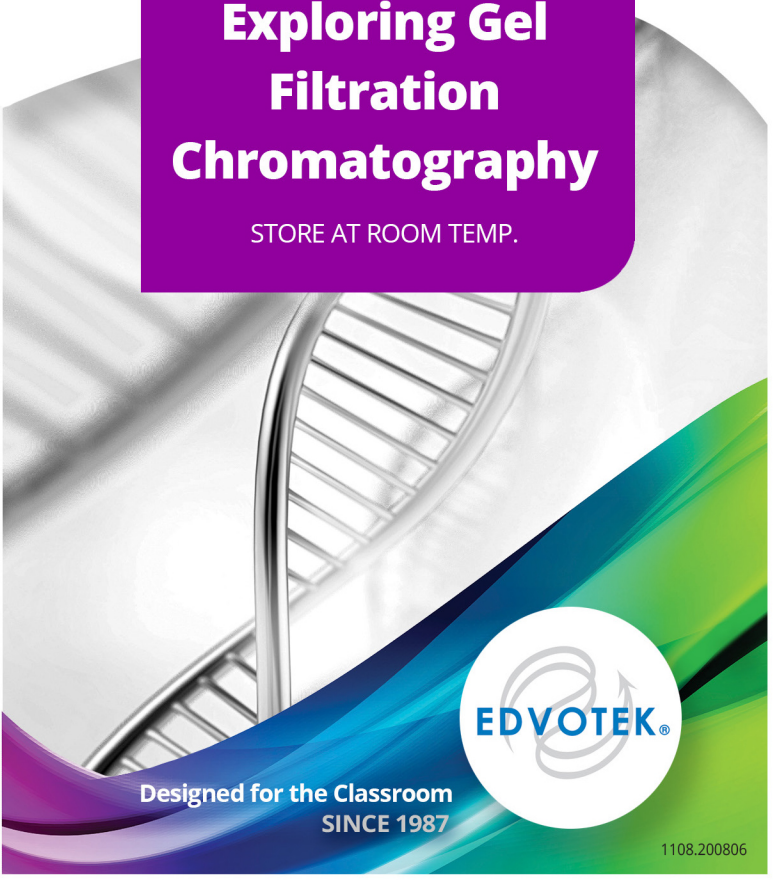
EDVOTEK® MyLab™ #1108

Exploring Gel Filtration Chromatography

STORE AT ROOM TEMP.



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OBJECTIVES

The objective of this experiment is to introduce the principles of gel filtration chromatography as a method that separates molecules according to their size and shape. A mixture of two molecules are separated in this experiment.

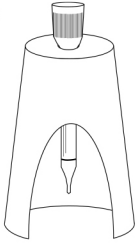
COMPONENTS

This experiment contains reagents and disposables for 3 experiments.

A Dye Sample Mixture
B Liquid Matrix
C Concentrated Elution Buffer
- Transfer Pipets
- Microtiter Plate
- Chromatography Column

REQUIREMENTS

- Purified Water
- Ring Stand with clamp or homemade column holder (large plastic cup with hole drilled through the bottom and front space removed for collecting fractions)



GENERAL SAFETY PRECAUTIONS

Parental or adult supervision required.

1. Designate a clean and uncluttered area for performing experiments.
2. Read all instructions before you begin.
3. Do not eat or drink. Do not apply make-up or contact lenses. Adult(s) should not smoke while performing experiments.
4. Wash your hands before and after performing the experiment.
5. Gloves and goggles should be worn routinely as good laboratory practice.
6. Disinfect the counter top or bench with 70% isopropyl alcohol (rubbing alcohol, or place clean newspaper over the area to be used.

SAFETY PRECAUTIONS

The solutions can be disposed down the drain. All other materials can be disposed in regular solid waste (trash).

WARNING: Choking hazard. Products contain small parts. Not appropriate for children under 5 years old. No human or animal products are used in any experiments.

GEL FILTRATION CHROMATOGRAPHY

Gel filtration chromatography (a.k.a molecular sieve chromatography) is a method that separates molecules according to their size and shape. The basic components of the gel filtration experiment are:

- The samples separated in chromatography experiments can be small molecules, proteins, and nucleic acid. In this experiment, the sample consists of a mixture of orange and blue dye molecules.
- The stationary phase of chromatography is the gel filtration matrix, which separates the molecules. The gel filtration matrix consists of microscopic beads that contain pores and internal channels that interact with molecules as they pass through the column.
- The column is a glass tube with a frit and elution spout fitted at the bottom. The frit is a membrane or porous disk that supports and retains the stationary phase in the column but allows water and dissolved solutes to pass.
- The elution buffer is the mobile phase of the chromatography which flows through the matrix and out of the column.

To prepare, we fill or pack the column with matrix to form the bed. The total volume of buffer between the beads when the column is packed is the void volume. The bed volume is equal to the volume of the beads plus the void volume. To separate the mixture, the sample is loaded on the top of the bed. The column is developed by adding the elution buffer to the column. As the buffer flows, it carries the molecules into the matrix where they are gradually separated into zones.

The separation of the mixture correlates to their molecular weight and shape. The larger the molecule, the more difficult it is for it to pass through the pores. Larger molecules tend to flow around and in between the beads. In contrast, smaller molecules spend more time in the maze of channels and pores in the bed. This means that larger molecules are eluted from the column before smaller molecules because they spend less time interacting with the beads (Figure 1). This is analogous to finding your way out of a complicated maze versus walking around the outside of the maze and avoiding the whole situation entirely.

The shape of the molecule also influences its flow through the column. Molecules with a more compact shape, such as a sphere, get stuck in the pores more easily than those with an elongated shape, like a rod. Therefore, a rod-like molecule elutes before a spherical one of the same molecular weight.

Each zone flows out of the column where they are collected for analysis. The partially or completely separated zones of molecules that are eluted from the column are called peaks. A peak consists of an increasing and decreasing concentration gradient of molecules.

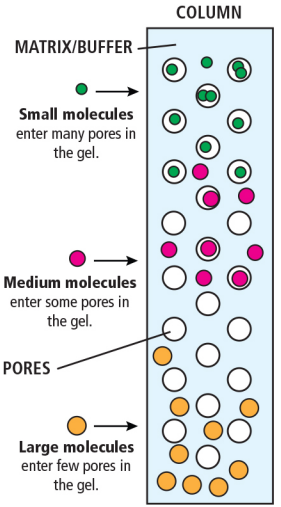


Figure 1

The gel filtration matrix separates a specific range of molecular weights known as its fractionation range. For example, consider a matrix with a fractionation range of 1000 to 100,000 daltons (Da). Molecules smaller than 1000 Da are not separated because they penetrate the beads with equal efficiency. These molecules take the maximum volume of buffer for elution. Molecules in the range of 1000 to 100,000 Da will enter the beads with varying efficiencies and be separated from one another. All molecules greater than 100,000 Da will not enter the beads and be eluted together in the void volume.

In this experiment, size exclusion chromatography is used to separate a mixture of colorful molecules. The orange molecule is a small dye with the molecular weight of 452 Da. The blue molecule is a rod-shaped polymer of glucose with a molecular weight of 2,000,000 Da. The fractionation range of the matrix is 4,000 to 150,000 Da. The smaller orange dye enters the pores of the beads and slowly works through them while the very large blue glucose polymer travels around the beads, moving much faster.

EXPERIMENTAL PROCEDURES: Packing the Column

1. Prepare the elution buffer by filing the buffer concentrate bottle to the neck of the bottle using purified water.

2. Mix the matrix thoroughly by swirling or gently stirring.

3. With a large transfer pipet, carefully pipet all of the mixed slurry into the column by letting it stream down the inside walls of the reservoir or pour the slurry into the column.

   If the flow of matrix is stopped by an air pocket, stop pouring and firmly tap the column until the air is removed and the slurry flows down. Then, continue pouring the slurry.

4. With a transfer pipet, add elution buffer to fill the reservoir.

5. Place an empty beaker under the column.

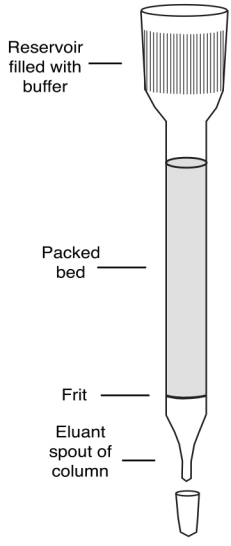


Figure 2

6. Remove the cap from the column spout.

7. Let the buffer flow through the column for approx. 10 min. The matrix will pack down into the column.

8. Place the cap onto the spout of the column.

9. The matrix is packed when it stops compressing.

EXPERIMENTAL PROCEDURES: Fraction Collection

1. Label the upper left well of the microtiter plate with a dot or a star. This is where you will collect the first fraction.
2. Carefully remove all the buffer from above the bed with a transfer pipet. The top of the bed should be exposed to air. Insert a pipet through the reservoir. Try to minimize disturbance of the bed while removing buffer.
3. Load approx. 150 µL (10 drops) of the sample onto the top of the bed with a transfer pipet. Let the sample drip down the inside walls of the column.
4. Place a small cup or jar under the column.
5. Remove the cap from the spout. The sample will slowly enter the bed. When it has completely entered the bed (the top of the bed will be exposed to air), replace the cap.
6. Carefully add several drops of buffer over the bed with a transfer pipet. Open the cap and allow the buffer to enter the column.
7. Continue adding buffer, several drops at a time, pausing to allow the buffer to enter the bed.
8. When the blue dye reaches near the bottom of the column, begin to collect 200 µL fractions in the microtiter plate.
9. As the dyes gradually separate in the column, periodically add fresh buffer to the reservoir to keep it full.
10. Continue collecting 200 µL fractions in each of the wells of the microtiter plate.
11. After all wells of column effluent (column fractions) have been collected, replace the cap onto the spout.
12. Identify the well with the greatest amount of blue dextran that eluted from the column.
13. Identify the well that has the greatest amount of orange dye that eluted from the column.

STUDY QUESTIONS

1. Draw and label the parts of a column. Include the amounts of buffer which is placed above/below the bed. Define: frit, matrix, and eluant.

2. What is the purpose of an elution buffer?

3. Which would elute from a column first: a spherical or a linear molecule? Why?

4. What is the stationary phase of the chromatography? The mobile phase?

TERMS AND CONDITIONS

- FOB: Washington, DC
- Safety Data Sheets are available on our web site and by request.

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EDVOTEK, INC.
1121 5th Street NW
Washington, DC 20001

1.800.EDVOTEK • Fax: 202.370.1501
info@edvotek.com • www.edvotek.com

ANSWERS TO STUDY QUESTIONS

1. (Refer to Figure 2.) Frit - porous disk which supports and retains the matrix in the column. Matrix - separation medium. Eluant - The liquid that flows out of the column.
2. As the mobile phase, the elution buffer transports the molecules to be separated through the matrix.
3. A linear molecule would elute from the column first. The molecule can not enter the beads as readily and passes around the matrix beads.
4. The stationary phase is the matrix and the mobile phase is the elution buffer.