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GEL CHROMATOGRAPHY: A REVIEW

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ABSTRACT

Gel filtration chromatography is a popular and versatile technique that enables the efficient separation of proteins and biological molecules in high yields. Here, the basics of the method are explained and conventional matrix types are contrasted. Selection of suitable operating conditions and application of the Method is also discussed

KEYWORDS: Gel-fi ltration chromatography, Gel-permeation, Gel-exclusion, Size-exclusion, Molecular-sieve, Operating conditions, Separations.

INTRODUCTION

History

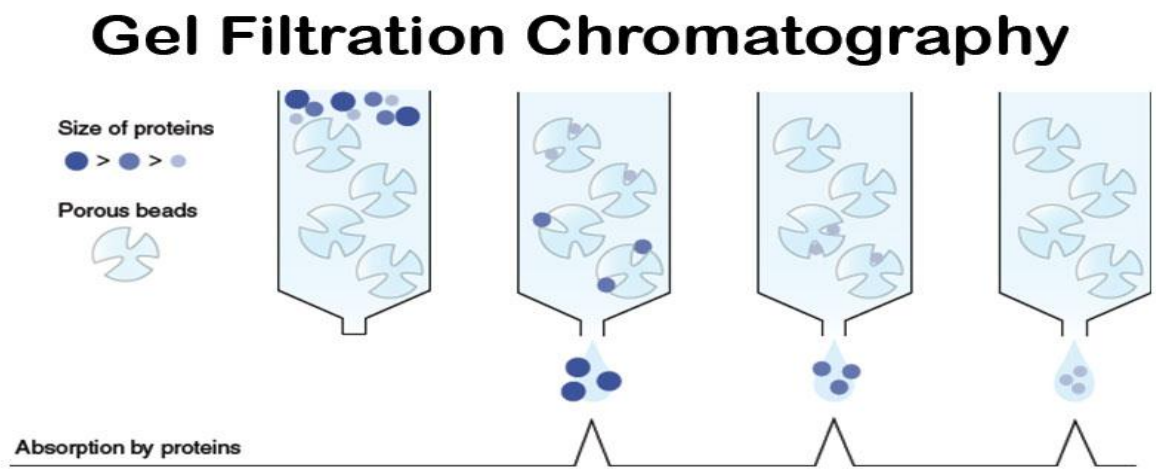
This method is often used to analyze polymers. SEC as a technique was first developed in 1955 by Lathe and Ruthven. Gel chromatography (GPC) is a form of size exclusion chromatography (SEC) that separates analytes by size, usually in an organic solvent. The term gel permeation chromatography was coined in 1964 by Dow Chemical Company J.C. Can be taken from the nose. This proprietary column technology was licensed to Waters Corporation in 1964, which commercialized the technology. GPC systems and consumables are also available. From the factory. Even for their analysis, it is often necessary to remove the polymer to purify the desired product.[1]

Gel filtration chromatography is a type of fractional chromatography used to separate molecules of different molecular sizes. This technique is also often referred to by various names such as gel permeation, gel extraction, size extraction, and molecular sieve chromatography. The basic principle of gel filtration is quite simple. Molecules are divided

between a mobile phase and a stationary phase, which consists of a porous matrix (defined as pores) as a function of their relative size. Such columns, usually constructed as beads, have two measurable fluid volumes: the internal volume consisting of the fluid between the beads and the fluid inside the bead. The external volume is usually called the void volume (V0), and the sum of the external and internal volumes is the total volume (Vt). For example, molecules larger than the pores of the stationary phase of the matrix will be expelled from the volume in the beads.[2]

Principal

To perform the separation, the gel filter media is packed into a column to form a packed bed. Porous matrix in the form of spherical particles is chosen for medium stability, chemical and physical, inertness (lack of reactivity and adsorptive properties). A packed bed is likened to a buffer that fills the pores of the matrix and the spaces between the particles. The liquid inside the pores is sometimes called the stationary phase, and this liquid is in equilibrium with the liquid outside the particles, called the mobile phase**.**



• *The stationary phase used is a porous polymer matrix filled with a solvent to use the pores as a mobile phase.*

• *Molecules in the sample that are adsorbed through a special column contain, for example, microporous packing material (gel).*

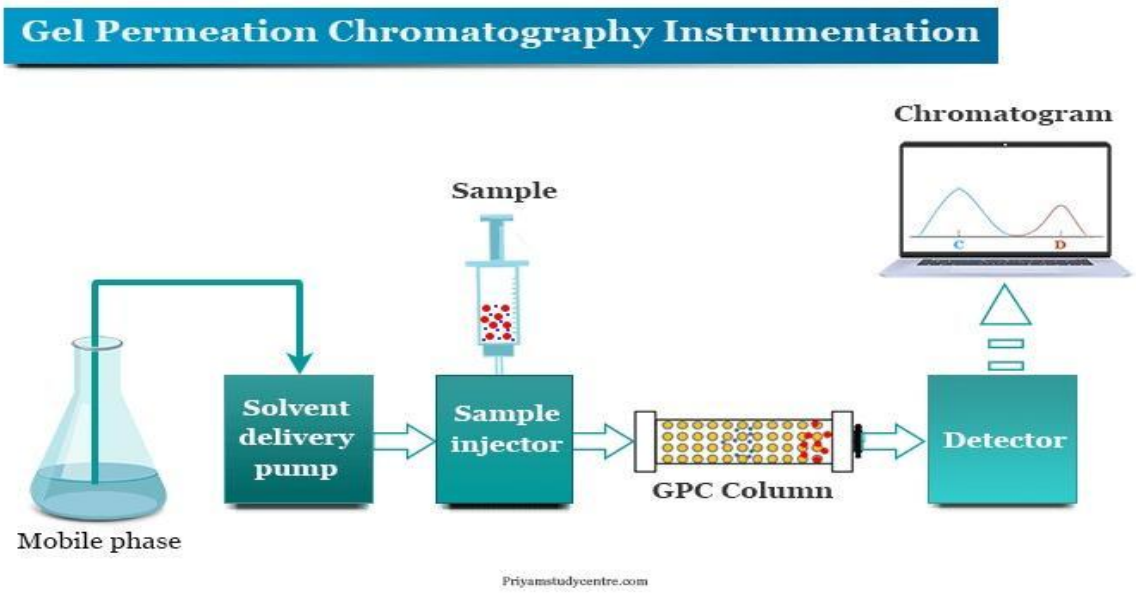
• *The separation principle is that molecules of a certain size are completely removed from the pores, while smaller molecules enter the pores partially or completely.*

• *Therefore, the mobile phase flow will cause large molecules to pass through the column unhindered without entering the gel matrix, while small molecules will be pushed back when they penetrate the gel.*[3]

Instrumentation

Good gel permeation chromatographic instrument contains the following important components[4,9]

1. Gels
2. Gel chromatography column
3. Eluent or mobile phase
4. Solvent delivery pump
5. Chromatographic detector



1. Gels

The choice of gel largely depends on the molecular size and chemical properties of analytes which can be separated by the GPC technique. Xerogels are commonly used as a stationary phase in gel permeation chromatography instruments. All the gels used in the GPC technique are organic in nature. Different types of gels which are available in the market have the trade names,

- BioGel p-2 (polyacrylamide)
- Sephadex G-10-200 (Dextran)
- Styrogel (modified polystyrene gels)
- Agarose

The gel agarose is sold under the trade names Sepharose and BioGel A. Such gels contain hydrogen bonding and are stable under the temperature range of 0 to 30 °C and pH range between 5 to 8. The gels used in the gel filtration technique have the following common properties,

- They are chemically inert.
- They are mechanically stable.
- They contain a uniform particle and pore size.

2. Gel chromatography column

The column used in the GPC instrument is filled with semi-permeable, porous polymer material and eluent allowed to flow under gravity. The gels used in the chromatographic column have a well-defined range of pore sizes. Washing the column before testing with buffer solution to remove any air bubbles.

3. Eluent

The eluent or mobile phase used in gel permeation chromatography should be a good solvent for polymers. Generally, organic solvents used as an eluent in the GPC instrument may include,

- tetrahydrofuran (THF)
- o-dichlorobenzene
- trichlorobenzene
- polyalkenes
- m-cresol
- o-chlorophenol

4. Pump

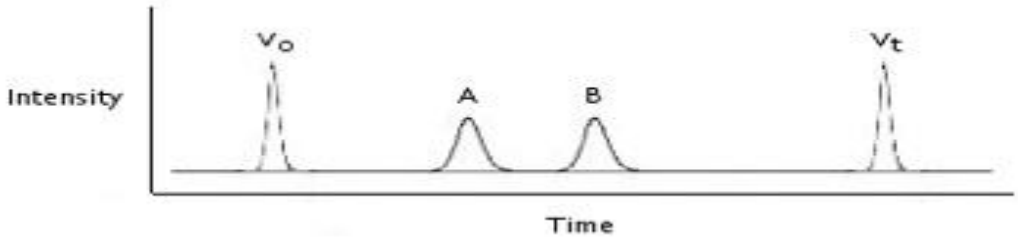
Piston or peristaltic pumps are used in the GPC technique for uniform delivery or constant flow rate of the mobile phase.

5. Chromatographic detector

As in other chromatographic methods, here also a suitable physical property like refractive index, absorbance, fluorescence intensity and other electrical properties of effluent can be measured by the detector.

There are many types of detectors used in the GPC analysis. They are divided mainly into two categories,

- The first type is concentration-sensitive detectors like UV absorption detectors, refractive index (RI) detectors, infrared (IR) absorption, and density detectors.
- The second category contains molecular weight-sensitive detectors like low-angle light scattering (LALLS) detectors and multi-angle light scattering (MALLS) detectors.



In GPC, the polymer concentration by weight can be continuously monitored by a detector. There are many types of detectors and they can be divided into two main categories. The first is concentration-sensitive detectors, which include UV absorption, differential refractometry (DRI) or refractive index (RI), and infrared (IR) absorption and density detectors. The second category is molecular weight sensitive detectors, which include low angle light scattering (LALLS) and multiangle light scattering (MALLS) detectors. The resulting chromatogram is the weight distribution as a function of the retention volume of the polymer.

The most sensitive detector Is the Differential UV Photometer and the most common detector is the Differential Refractometer (DRI). When characterizing copolymers, two detectors must be in sequence. At least two of those detectors must be concentration detectors to accurately determine the composition of the copolymer. Detection of most copolymer compositions is performed using UV and RI detectors, but other combinations can be used.

Methods of Gel Filtration Chromatography[5]

A. Almost all gel permeation chromatography is done in column chromatography. The test model is very similar to other liquid chromatography methods. The sample is dissolved in a suitable solvent, usually organic, in the case of GPC, which is then filtered before being injected into the column. Milk is part of a multi-component mixture. The use of a pump ensures a continuous supply of fresh eluent to the column. Most analytes require a detector to

be invisible. Multiple detectors are often used to obtain additional information about polymer samples.

B. The stationary phase for GPC is a gel. The pore size of the gel must be carefully controlled in order to use the gel in a specific separation. The absence of ionic groups and the low affinity of the substance for dissociation in a given solvent are also desirable properties for gel-forming solvents.

C. A microporous packing material is used to pack the GPC column. The gel is poured into a column called a filter column.

D. Element (solid phase) must be a high solvent for the polymer and the polymer has a high detector response, wetting the coating surface. Tetrahydrofuran is the most common element in room temperature (THF) soluble GPC polymers.

E. Piston or peristaltic pumps are two types of pumps available for GPC to uniformly distribute a small volume of liquid.

F. In GPC, the detector will continuously monitor the weight of the polymer in the elongating solvent. There are several different detectors that can be divided into two groups. UV absorption, differential refractometer (DRI) or refractive index (RI) detector, infrared absorption (IR) and density detector are the first type of concentration sensitive detector.

Types of Gel Filter Chromatography[3]

- Team Division

Part of the model is divided into two large groups according to their size. Band separation can be used to remove high or low molecular weight contaminants (such as phenol red from culture fluids) or for salts and buffers.

- High resolution biomolecular imaging

Model fragments are separated based on differences in molecular size. High-resolution fractionation can be used to isolate one or more components, separate monomers from aggregates, determine molecular weight, or analyze molecular weight distribution**.**

SEPARATION PROCEDURE[6]

Apply software for FILTER GEL FILTER.

Swelling gel: Some resins come in powder form. It must first be sonicated in the desired eluent or buffer for swelling.

Column packing: prepare the gel + buffer and pour it into the buffer-filled column.

Washing the column: After coating, put some buffer in the column to remove air bubbles and check the uniformity of the column.

Loading for problem: The sample must be injected into the resin in solution form using a syringe.

Sample Receipt and PROCEDURE: Particles are collected until the sample exits the column

Applications of Gel Filtration Chromatography[3]

• gel filtration plays an important role in the purification of enzymes, polysaccharides, nucleic acids, proteins and other biological macromolecules.

• Gel filtration can also be used to facilitate the refolding of denatured proteins by careful control of varying buffer conditions.

• Used in protein splitting experiments.

• The gel filter method is also used to determine molecular weight.

• Size separation of sugar, protein, peptide, resin, etc.

• Can be used to determine the quaternary structure of purified proteins.

Advantages of Gel Filtration Chromatography[3,7]

- ❖ Gel filtration is a suitable technique for handling biomolecules that are sensitive to changes in pH, concentrations of metal ions or co-factors, and harsh environmental conditions.
- ❖ An important advantage of gel filters is that they can meet the requirements for further purification, analysis, or storage without changing the type or resolution of the sample.
- ❖ Essays can be carried out using the necessary ions or cofactors, detergents, urea, guanidine hydrochloride, at high or low ionic strength, at 37 °C or in a cold room according to the test requirements.
- ❖ Unlike ion exchange or affinity chromatography, the molecules are not bound to the chromatographic medium, so the composition of the buffer does not directly affect the resolution (the degree of separation between the peaks).
- ❖ Short analysis time.
- ❖ Separate clearly.
- ❖ Narrow bandwidth and good sensitivity.

- Ample Example No loss.
- A small number of cellular phases.
- The flow rate can be determined.
- Short analysis time.
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Disadvantages[7]

- G is the finite number of GPC tasks that can be solved in a short time.
- Dust and other particles must be filtered before using the instrument to prevent damage to the column and interference with the detector.
- The molecular weight of most chains can show nothing but a broad peak for GPC cleavage.

Limitations of Gel Permeation Chromatography[8]

• A limited number of GPC tasks that can be solved in a short period of time.

• Filtering must be done before using the device to prevent dust and other particles from damaging the column and interfering with the detector.

• The molecular weight of most chains will show nothing but a broad peak for GPC cleavage.

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