Sep-Pak® cartridge snugly onto the syringe tip.

c.Hold the assembly with the open barrel up and add the appropriate amount of solvent to the

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syringe barrel.

d.Insert the piston and use it to push the contents of the barrel slowly through the cartridge. e.Remove the cartridge from the syringe tip.

f.Remove the piston and repeat Steps b through e as needed.

Notes:The flow rate in Steps 3, 5 and 6 below ("Doing the Activity") should not exceed 5 mL per minute.

·If a squeeze bottle is used,

a.Snugly attach the tip of the filled squeeze bottle to the longer end of the cartridge. b.Squeeze the bottle to push the contents slowly through the cartridge.

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 following Steps 1 and 2 below ("Doing the Activity").

DOING THE ACTIVITY

1.Pre-wet the cartridge by passing about 10 mL of undiluted rubbing alcohol (70% isopropyl alcohol) through the cartridge.

2.Flush the cartridge with 5 mL of water.

3.Using a syringe or squeeze bottle as described above, slowly load about 1 mL of the grape drink sample into the SeP-Pak® cartridge. Note that a band of color is concentrated near the inlet of the cartridge.

If using a dropper bottle, count the drops to make 1 mL (20 drops = 1 mL).

4.Elute the polar additives (that is, the sugar, if present, and citric acid) by passing about 5 mL of water through the cartridge, collecting the fraction in a small vial labeled "Polar Additives."

Note:Elution is a term used to describe flushing the cartridge with a solution that removes one of the components in the mixture from the cartridge.

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5.Slowly elute the red food coloring with about 4 to 5 mL of the 8.25% isopropyl alcohol solution. Collect the fraction in a small vial labeled "Red Food Coloring".

6.Slowly elute a combination of red and blue food colorings with about 4 to 5 mL of the 17.5% isopropyl alcohol solution. Collect the fraction in a small vial labeled "Red and Blue Food Coloring".

7.Slowly elute the blue food coloring with about 3 to 4 mL of the 35% isopropyl alcohol solution. Collect the fraction in a small vial labeled "Blue Food Coloring".

8.Elute the flavoring oils and other nonpolar additives with about 5 mL of 70% isopropyl alcohol (undiluted rubbing alcohol). Collect the fraction in a small vial labeled "Nonpolar Additives".

Note: This serves to clean the cartridge.

9.Subsequent separations with this wet cartridge can begin with Step 2. If the Sep-Pak® is allowed to dry out, be sure to start at Step 1 again.

10.Allow the solvent in each vial of extract to evaporate and observe the concentrated powder or oils that result.

11.Add 3-4 mL of water to each dried extract. Recombine the parts. Compare this reconstituted mixture with the original Kool-Aid®.

VARIATION A

A different series of bands will elute if you skip step 5 in "Doing the Activity".

Note: If you choose this variation, you will not need to prepare the 8.25% isopropyl alcohol solution described in step 4 in "Getting Ready".

VARIATION B

To observe other series of bands, vary the type of drink, the sample size, and the concentration of the solvents.

EXPLANATION

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The grape drink used in this experiment has four major components: polar additives, which include sugar and citric acid, red food coloring, blue food coloring, and nonpolar components which include the flavoring oils. Each of these components has some affinity for the solid in the Sep-Pak®, but can be removed by solvents which have even more affinity for them. When you elute the cartridge with a series of solvent mixtures with varying polarity, the components can be separated. The most polar fraction (sugar and citric acid) is eluted with water, which is the most polar solvent. The least polar fraction (the flavoring oils) is eluted with 70% isopropyl alcohol (undiluted rubbing alcohol), the least polar solvent used.

The food colorings are separated with different ratios of rubbing alcohol to water; the higher the percentage of rubbing alcohol, the lower the polarity of the solvent. Each dilution has a different polarity and thus makes the separations possible.

The purple color of Kool-Aid~ grape drink is due to a mixture of Blue #1 and Red #40 food dyes. Polar water has no great affinity for the dyes, so upon contact with the Sep-Pak® cartridge the dyes are adsorbed by the cartridge and removed from the grape drink solution. The lower the percentage of isopropyl alcohol in the solution, the greater the polarity of the solution. The molecular weight of the red dye is about half the molecular weight of the blue dye, and the red dye is more polar. The more polar red dye is eluted with the 8.25% isopropyl alcohol solution. Increasing the amount of alcohol to 35% makes the solution less polar and elutes the less polar blue dye.

CURRICULUM INTEGRATION

Some suggested uses of this activity include units on separation technologies, solutions, and polar and nonpolar molecules.

<u>REFERENCES</u>

Bidlingmeyer, B. A. and Warren Jr., F. V. (1984). An Inexpensive Experiment for the Introduction of High Performance Liquid Chromatography. <u>Journal of Chemical Education, 61</u>, 716-720.

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