Kool Chromatography

Column Chromatography Demonstration Kit

Introduction

Separate different colored dyes in grape Kool-Aid® using column chromatography, a popular method used in research and industry to separate, isolate, and purify components of mixtures.

Chemical Concepts

- Polarity
- Column chromatography

Materials Needed

Isopropyl alcohol solution, 70%, 500 mL*
Sep-Pak® C18 cartridge*
Grape Kool-Aid®, 1 packet*
Beakers, 600-mL, 2
Graduated cylinder, 100-mL
Microplate, 6-well*
Overhead projector
Syringe with luer lock tip, 12-mL*

**Materials included in kit.*

Safety Precautions

Isopropyl alcohol solution is a flammable liquid; keep away from open flame. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron.

Preparation

1. To prepare 500 mL of a 25% isopropyl alcohol solution, add 180 mL of 70% isopropyl alcohol solution to a 600-mL beaker and dilute to the 500-mL mark with distilled or deionized water.
2. To prepare 500 mL of a 5% isopropyl alcohol solution, add 35 mL of 70% isopropyl alcohol solution to a 600-mL beaker and dilute to the 500-mL mark with distilled or deionized water.
3. Prepare the Kool-Aid® according to the package instructions. Do not add sugar. The resulting solution is approximately 0.3 g of Kool-Aid powder per 100 mL of distilled or deionized water.
4. If the syringe has a tip cover, remove it before performing this demonstration.

Procedure

1. Pretreat the column by drawing 10 mL of the 70% isopropyl alcohol solution into the syringe. Twist the Sep-Pak® C18 cartridge snugly into place on the luer lock tip of the syringe. Using the plunger, expel the isopropyl alcohol solution out of the syringe back through the column.
2. Repeat Step 1 using 10 mL of distilled or deionized water in place of the 70% isopropyl alcohol solution.
3. Place the 6-well microplate on the overhead and pour grape Kool-Aid® into one of the wells. Remove the cartridge from the syringe and draw 10 mL of the grape Kool-Aid from the microplate into the syringe.
4. Place the cartridge back on the syringe and force the Kool-Aid through the column and into a clean well on the microplate. Notice the clear solution that elutes (or exits) from the column.

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5. Again remove the cartridge from the syringe. If there is any grape Kool-Aid left in the syringe, rinse the syringe with 5% isopropyl alcohol first. Draw 10 mL of 5% isopropyl alcohol solution into the syringe and place the cartridge back on the syringe.
6. Force the 5% isopropyl alcohol solution through the column into a clean well on the microplate. Note the red-colored solution that exits the column.
7. Remove the cartridge from the syringe and draw 10 mL of 25% isopropyl alcohol solution into the syringe. Replace the cartridge.
8. Force the 25% isopropyl alcohol solution through the column into a clean well on the microplate. Note the blue-colored solution that elutes from the column.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Flush all solutions down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Tips

- The Sep-Pak® C18 cartridge has a short end and a long end. The cartridge can be used either direction. It is important to keep the flow going in one direction.
- The Sep-Pak C18 cartridge can be used to separate many mixtures of varying polarity. The demonstration becomes more visually appealing if the mixture has at least two colors that elute separately.
- An air pocket in the syringe will not affect the outcome of the demonstration.
- To store or reuse the Sep-Pak C18 cartridge, first clean the column by rinsing it with 10 mL of 70% isopropyl alcohol solution, then rinsing with 10 mL of distilled or deionized water. If cleaned properly after each use, the Sep-Pak C18 cartridge can be reused indefinitely.

Discussion

The ingredients of grape Kool-Aid® include sugar, citric acid, red dye, ascorbic acid, and blue dye. As the Kool-Aid passes through the very non-polar Sep-Pak C18 column, the polar molecules, such as citric acid, preferentially adhere to the polar solvent—water. The non-polar molecules, such as the dyes, spend very little time adhering to the polar solvent and therefore stay in the non-polar column. The 5% isopropyl alcohol solution is slightly non-polar. As the dilute alcohol solvent is passed through the column, the red dye, which is also slightly non-polar, is still more attracted to the solvent than it is to the column. The blue dye, however, is more non-polar than the red dye and is still attracted more strongly to the column than it is attracted to the solvent. Therefore, only the red dye is eluted from the sample by the 5% isopropyl alcohol solution. The 25% isopropyl alcohol solution is more non-polar than the 5% isopropyl alcohol solution. The more non-polar mixture now attracts the blue dye away from the column, causing it to flow out of the cartridge with the solvent.

Column liquid chromatography (LC) is often used in industry to separate mixtures and detect trace components of a mixture. High performance liquid chromatography (HPLC) has become the instrument-of-choice for many quantitative analyses. This demonstration and the concepts and processes involved can be compared directly to HPLC. As with HPLC there is a solvent delivery system (the syringe), an injector (the syringe), a column (Sep-Pak cartridge), and a detector (the human eye).

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Systems, order, and organization
Evidence, models, and explanation
Content Standards: Grades 9–12
Content Standard B: Physical Science, structure and properties of matter

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Answers to Worksheet Questions

1. Describe what happened in this demonstration.

   Water was drawn into a syringe, and then forced back out the syringe via an attached column cartridge. Grape Kool-Aid was then passed into the syringe and out through the column. The solution that left the syringe was colorless. Then 5% isopropyl alcohol was passes through the column. This time the exiting solution was red. Finally, 25% isopropyl alcohol was passed through the column. The exiting solution was blue.

2. The Sep-Pak C18 cartridge is very non-polar. Rank the three solutions used to separate the Kool-Aid, water, 5% isopropyl alcohol, and 25% isopropyl alcohol, in terms of their polarity from the most polar to the least polar.

   Water is the most polar of the solutions. 5% isopropyl alcohol is slightly less polar than water, and 25% isopropyl alcohol is the least polar, or the most non-polar, of the three solutions.

3. The ingredients of grape Kool-Aid are sugar, citric acid, ascorbic acid, blue dye, and red dye. Water, 5% isopropyl alcohol, and 25% isopropyl alcohol were passed through the column in that order. Based on what you know about the polarity of the solutions, explain what you observed during the demonstration.

   When the polar solvent, water, was passed through the column, the polar molecules naturally preferred the water to the cartridge. But the red and blue dyes, which are more non-polar, stayed in the column. When 5% isopropyl alcohol, which is slightly non-polar, was passed through the slightly non-polar red dye molecules adhered to the solvent. When, at last, the 25% isopropyl alcohol, the least non-polar of the solvents, passed through, the very non-polar blue dye was more attracted to this solution than the cartridge, and exited the cartridge with the alcohol.

4. High-performance liquid chromatography, also known as HPLC, is often used for quantitative analyses. HPLC requires the use of a solvent delivery system, an injector, a column, and a detector. This demonstration is comparable to HPLC. Therefore, what is the equivalent of each of those materials in this demonstration procedure?

   The syringe served both as the solvent delivery system and the injector. The Sep-Pak cartridge was the column, and people, primarily their eyes, were the detectors in this demonstration.

References

Vonderbrink, S. A. *Laboratory Experiments for Advanced Placement Chemistry;* Flinn Scientific: Batavia, IL, 1995; pp 149–153.

Bidlingmeyer, B. A.; Warren, F. V. *J. Chem Educ.* **1984,** *61,* 716–720.

The *Kool Chromatography—Column Chromatography Demonstration Kit* is available from Flinn Scientific, Inc.

Catalog No.	Description
AP8950	Kool Chromatography— Column Chromatography Demonstration Kit

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Name: _____

Kool Chromatography Worksheet

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