HiPer® Gel Filtration Chromatography Teaching Kit

Product Code: HTC004

Number of experiments that can be performed: 5

Duration of Experiment:

Protocol: 1 hour (approximately)

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store the Gel Filtration Column, Gel Filtration Buffer and the Sample at 2-8°C

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Aim:

To learn the technique of chromatographic separation of a mixture of biomolecules by Gel Filtration Chromatography using Sephadex G-25 column.

Introduction:

Chromatography is a method to separate and analyze a complex mixture. This separation process consists of two phases: a stationary phase and a mobile phase. The mobile phase consists of the mixture to be separated which percolates through the stationary phase. Gel filtration chromatography or size exclusion chromatography involves the separation of macromolecules on the basis of their molecular size. In this chromatography, the sample consisting of a mixture of large and small molecules, is separated by allowing the sample to pass through porous gel beads.

Principle:

Gel filtration or size exclusion chromatography is a chromatographic procedure through which molecules are separated from a mixture according to their molecular size and shape. The stationary phase or gel matrix is composed of beads containing pores that span a relatively narrow size range which defines the size of the macromolecules that are to be separated or fractionated. The molecules which are too large to enter the bead pores are excluded and thus elute first from the column (as shown in Figure 1). These large molecules elute as the first peak in a chromatogram and this is called Total Exclusion. Intermediate molecules that can enter the pores of the beads will have a longer retention time depending upon their size and shape. As a result different molecules have different total transit times through the column and this portion of a chromatogram is called the Selective Permeation Region. Molecules that are smaller than the pore size can penetrate through the beads, retain (included) for a longer time and elute as the last peak in the chromatogram. This is called the Total Permeation Limit.

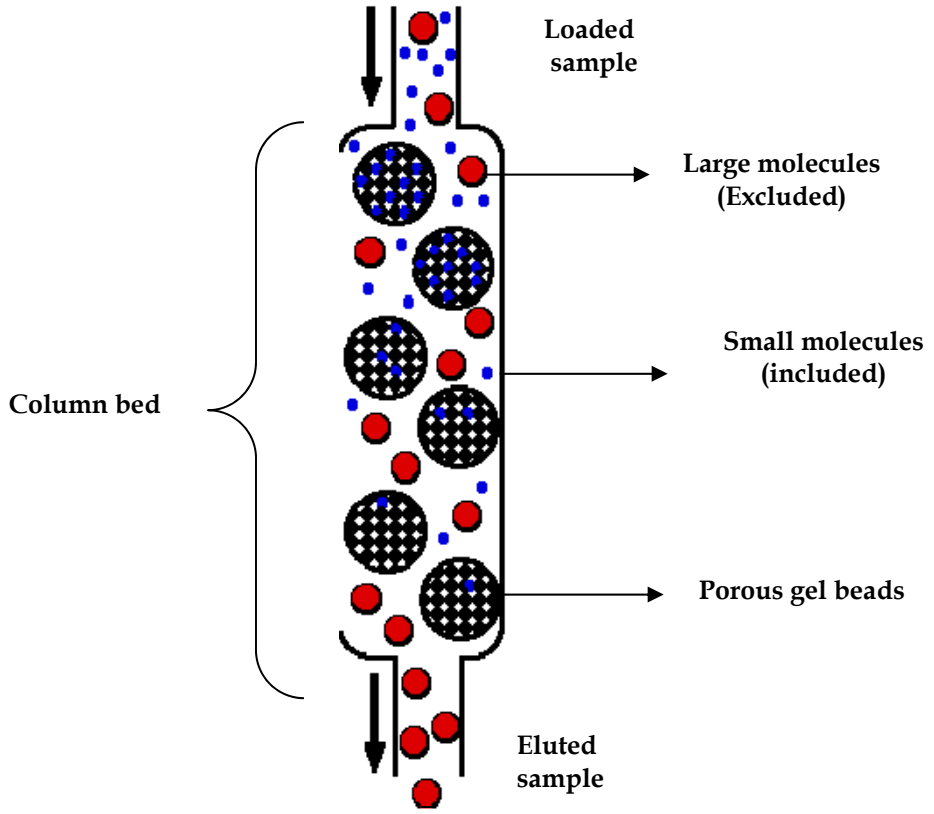


Fig 1: Schematic Diagram of Gel Filtration Chromatography

In Gel Filtration Chromatography the gel matrix used should be chemically and physically stable and is selected on the basis of bead size and the size of the molecules to be separated. Moreover, the gels have to be uncharged and inert i.e. don't react with the materials being fractionated. The gels used as molecular sieves consist of cross linked polymer. Most often/ frequently the following three types of gels are used:

1. **Dextran:** This is a homopolysaccharide of glucose and its trade name is Sephadex. It is prepared with various degrees of cross-linking to control the pore size. It comes as dry beads and should be swelled in water before use. This is mainly used for the separation of small peptides and globular proteins.

2. **Polyacrylamide:** This comes under the trade name of bio-gel P and is prepared by cross linking acrylamide and N, N'-methylene-bisacrylamide. Its pore size is determined by the degree of cross linking and this is available in varying range of pore sizes.

3. **Agarose:** Agarose is a linear polymer of D-galactose and 3, 6 anhydro-1-galactose and its pore size is determined by its concentration in the gel. The pore size of agarose gels is much larger than sephadex or bio-gel P. It is soluble in boiling water and forms a gel upon cooling. This is useful for the separation of large globular proteins and DNA.

Sephadex® G-25, a polymer of dextran, is a very common gel filtration material. It has the capacity to separate molecules with molecular weights from 1000 to 5000 Da. Molecules with molecular weights more than 5000Da will be excluded from the beads and those with molecular weight less than 1000 Da will be completely included. Therefore, a mixture containing molecules of varying size can be fractionated on a Sephadex G-25 column by gel filtration chromatography.

Kit Contents:

This kit can be used to fractionate three different biomolecules from a mixture by performing gel filtration chromatography.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity 5 expts	Storage
1	TKC267	Gel Filtration Column (2 ml)	2 Nos.	2-8ºC
2	TKC268	Gel Filtration Buffer	125 ml	2-8ºC
3	TKC269	Sample	1.2 ml	2-8ºC

Materials Required But Not Provided:

Glass wares: Test tubes
Other requirements: Column stand, Micropipette, Tips

Storage:

HiPer® Gel Filtration Chromatography Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Gel Filtration Column, Gel Filtration Buffer and the Sample at 2-8ºC.

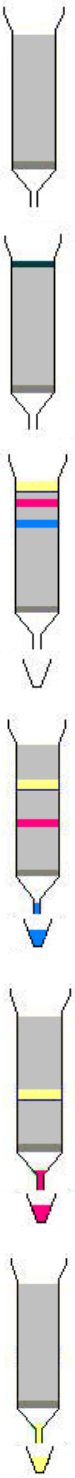
Important Instructions:

1. Before starting the experiment the entire procedure should to be read carefully.
2. Allow all the components to come to room temperature before starting the experiment.
3. Never let the column become dry.
4. When using the column open the top cap first and then the bottom cap and to stop the flow of the column fix the bottom cap first and then the top cap.
5. Store the column at 2-8°C after each use.
6. A column can be reused only 2 – 3 times.

Procedure:

1. Fix the column vertically to a column stand. Allow the column to come to room temperature.
2. Add 4 ml of Gel Filtration Buffer to the column and allow it to drain out completely. This is done for the equilibration of the column.
3. Load 0.2 ml of ready-to-use sample onto the column very carefully.
4. Add 0.2 ml of Gel Filtration Buffer after the sample sinks completely.
5. Allow the Gel Filtration Buffer to drain out completely.
6. Add additional Gel Filtration Buffer to the column till all the coloured biomolecules are eluted out.
7. Use different tubes for collection of the coloured fractions.
8. Store the column at 2-8°C for next use after fixing the top and bottom caps

Flow Chart:



Allow the Sephadex column to come to room temperature

Load the sample on the column

Individual components of the sample start to separate

Blue coloured sample is eluted first

Pink coloured sample is eluted second

Yellow coloured sample is eluted last

Observation and Result:

Observe the order in which the coloured molecules are separated within the column and finally eluted out. Note down the order as in the following table:

Order of elution	Colour of the eluted sample
First	
Second	
Third	

Interpretation:

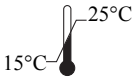
The gel matrix of the column is Sephadex G-25 which has the capacity to separate molecules with molecular weights from 1000 to 5000 Da. The blue coloured component is blue dextran which has a molecular weight of 2000 kDa. For this reason it can't enter the beads and elutes first. The pink coloured component is vitamin B12 which has a molecular weight of 1357 Da. These molecules enter the pores but have a shorter retention period and collected as a second fraction. The yellow coloured component is Paranitrophenol which has a molecular weight of 139 Da. For this reason they enter the pores of the beads and retain in the gel for a longer period of time and elutes as the last fraction.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Poor resolution of the coloured fractions	Column is not stored properly	Always store the column at 2-8°C after the experiment. Do not use the column more than 2-3 times. Bring all the components to room temperature one hour before use
2	Flow rate is low	The column has got some air gap	Never let the column go dry

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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