7. GEL FILTRATION

7.1 Introduction

Gel filtration is a technique of separation of components of a mixture based on their molecular size by passing them through a column containing aqueous or non-aqueous gels. Gel filtration is also known as *gel permeation chromatography, size exclusion chromatography, gel chromatography* and *molecular sieve chromatography*.

Gel filtration uses porous polymeric materials as stationary phase, which have permanent pores and cavities in their structure. These gels prior to their packing in the column are generally saturated with a solvent (water), which causes them to swell. After such gel has been packed in the column, the sample is applied via suitable means. Those molecules whose sizes are larger than the pore size of the gels pass quickly through the column, whereas smaller sized molecules enter into the pores and get retained. Thus, the substances elute from the column based on their decreasing molecular size.

Advantages

1. The procedure is simple and is unaffected by the composition of the sample to be analyzed or by the temperature of analysis.
2. Gels with suitable pore sizes can be employed for obtaining desired separation, thus making the technique useful for a variety of components with a range of sizes.
3. Biological materials or samples which are sensitive to temperature or other factors and degenerate easily, can be analyzed using this mild technique.
4. The gels can be reused any number of times as they remain stable without undergoing any change in their properties even upon usage.

Disadvantage

The separation is based upon size and shape and not on molecular weight. Thus, if the same compound exists as branched and unbranched molecules, then it shows different elution properties.

7.2 Principle

The stationary phases in gel filtration are beds of porous polymeric substances. These are capable of absorbing water or any other suitable solvent and thereby undergo swelling. Since gels are a network of cross-linked polymer chains due to swelling the space between these polymer chain increases. The average size of the pores thus formed is dependent upon the amount of solvent that has been absorbed by the gel. For every gel, there exists a critical size of a molecule which can penetrate through it. Those ions or molecules which are larger than the critical size cannot penetrate through the column and get eluted first. Smaller ions or molecules can penetrate through these pores and thus their movement is retarded. Thus, the difference in the particle size of the sample results in different speeds of elution.

7.3 Theory

The total volume of column that has been occupied by gel after its swelling is given by following equation,

$$V_t = V_g + V_l + V_0$$

Where,

V_t = Total volume occupied by the gel

V_g = Volume occupied by the solid matrix of the gel

V_l = Volume of solvent held in the pores of the gel

V_0 = Volume of liquid between the gel beds.

V_0 also refers to the volume of solvent required to carry those large-sized components through the column that cannot enter the pores of the gel when no mixing or diffusion takes place, however some diffusion or mixing always occurs making the components to appear in a gaussian shaped band with maximum concentration at V_0. For smaller components the band maximum will appear at the end of the column as they can easily enter the pores of the gel where $V_l + V_0$ represent the total column volume. Intermediate molecules that are not too large or small occupy a fraction (K_d) of V_l where the elution volume (V_e) is given by,

$$V_e = V_0 + K_d V_l$$

1. For molecules that are too large to enter the pores of the gel $K_d = 0$ and $V_l = V_0$.
2. For molecules that are too small and can easily enter the pores without any hindrance, $K_d = 1$ and $V_e = V_0 + V_l$

7.4 Procedure

1. Column

The columns used in gel filtration are broader and longer when compared to those used for adsorption or partition chromatography and they give high resolution. The diameter of the columns used ranges from 10-200 mm while columns 100 cm in length are preferred for laboratory purpose. The column contains glass wool or filter paper at its lower end which is covered with a thin layer of glass beads, sand or quartz. Presence of these materials allows the mobile phase to flow out but prevents the exit of stationary phase.

2. Gel

The ideal properties of gel are as follows,

1. Particle size of the gel should be uniform and it should be mechanically stable.
2. Gel material should be chemically non-reactive.
3. Pore size of the gel matrix should be uniform.
4. The amount of ionic groups in the gel should be less.
5. Gels should possess less swelling ability to ensure even packing of the column and to avoid its flow out of the column.

Types of Gels

(a) Soft Gels: Soft gels have high swelling ability and absorb large amounts of solvents to become much larger than their original size, resulting in increase of porosity which is dependent upon the volume of solvent absorbed. In order to get good separation efficiency, the speed of flow of solvent should be low.

E.gs: Dextran, Sephadex.

(b) Semi-rigid Gels: They are available in a variety of pore sizes and can swell upto 1.1 to 1.8 times their original dry volume. They can be used even under high pressure conditions and are made wettable by their chemical or physical modifications.

E.gs: Cross-linked polystyrene, ion-exchange resins and polyvinyl acetate gels.

(c) Rigid Gels: Columns prepared with rigid gels show high permeability. These gels have uniform pore size which remains constant for entire material. These gels do not swell and do not loose their stability even at higher temperatures.

E.gs: Glass, silica gel.

Choice of Gel

Selection of gel is based on the desired type of separation. Separation is of two types, *group separation* or *desalting* and *fractionation*. Group separation or desalting is the separation of high molecular weight substances i.e., larger molecular weight substances are eluted first while the lower molecular weight substances are retained in the gel matrix. Examples of gels used in desalting include bio-gel P-6, bio-gel P-10, Sephadex G-25, Sephadex G-50.

Fractionation is the separation of sample containing molecules of a narrow range of molecular weights, within the gel matrix as per different pore sizes. Examples of gels used in fractionation include Sephadex-G 25, Sephadex G-100, sephadex G-500.

Particle Size of Gels

Smaller particles with size less than 40 μ give good resolution and can be obtained by sieving commercially available material. Gel powder with particle size of 70 μ is used for general purpose separation.

Gel Preparation

Weighed quantity of gel powder is allowed to swell by addition of excess amounts of desired mobile phase, until it reaches equilibrium. This may take a longer time, therefore the slurry is heated to a temperature of 100°C by using a boiling water bath, so that the equilibrium is attained in about 2 days. This method helps in preventing the microbial growth and also helps in the removal of air bubbles from the slurry. The slurry is allowed to cool to room temperature before packing it in the columns.

3. Packing of Columns

Addition of mobile phase after packing the column with dry gel may result in breaking of the column due to swelling property of gels. Therefore, special methods are required to pack these columns. One of the methods include joining another container to the top of the column, followed by filling the column with mobile phase and addition of gel slurry from the top. The gel is allowed to settle down, swell and air bubbles in it are removed by application of vacuum. The supernatant liquid is drained after complete sedimentation of the gel in the column. The mobile phase may be kept at slightly higher temperature than the column (15-30°C), by heating the solvent reservoirs using an incandescent lamp to prevent the formation of air bubbles.

In case of hard gels like agarose gels, these are mixed with a suitable buffer solution and heated before packing as they are too thick to be packed directly into the column. The gel is then subjected to deaeration under vacuum and is finally packed.

4. Preparation of Sample

Volume of the sample required depends upon whether the work is analytical or involves group separation (desalting). For analytical work, a sample size of 1-3% of total gel bed volume is sufficient whereas for desalting technique, sample size of 25-30% of the total column bed volume is used. If the sample size is small, the eluted component will be still less in concentration.

5. Sample Application

Pipettes with bent tip (for normal viscosity samples) or valve loops (for viscous samples) are used to introduce the sample over the top of the bed, followed by washing the surface with mobile phase to ensure that complete sample has passed into the bed. Special units are present for introduction of samples in case of commercially available plunger type columns.

6. Solvents or Mobile Phase

An ideal solvent system used should possess the following properties,

(a) It should be compatible with the sample, detector and the integrated system hardware used.

(b) It should dissolve the sample.

(c) It should wet the gel slurry, but should not get adsorbed over it.

(d) It should have the ability to swell the soft gels when used with them.

(e) It should have proper viscosity to obtain good resolution.

An electrolyte may also be added to the mobile phase to obtain better elution characteristics of the components.

7. Detectors

Various detectors like differential refractometers, UV-visible spectrophotometers, flame ionization detectors, electrical conductivity detectors etc., can also be used for the analysis of separated components.

7.5 Applications

1. Gel filtration is used in desalting protein solutions, for molecular size determination of components, separation and purification of large molecules like protein, studying the plasma-binding property of drugs etc.
2. It can be used in the removal of impurities and undesired substances from samples of large biomolecules like antibodies, enzymes, hormones, proteins, viruses etc.
3. It can be used for molecular weight determination of proteins having similar shape as they show a direct and approximately linear relationship between the volume of column eluate and molecular weight over a specific range of molecular weights.
4. Concentrated solutions containing high molecular weight compounds can be analyzed by addition of dry and coarse sephadex G-25.
5. Gel filtration is extensively used in the separation of asphalts, butyl rubbers, liquids, polyethylenes, polypeptides, polystyrenes, proteins, silicon polymers, sugars etc.,
6. Sephadex G-25 and G-50 which are tightly cross linked gels with small pore size are used in removing low molecular weight molecules from high molecular weight natural molecules.
7. Gel permeation can be used to study complex, biochemical or highly polymerized molecules.

(sephadex G-25) G-25 G-50

removing low. eluate and

asphalts butyl rubbers