IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A Review ON Size Exclusion Chromatography

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Abstract :

Size exclusion chromatography (SEC) may be a historical technique widely employed for the detailed characterization of therapeutic proteins and may be considered as a reference and powerful technique for the qualitative and quantitative evaluation of aggregates. the most advantage of this approach is that the mild mobile phase conditions that let the characterization of protein with minimal impact on the conformational structure and native environment. Despite the very fact that the chromatographic behavior and peak shape are hardly predictable in SEC, some generic rules are often applied for SEC method development. Recent developments are reviewed in size exclusion chromatographic calibration methodologies, including direct calibration by using narrow and broad polymer standards and various instrumental methods (nuclear resonance, mass spectrometry, light scattering) also as universal calibration with and without viscometer detectors, for easy and sophisticated polymers. the utilization and number of bio therapeutics has increased significantly. For these largely protein-based therapies, the quantization of aggregates is of particular concern given their potential effect on efficacy and immunogenicity. This requirement has renewed interest in size-exclusion chromatography (SEC).

Key words: Size Exclusion Chromatography, Biomolecules, Macromolecules.

Introduction:

Size exclusion chromatography (SEC) is employed for semi-preparative purification and various analytical assays. Grant Henry Lathe and Colin R Ruthven was the pioneer of the dimension's exclusion chromatography who started this system for the separation of analyte of various size with starch gels because the matrix, later Jerker Porath and Per Flodin introduced dextran gel. (1)

Size exclusion chromatography (SEC), also referred to as molecular sieve chromatography, may be a

chromatographic method during which molecules in solution are separated by their size, and in some cases relative molecular mass . it's usually applied to large molecules or macromolecular complexes like proteins and industrial polymers. (2) In 1959, the molecular sieving principle was applied for the separation of biochemical polymers on dextran gels, and it had been called gel filtration chromatography. It uses the aqueous based eluents with salts. In 1961, an equivalent principle was applied for the relative molecular mass determination of synthetic polymers is named gel permeation chromatography. It uses primarily organic solvents. (3)

In Size exclusion chromatography (SEC), the upper the relative molecular mass of the molecule, the greater its hydrodynamic radius, which ends up in faster elution. At an equivalent time, if an analyte molecule interacts with the stationary phase thus increasing the retention of larger molecules, which can confound separation of molecules based solely on their hydrodynamic radius. These two processes produce opposite effects and analysis of the polymer relative molecular mass and relative molecular mass distribution would be impossible. This brings specific requirements to the choice of the column packing and therefore the mobile phase, where the mobile phase molecules should interact with the surface of the stationary phase stronger than the polymer, thus the preventing its interaction with surface.(3)

Principle:

Size exclusion chromatography (SEC) is that the separation of mixtures supported the molecular size (more correctly, their hydrodynamic volume) of the components. Separation is achieved by the differential exclusion or inclusion of solutes as they undergo stationary phase consisting of heteroporous (pores of various sizes) cross linked polymeric gels or beads. the method is predicated upon different permeation rates of every solute molecule into the inside of gel particles. Size exclusion chromatography involves smooth interaction with the sample, enabling high retention of biomolecular activity. For the separation of biomolecules in aqueous systems, SEC is mentioned as gel filtration chromatography (GFC), while the separation of organic polymers in non-aqueous systems is named gel permeation chromatography (GPC). (1)

the essential principle of size exclusion chromatography is sort of simple. A column of gel particles or porous matrix is in equilibrium with an appropriate mobile phase for the molecules to be separated. Large molecules are completely excluded from the pores will undergo the space in between the gel particles or matrix and can come first within the effluent. Smaller molecules will get distributed in between the mobile phase of in and out of doors the molecular sieve and can then pass through the column at a slower rate, hence appear later in effluent.

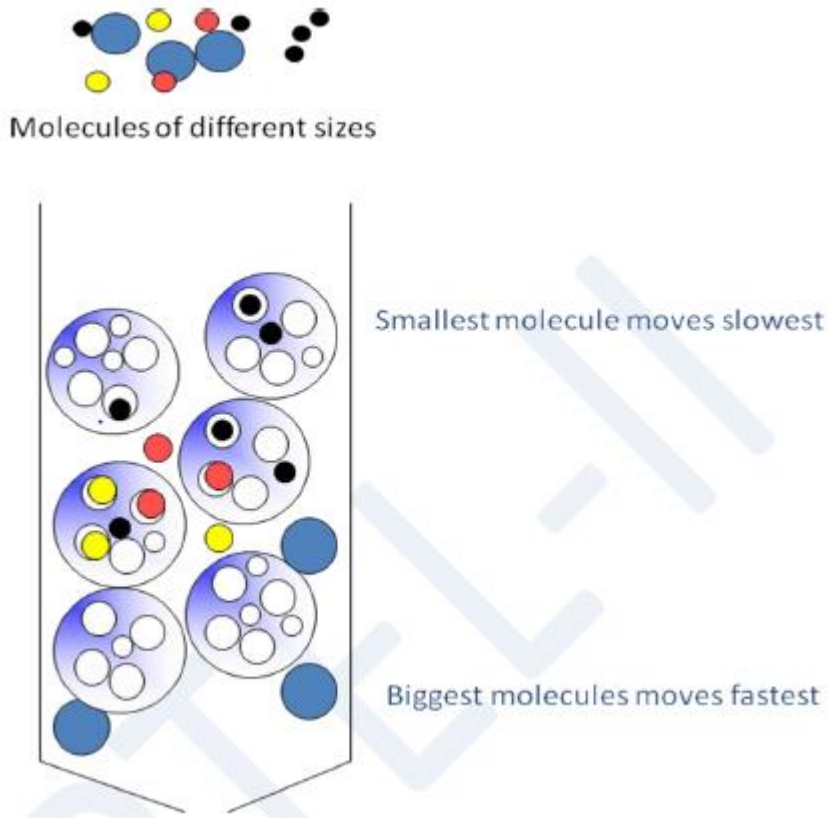

Fig.1 Theory of Size exclusion chromatography

Mechanism of Size Exclusion Chromatography (1)

Size exclusion also referred to as gel filtration chromatography may be a case of liquid-liquid partition chromatography, during which the solute molecules are get distributed in between two liquid phases, (i) liquid within the gel pores and (ii) liquid outside the gel.

The size exclusion might be explained by Steric Exclusion Mechanism. because the gel particles contain range of pore sizes, small molecules can enter in sizable amount of pores while the massive molecules will get small number of pores into which they will enter. Thus the various fractions of total pore volume are accessible to molecules of various sizes. Thus, molecules with different sizes will differ in distribution coefficient between these two liquid phase. because the small molecules can enter in additional pores while larger molecules can enter in pores only larger than the molecular size.

the whole volume (Vt) of a column full of a gel that has been swelled by solvent is given by,

$$Vt = Vg + VI + Vo$$

Where Vg is that the volume occupied by the solid matrix of gel, Vi is that the volume of solvent held within the pores or interstices and Vo is that the free volume outside the gel particles. When mixing or diffusion occurs, the diffusion equilibrium and therefore the retention volume (VR) of the given species is given by

$$VR = V(int.) + Kd\ V(int.)$$

where distribution coefficient (Kd) is given by Kd = Vi(acc) / V(total)

where Vi(acc) is that the accessible pore volume. V(total) is that the total pore volume and V(int.) is that the interstitial volume?

The other proposed mechanism is Secondary Exclusion Mechanism. This mechanism states that when a sample containing a mix of small and enormous molecules is applied to a gel filtration column, the tiny molecules diffuse rapidly into the pores of gel, whereas large molecules will find relatively few unoccupied pores and move further down the column till they find the unoccupied pores. This leads to the enhancement of separation of small and enormous molecules.

Experimental conditions:

1. Mobile phase and Temperature:

The mobile phase should be completely dissolving the polymer sample during a continuous solution phase, it must be low in viscosity for the SEC system to work in normal pressure range, and it must effectively prevent the polymer molecules from interacting with the stationary phase. Temperature may be a useful parameter to regulate when one or more of those conditions haven't been met but where one is constrained to use an exact mobile phase. Some polymers like polyesters, and polylefins may dissolution only at elevated temperatures. The viscosity of inherently viscous mobile phase might be lowered by raising the temperature.

2. Stationary Phases:

When selecting an optimum stationary phase there are additional criteria to be met; the packing shouldn't interact chemically with the solute i.e. sample it must be completely wetted by the mobile phase but shouldn't suffer adverse swelling effects, it must be stable at the specified operating temperature, and it must have sufficient pore volume and an adequate range of sizes to resolve the sample relative molecular mass distribution. For the high performance SEC, either semi rigid polymeric gels or modified, rigid silica particles are used. The pore size should be in range of 60 to 4000 A°. High performance packing materials have particle size within the range of 5 to 10 µm with efficiencies of several thousand theoretical plates per 15cm column.

3. Sample Size and Mobile Phase flow :

Sample size is defined by both the quantity of the aliquot injected also as by the concentration of the sample solution. the utilization of excessively large sample volumes can cause significant band broadening, leading to loss of resolution and errors in relative molecular mass measurement. The optimum injection volume is going to be function of the dimensions and number of the columns but are going to be range between 25 to 200µL. The flow 1 mL/min are most ordinarily employed for Sets of SEC column and for the only column separation, a flow of 0.5 ml/ min is employed.

4. Detectors :

the foremost common sort of universal detectors far and away is that the differential index of refraction (DRI) detector. The differences in index of refraction between a moving sample containing stream and static reference of mobile phase employing a split optical cell. It responds well at a moderate concentration level to most polymeric samples provide that they're different in index of refraction from the mobile introduce which they're dissolved. Despite temperature independence of the SEC separation phenomenon, the DRI is very temperature sensitive as a results of the strong temperature dependence of index of refraction. Thus one normally maintains the DRI during a constant temperature oven together with the columns and injector. The temperature is in any case 5-10°C above ambient.

Other common sorts of concentration detectors are the ultraviolet (UV) and infrared (IR) detectors.IR detector is slightly more sensitive than the DRI detector while the UV detector is several orders of magnitude more sensitive.

Factor affecting filtration: (2)

The particles in solution don't have a hard and fast size, leading to the probability that a particle that might rather be hampered by a pore passing right by it. Also, the stationary-phase particles aren't ideally defined; both particles and pores may vary in size. The stationary phase can also interact in undesirable ways with a particle and influence retention times, though care is taken by column manufacturers to use stationary phases that are inert and minimize this issue.

Applications: (6)

Size exclusion chromatography has been used with great success within the separation of the sugars, polypeptides, protein, liquids, butyl rubbers, polystyrenes, silicon polymers, etc. Size exclusion chromatography (SEC) has been mainly applied to studies of complex, biochemical or highly polymerized molecules.

The main application of SEC is that the separation and characterization of molecules of various relative molecular mass. It become possible to separate molecules of comparable molecular weights by proper selection of the acceptable gel and column length. Another important application is that the separation of huge molecules of the biological origin from inorganic and ionizable species.

The main application of the dimensions exclusion chromatography are as follows:

1. Purification:

The main application of exclusion chromatography is within the purification of biological macromolecules. Viruses, enzymes, hormones, antibodies, nucleic acids and polysaccharides have all been separated and purified by the utilization of appropriate gels or glass granules.

2. relative molecular mass determination:

The effluent volumes of globular proteins are largely determined by their relative molecular mass it's been shown that over a substantial relative molecular mass effluent volume is approximately a linear function of the logarithm of the relative molecular mass .

3. Solution concentration :

Solution of high relative molecular mass substances are often concentrated by the addition of dry sephaex G-25 (coarse). Water and low relative molecular mass substance remain in solution. After ten min. the gel is removed by centrifugation, leaving the high molecular material during a solution whose concentration has increased but whose pH and ionic strength are unaltered.

4. Desalting:

By use of a column of sephadex G-25, solution of high relative molecular mass compounds might be desalted. The high relative molecular mass substance moves with the void volume while lower relative molecular mass components are distributed between the mobile phase and crawl.

5. Protein building studies:

Size exclusion chromatography is use to review the reversible binding of aligand to a macromolecules like protein including receptor proteins.

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