Amp It Up! Engineering/Technology and Industry Lesson Extension

Teacher Name(s), School and District:
Donna Brewster, Beverly High School, Beverly, MA

Course Name: Honors Chemistry

Lesson/Unit Name:
This lesson focuses on how solutions can be separated using liquid chromatography

Science or Education Topic(s):

Common Core Standard, ELA

CCSS.ELA-LITERACY.W.9-10.2.D
Use precise language and domain-specific vocabulary to manage the complexity of the topic. of the discipline in which they are writing.

CCSS.ELA-LITERACY.W.9-10.4
Produce clear and coherent writing in which the development, organization, and style are appropriate to task, purpose, and audience. (Grade-specific expectations for writing types are defined in standards 1-3 above.)

Massachsetts State Frameworks – Content Standards, Chemistry:

1. Properties of Matter

Central Concept: Physical and chemical properties reflect the nature of the interactions between molecules or atoms, and can be used to classify and describe matter.

7. Solutions, Rates of Reaction, and Equilibrium

Central Concepts: Solids, liquids, and gases dissolve to form solutions. Rates of reaction and chemical equilibrium are dynamic processes that are significant in many systems (e.g., biological, ecological, geological).

Massachsetts State Frameworks – Science Inquiry Skills

SIS2. Design and conduct scientific investigations.
SIS3. Analyze and interpret results of scientific investigations.
SIS4. Communicate and apply the results of scientific investigations.

Massachsetts State Frameworks – Content Standards, Technology/Engineering:

1. Engineering Design

Central Concepts: Engineering design involves practical problem solving, research, development, and invention/innovation, and requires designing, drawing, building, testing, and redesigning. Students should demonstrate the ability to use the engineering design process to solve a problem or meet a challenge.

When Taught:
This will be taught during the 3rd quarter, during the discussion of solutions

Abstract:

During this lesson, students will be introduced the the concept of chromatography as a tool for separating a mixture. Students will first read about chromatography as a jig saw activity. Following the reading, a brief overview of how liquid chromatography is used in industry in the production of conjugated antibodies. During this overview, the work of Cell Signaling will be introduced, showing students how industry actually uses chromatography to research and produce conjugated antibodies for the detection and treatment of cancer. An overview of the steps of liquid chromatography will be presented, followed by the students doing a separation of grape Kool-Aid. Finally, students will do a lab write-up on their separation.

Objectives and assessment:

Objectives *By the end of this lesson/unit, the students will be able to:*	**Assessment** *How was the objective assessed? List the example of formative or summative assessment.*
Identify chromatography as a tool for separating components of a mixture	Jig Saw reading
Distinguish between three types of chromatography in terms of their uses	Jig Saw reading
Investigate the use of chromatography in the separation on a mixture	Collection of data during lab
Draw conclusions about the characteristics of the components of a mixture based on experimental data	Lab write-up and conclusion

Engineering/Technology Link: Please check the appropriate box(es) in question 1. And provide a brief answer to question 2.:

1. How did you *introduce* engineering/ technology concepts or the company/industry focus in your course?
    - ✓ Defined terms (science, engineering, technology)
    - ✓ Overview of the company
    - ✓ Challenge based on 'industry specific' area of focus (manufacturing process, quality control, measurement, development, teamwork etc.)

2. After introducing the concepts, following is a brief overview of what students will do during this lesson:

Students will explore industry uses of chromatography with emphasis on production of conjugated antibodies. Activities include:

- Reading on chromatography as a jig saw
- Power point presentation on use of liquid chromatography for purification of conjugated antibodies

Students will use liquid chromatography in a hands-on activity in order to separate grape Kool Aid into fractions. Students will:

- Preform lab
- Complete lab report

Level of Inquiry:

- ✓ *Structured inquiry*: Instructor provides question and procedure. Students determine the results based on given procedures.

Lesson Extension Plan:

Title/Topic: Liquid Chromatography and separation of mixtures
Time (minutes): two 84 minute blocks
Company Name and brief Description: Cell Signaling **Research in the field of applied systems biology research, particularly as it relates to cancer, and production of antibodies for multiple applications.**
Overview of the Lesson During this lesson, students will learn about chromatography as a means of separating components of a liquid
Standard(s)/Unit Goal(s) to be addressed in this lesson: Solutions are homogeneous mixtures that can be separated based on the physical properties
Essential Question(s) addressed in this lesson: How can scientists separate mixtures and retrieve different fractions of a mixture?
Objectives • Identify chromatography as a tool for separating components of a mixture • Distinguish between three types of chromatography in terms of their uses • Investigate the use of chromatography in the separation on a mixture • Draw conclusions about the characteristics of the components of a mixture based on experimental data
Link to Industry: • Students will become award of the role of Cell Signaling in the field of disease research and antibody production • Students will learn about the use liquid chromatography in producing high-quality, pure conjugated antibodies
What students should know and be able to do before starting this lesson • The difference between types of matter, in particular, homogeneous and heterogeneous mixtures • Molecular geometry and effect on polarity of a molecule

- Effects of polarity on dissolvability of solutes

Instructional Materials/Resources/Tools

- Reading on chromatography
- Graphic organizer for reading
- Power point presentation on use of chromatography
- Lab on liquid chromatography
- C-18 Sep-pac cartridges
- Isopropyl alcohol (in various concentrations)
- Grape Kool-aid
- 10-mL and 1-mL syringes
- graduated cylinders
- beakers

Lesson Delivery

Lesson Opening

Students will do a jig saw reading on liquid chromatography

During the Lesson (activities/labs/challenges)

1. Students will focus on four parts of a reading, and present their findings to their groups
   - What is Chromatography?
   - How does chromatography work?
   - What are the different types of chromatography?
   - What is chromatography used for?
2. Power Point presentation on the use of liquid chromatography by Cell Signaling in the production of conjugated antibodies
3. Students will do lab on separation of Grape Koolaid by liquid chromatography
4. Students will complete a lab write-up, including how liquid chromatography is used by industry.

Assessment

Student Assessment:

Students will be assessed on their lab using a rubric

Additional resources and assessments: List the attachments here.
Attachments should include handouts, readings (with references), lab write-ups, rubrics, exams/quizzes, and/or other similar materials.

Reading: "How Does Chromatography Work?" *Explain That Stuff*. Web. 16 Apr. 2016.

Chromatography

by Chris Woodford. *Last updated: July 12, 2015.*

Most of us have got our papers wet at some time or another, but have you ever noticed what happens to the ink as the water spreads? It doesn't always smudge and blur, as you might expect. Sometimes it splits up into weird colored streaks that creep across the page. When that happens, you're seeing **chromatography** in action. In this case it's totally accidental, but we can also use it by design to split up mixtures and other substances into their components. Chromatography is actually one of the most useful analytical techniques chemists have at their disposal, helpful in everything from identifying biological materials to finding clues at crime scenes. What is it and how does it work? Let's take a closer look!

Photo: Injecting a sample into a gas chromatography machine. Photo by courtesy of NASA Kennedy Space Center (NASA-KSC).

What is chromatography?

Chromatography is a pretty accurate description of what happens to ink on wet paper, because it literally means "color writing" (from the Greek words *chroma* and *graphe*). Really, though, it's a bit of a misnomer because it often doesn't involve color, paper, ink, or writing. Chromatography is actually a way of separating out a mixture of chemicals, which are in gas or liquid form, by letting them creep slowly past another substance, which is typically a liquid or solid. So, with the ink and paper trick for example, we have a liquid (the ink) dissolved in water or another solvent creeping over the surface of a solid (the paper).

Photo: Accidental chromatography: A poster's ink runs in the rain. Photo by courtesy of Anders Sandberg published on Flickr under a Creative Commons Licence.

The essential thing about chromatography is that we have some mixture in one state of matter (something like a gas or liquid) moving over the surface of something else in another state of matter (a liquid or solid) that stays where it is. The moving substance is called the **mobile phase** and the substance that stays put is the **stationary phase**. As the mobile phase moves, it separates out into its components on the stationary phase.

We can then identify them one by one.

How does chromatography work?

Think of chromatography as a race and you'll find it's much simpler than it sounds. Waiting on the starting line, you've got a mixture of chemicals in some unidentified liquid or gas, just like a load of runners all mixed up and bunched together. When a race starts, runners soon spread out because they have different abilities. In exactly the same way, chemicals in something like a moving liquid mixture spread out because they travel at different speeds over a stationary solid. The key thing to remember is that chromatography is a *surface* effect.

As the liquid starts to move past the solid, some of its molecules (energetic things that are constantly moving about) are sucked toward the surface of the solid and stick there *temporarily* before being pulled back again into the liquid they came from. This exchange of molecules between the surface of the solid and the liquid is a kind of adhesive or gluing effect called **adsorption** (with a *d*—don't confuse it with absorption, with a *b*, where molecules of one substance are *permanently* trapped inside the body of another). Now remember that our liquid is actually a mixture of quite a few different liquids. Each one undergoes adsorption in a slightly different way and spends more or less time in either the solid or the liquid phase. One of the liquids might spend much longer in the solid phase than in the liquid, so it would travel more slowly over the solid; another one might spend less time in the solid and more in the liquid, so it would go a bit faster. Another way of looking at it is to think of the liquid as a mixture of glue-like liquids, some of which stick more to the solid (and travel more slowly) than others. This is what causes the different liquids within our original liquid mixture to spread out on the solid.

For chromatography to work effectively, we obviously need the components of the mobile phase to separate out as much as possible as they move past the stationary phase. That's why the stationary phase is often something with a *large surface area*, such as a sheet of filter paper, a solid made of finely divided particles, a liquid deposited on the surface of a solid, or some other highly *adsorbent* material.

What are the different types of chromatography?

There are many different ways of using chromatography. These are some of the best known:

Paper chromatography

This is the "spot of ink on paper" experiment you often do in school (also the effect we described at the start when you get your papers wet). Typically you put a spot of ink near one edge of some filter paper and then hang the paper vertically with its lower edge (nearest the spot) dipped in a solvent such as alcohol or water. Capillary action makes the solvent travel up the paper, where it meets and dissolves the ink. The dissolved ink (the mobile phase) slowly travels up the paper (the stationary phase) and separates out into different components. Sometimes these are colored; sometimes you have to color them by adding other substances (called **developers** or developing fluids) that help you with identification.

Photo: Simple paper chromatography. Draw some blobs of ink on paper (Crayola washable children's fiber tips are perfect), roll the paper into a cylinder, and place it in a wine glass with a small amount of water. As the water creeps up the paper, the colors will separate out into their components. That's chromatography in action!

Column chromatography

Instead of paper, the stationary phase is a vertical glass jar (the **column**) packed with a highly adsorbent solid, such as crystals of silica or silica gel, or a solid coated with a liquid. The mobile phase is pumped at high pressure through the column and splits into its components, which are then removed and analyzed. In **liquid-column chromatography**, the mixture being studied is placed at one end of the column and an extra added substance called an **eluant** is poured in to help it travel through. **Thin-film chromatography** is a variation of this technique in which the "column" is actually a film of glass, plastic, or metal coated with a very thin layer of adsorbent material

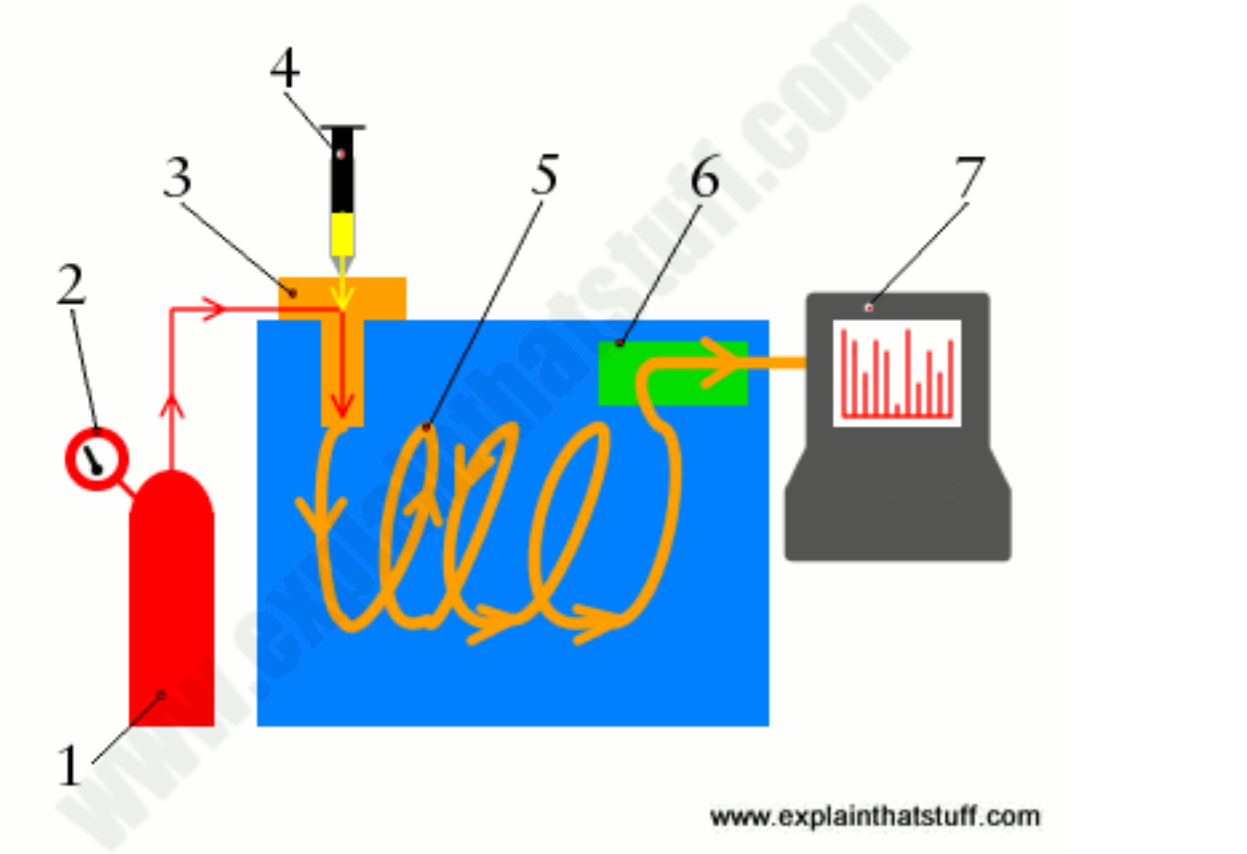
Gas chromatography

So far we've considered chromatography of liquids traveling past solids, but one of the most widely used techniques is a type of column chromatography using gases as the mobile phase. Gas chromatography is a largely automated type of chemical analysis you can do with a sophisticated piece of laboratory equipment called, not surprisingly, a gas chromatograph machine.

Photo: Gas chromatography is largely automated, but it still takes a trained operator to work one of these machines. Photo by courtesy of NASA Kennedy Space Center (NASA-KSC).

First, a tiny sample of the mixture of substances being studied is placed in a syringe and injected into the machine. The components of the mixture are heated and instantly vaporize. Next, we add a carrier (the eluant), which is simply a neutral gas such as hydrogen or helium, designed to help the gases in our sample move through the column. In this case, the column is a thin glass or metal tube usually filled with a liquid that has a high boiling point (or sometimes a gel or an adsorbent solid). As the mixture travels through the column, it's adsorbed and separates out into its components. Each component emerges in turn from the end of the column and moves past an electronic detector (sometimes a mass spectrometer), which identifies it and prints a peak on a chart. The final chart has a series of peaks that correspond to all the substances in the mixture. Gas chromatography is sometimes called **vapor-phase chromatography (VPC)** or **gas-liquid partition chromatography (GLPC)**.

Here's a very simplified overview of what happens in the gas chromatography process:



1 The **eluant** (carrier gas) is introduced from a gas cylinder outside the machine. It's called the carrier because that's exactly what it does—carry the sample we're studying through the machine. In gas chromatography, the carrier gas is the mobile phase.
2 The **rate of flow** of the carrier is carefully controlled to give the clearest separation of the components in the sample.
3 The carrier enters the machine through an **inlet port/splitter**.
4 The sample being measured is injected into the carrier gas using a **syringe** and

instantly vaporizes (turns into gas form).

5 The gases that make up the sample separate out as they move along the **column** (orange), which is the stationary phase. The column is a very thin (capillary) tube, sometimes as much as 30–60m (100-200ft) long, coiled and entirely contained inside an oven (blue) that keeps it at a high enough temperature to ensure that the sample remains in gas form. The temperature of the oven can be carefully controlled.

6 As the sample separates out and its constituent gases travel along the column at different speeds, a **detector** senses and records them. Various different detectors can be used, including flame ionization detectors, thermal conductivity detectors, and mass spectrometers (usually separate machines).

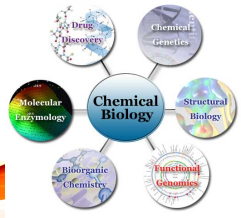
7 The data analyzer/recorder attached to the machine draws a **chromatogram** (chart) with peaks corresponding to the relative amounts of the different chemicals in the sample.

What is chromatography used for?

Chromatography was developed in Russia in 1906 by an Italian-born botanist named Mikhail Tswett (sometimes spelled Tsvet; 1872–1919), who used it for studying plant pigments such as chlorophyll. During the 20th century, chemists found chromatography was a superb technique for studying and separating all kinds of complex mixtures. It's now widely used in forensic science (for identifying samples taken from crime scenes), in pollution monitoring (for identifying small concentrations of unknown pollutants in air and water samples), and for studying complex mixtures in such things as food, perfume, petrochemical, and pharmaceutical production. One of chromatography's big advantages is that it works with tiny samples and low concentrations (particularly helpful when it comes to such things as forensic science and drug or pollution testing).

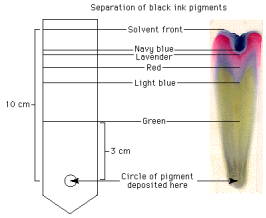
Photo: What's your poison? A sample of vehicle exhaust is injected into a gas chromatography machine so the pollutants it contains can be analyzed. Photo by Warren Gretz courtesy of US DOE/NREL (US Department of Energy/National Renewable Energy Laboratory).

CHROMATOGRAPHY



WHAT IS CHROMATOGRAPHY?

- Chromatography is the separation of a mixture by passing it in solution
  - The parts of the mixture separate at different rates
- Paper chromatography
- Liquid chromatography
- Gas chromatography

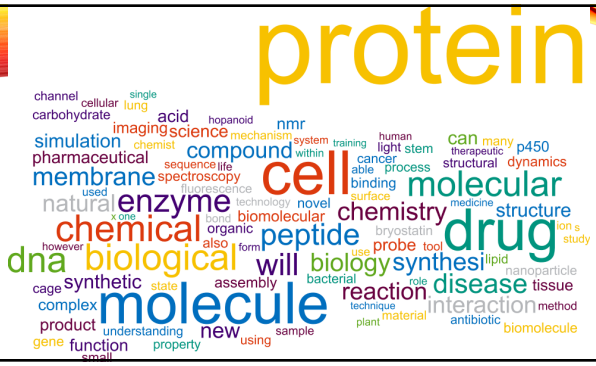


READ ABOUT CHROMATOGRAPHY

Explain that stuff!!

- What is Chromatography?
- How does chromatography work?
- What are the different types of chromatography?
- What is chromatography used for?

http://www.explainthatstuff.com/chromatography.html

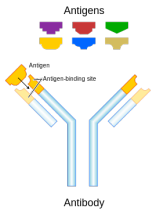


Cell Signaling TECHNOLOGY®

- A company right in your back yard!
- Active in the field of applied biological research
- What do they do
  - Research cancer
  - Produce antibodies to help detect cancer
  - Antibodies can then be used to figure out the best treatments for a particular cancer
- Cell Signaling uses liquid chromatography to purify their antibodies … then they sell them

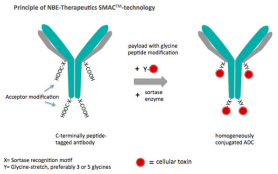
WHAT ARE ANTIBODIES?

- Antibodies are a defense mechanism in our bodies
- Antibodies are proteins made by our bodies
- Function: they find and destroy foreign objects in our bodies, such as bacteria and viruses



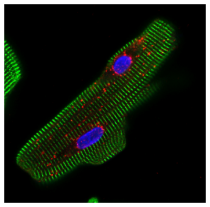
CELL SIGNALING MAKES CONJUGATED ANTIBODIES

- Using chemistry, antibodies are "tagged"
- Why tag antibodies?
- The antibodies detect certain tissues in your body
  - The tag allows the cells to be imaged

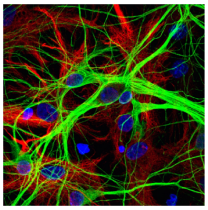


EXAMPLES OF FLUORESCENCE IMAGING

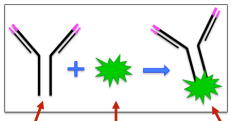
Heart cells



Nervous tissue



TAGGING AN ANTIBODY

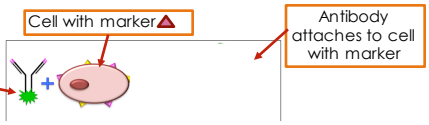


antibody

Fluorescent tag

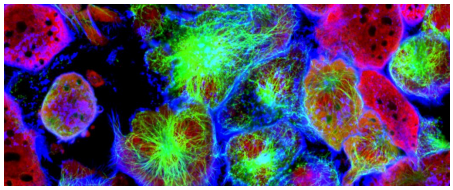
Conjugated antibody

CONJUGATED ANTIBODY AT WORK



Nothing happens here – the marker is not on the cell

CANCER CELLS WITH FLUORESCENT ANTIBODIES

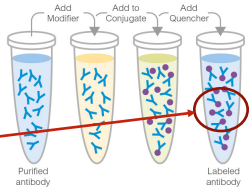


WHAT IS USED TO TAG ANTIBODIES?

- Phycoerythrin is a red pigment found in red algae
- This pigment is used to "tag" antibodies

TAGGING AN ANTIBODY WITH PHYCOERYTHRIN

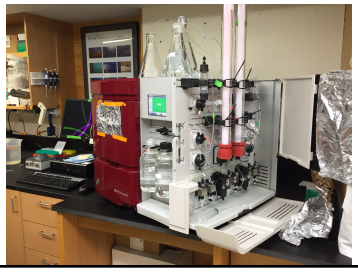
- The resulting mixture contains
  - Antibodies (smaller mass)
  - Phycoerythrin (very large mass)
  - Antibody-phycoerythrin complex
- Need to separate the antibody-phycoerythrin complex from the rest!
  - That can be sold by Cell Signaling
- Separation occurs by **liquid chromatography**
- This complex can then be sold $$$



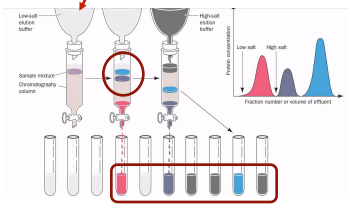
GUESS HOW MUCH THESE TAGGED ANTIBODIES COST?

- One bottle that contains 100 µL (that's about 2 drops)…
- About $500!
- 1 teaspoon holds 50 bottles!!

LIQUID CHROMATOGRAPHY!



HOW DOES IT WORK?



Mixture enters the chromatography column

As the mixture flows through the column, the mixture separates into fractions

The fractions are then collected, saving the part of the mixture you want

For Cell Signaling, this would be the purified tagged antibody

YOUR TURN

- In our lab you will be separating grape koolaid using liquid chromatography

Liquid Chromatography

Chromatography is a process used to separate the components of a mixture. A mixture is injected into a chromatography column, where it lands on a substrate, also known as the stationary phase. The stationary phase may be polar, attracting polar substances, or nonpolar, attracting nonpolar substances. When a mixture is injected into a chromatography column, the substances in the mixture cling to the stationary phase. Next, a solvent is injected into the column. The solvent is called the mobile phase. As the solvent moves along the stationary phase, it will carry the components with it. When and how quickly the substances are carried out of the column by the solvent depends on the polarity of the substances and their solubility in the solvent. If the solubilities and/or polarities of the individual parts of the mixture are significantly different, the substances in the mixture will separate from each other as the mixture travels along the substrate. The substance that is the most strongly attracted to the solvent will be the first to leave the chromatography column. The substrate (stationary phase) and the solvent (mobile phase) can be in any phase, depending on the properties and concentrations of the components in a given mixture. Therefore, there is solid, liquid, and gas chromatography.

In this experiment, you will use liquid chromatography to separate the dyes, FD&C Blue and FD&C Red that are found in grape-flavored Kool-Aid®, from the other ingredients in the dry drink product. You will use a special column, called a C18 Sep-Pac® for the experiment. This column contains a silica solid with a C18 hydrocarbon bonded to it, which renders the solid nonpolar.

During this lab you will conduct a step gradient separation. In this process, three solvents are used (each of a different polarity and concentration) to separate most all of the substances in the mixture.

OBJECTIVES

In this experiment, you will

- Conduct a step gradient, liquid-chromatographic separation.

Prelab

1. List the following substances from most polar to least polar: water, isopropyl alcohol, C18 hydrocarbon

2. When separating the dyes of kool-aid, the fraction that elutes first will be more or less polar than the others? Explain how you can tell.

MATERIALS

C18 Sep-Pac cartridge
10 mL syringe with male Luer® tip
or 50 mL dropper bottle with tip
or 100 mL wash bottle
1 mL syringe with male Luer tip
70% isopropanol (2-propanol)
Grape Kool-Aid drink mix, unsweetened
distilled water
four 50 mL beakers

two 10 mL graduated cylinders
two 25 mL graduated cylinders
three 100 mL beakers

PROCEDURE

1. Prepare the solvents (mobile phase).
   a. Mix 3.5 mL of 70% isopropanol with 46.5 mL of distilled water into a 100 mL beaker to make a 5% isopropanol solution.
   b. Mix 20 mL of 70% isopropanol with 30 mL of distilled water into a 100 mL beaker to make a 28% isopropanol solution.
   c. Obtain a dropper bottle of distilled water as the third solvent for the step gradient separation.

2. Pretreat the C18 Sep-Pac liquid chromatography cartridge.
   a. Obtain a C18 Sep-Pac cartridge and cut off the exit tube (the short end). This will help keep the two food dyes separated.
   b. *If you are using a 10 mL syringe*, fill it with 10 mL of undiluted 70% isopropanol. Attach the tip of the syringe to the long end of the Sep-Pac cartridge and inject the isopropanol into the column at a rate of 5-10 mL per minute. Collect the eluate into a 10 mL graduated cylinder to help you monitor the flow rate of the isopropanol.
   c. *If you are using a wash bottle or a dropper bottle*, fill the bottle with 70% isopropanol and firmly attach the top of the bottle to the long end of the column. Pump 10 mL of isopropanol slowly through the column.
   d. Wash the Sep-Pac cartridge with 10 mL of distilled water.

3. Use a 1 mL syringe to draw up 1 mL of your sample of grape Kool-Aid. Slowly inject the 1 mL of Kool-Aid into the Sep-Pac cartridge. Collect and discard the effluent that washes out of the column as you inject the sample.

4. Elute the components of the grape Kool-Aid sample and separate by the step gradient process.
   a. Obtain four 50 mL beakers. Label the beakers, 1–4.
   b. Set up Beaker 1 to collect the first eluate. Pass 5 mL of distilled water through the column to elute the polar components of the mixture and collect them in the first of four 50 mL beakers.
   c. Set up Beaker 2 to collect eluate from the column. Pass 5 to 10 mL of 5% isopropanol solution through the column to elute the red dye.
   d. Set up Beaker 3 to collect eluate. Pass 5 to 10 mL of 28% isopropanol solution through the column to elute the blue dye.
   e. Set up Beaker 4 to collect eluate. Pass 8 mL of 70% isopropanol solution through the column to elute flavor oils and other nonpolar ingredients.

5. Place the four beakers of eluate in a hood, or well-ventilated area away from open flames, and allow the solvents to evaporate. When the beakers are dry, observe the contents of each beaker and record your observations.

6. All of the solutions may be discarded down the sink. The Sep-Pac cartridge is reusable.

DATA TABLE

Fraction	observations

DATA ANALYSIS

1. Describe the contents of the four 50 mL beakers in which you collected the various ingredients of the grape Kool-Aid mix. Estimate the relative amounts of the substances.

2. Describe how the solvents worked as the mobile phase of the liquid chromatography experiment. Why was it necessary to use different concentrations of aqueous isopropanol in the step-gradient separation?

CONCLUSION (your conclusion should be 300 words or less)

- Claim: restating the purpose of the lab, was the purpose accomplished?
- Evidence: referring to your observations from the lab, back up your claim
- Reasoning: explain the results of your lab
- Going further: Why is it important for Cell Signaling to provide their customers with highly specific and thoroughly validated antibodies? How does liquid chromatography help Cell Signaling provide their customers with highly specific and thoroughly validated antibodies?