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A Review on Gel Permeation Chromatography and Its Pharmaceutical Applications

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ABSTRACT: Gel permeation chromatography (GPC) is a powerful analytical technique it is based on their elution from a column filled with a porous gel used to separate dissolved molecules by size. It is also a type of molecular sieving chromatography, where samples are separated into their constituent parts by dissolving the sample in a mobile phase(solvent) and passing it through a porous column packing. Gel permeation chromatography is routinely usedfor encompasses many biochemical applications and used for chemical analysis of large molecule species (polymers). Gel permeation chromatography is the leading method for measuring the molecular weight distributions of complex polymers. This article gives areview on pharmaceutical application of gel permeation chromatography.

KEY WORDS: Gel permeation chromatography, analytical technique, porous gel, sieving chromatography.

I. INTRODUCTION:

Chromatography is a division strategy utilized for substance investigation. Chromatography is Used to isolate combinations in a solitary advance and measure the measure of each part Also, their overall extents in this way, presently it is acknowledged as presumably the most impressive and Adaptable scientific strategy accessible. There are numerous sorts of chromatography Strategies are utilized among them two are ordinarily utilized they are gas Chromatography and fluid chromatography. [1] Gel penetration chromatography comes Under fluid chromatography. As a method, SEC was first evolved in 1955 by Lathe and Ruthven. [2]The gel Saturation chromatography was created by j. c Moore. [3] The term gel saturation Chromatography can be followed back to J.C. Moore of the Dow Chemical Company who Examined the method in 1964 and the exclusive segment innovation was authorized To Waters Corporation, who consequently marketed this innovation in 1964.

Gel penetration chromatography (GPC) now and again alluded to as gel filtration Chromatography (GFC)or strainer size rejection chromatography is a Partition procedure Also, Subset of high-performance fluid chromatography whereby polymer atoms are isolated dependent on their hydrodynamic volumes. This procedure is utilized for the detachment of proteins, polysaccharide, chemical, and engineered polymer. It has found broad use in deciding the atomic weight circulation of High sub-atomic weight polymers. In the early improvement of this method cross connected polydextran gets having changing pore sizes (sephadex, Pharmacia, Sweden) were utilized as the fixed stage in the previous few decades gel saturation chromatography (GPC) has gotten perhaps the most significant method for the partition GPC was first applied to the assurance of the chain length of fructans by Pollock et al . Gel penetration chromatography is basically a type of fluid chromatography in which solute atoms are specifically impeded as a consequence of their pervasion into dissolvable filled pores in the section pressing.

II. METHOD OF WORKING:

A partition dependent on size, likewise called size avoidance chromatography. GPC isolates in light of the hydrodynamic volume or size of the analytes. [4] This varies from other division procedures which rely on substance or actual associations to isolate analytes. The segment is loaded up with gel. Gel goes about as a fixed stage. Gel is made up of permeable dabs. Gel utilized polystyrene, dextran, polyacrylamide gels, agarose gels (all have permeable design).

The absolute volume involved by gel in the segment. Absolute volume Vt = Vg (volume of gel dabs) + Vi (inward volume) + Vo (free volume outside tthe particles) Vt = Vg + Vi + Vo Analytes

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that are not held are eluted with the free volume outside of the particles (Vo), while analytes that are totally held are eluted with volume of dissolvable held in the pores (Vi). The allout volume can be considered by the accompanying condition, where Vg is the volume of the polymer gel and Vt is the complete volume. In the event that the example particles are little, they can without much of a stretch enter the pores of the globules and if test particles are huge, they neglect to enter the pores of the dabs.

The highest point of the section is appended with siphon which ceaselessly siphon portable stage in the segment. Base is associated with the indicator, the identifier the finder might be 1. Refractive list 2. UV retention 3. IR ingestion. Identifier is chosen dependent on the example. At the point when the example alongside portable eliminate passed the section enormous particles outside the dabs effectively pushes ahead where little atoms which are caught in the pores of the dabs sets aside longer effort to move.

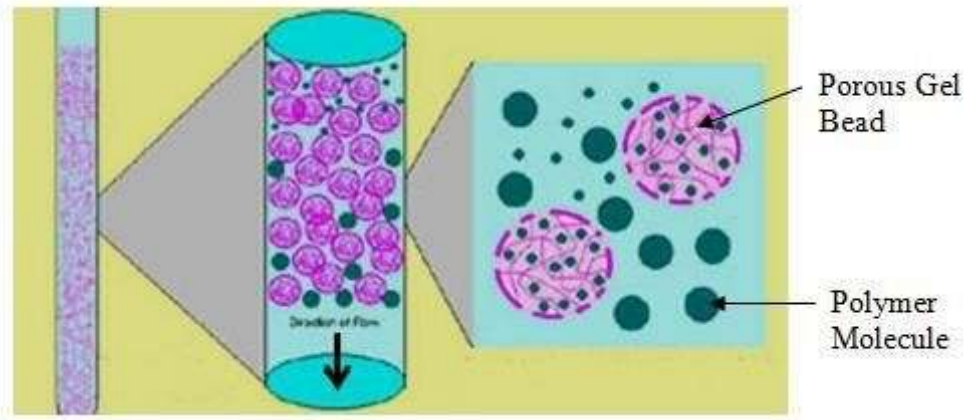

Figure 1: gel permeation chromatography

III. INSTRUMENTATION OF GEL PERMEATION CHROMATOGRAPHY:

3a. Instrumentation:

Gel permeation chromatography is almost conducted in chromatography columns. The experimental design of gel permeation chromatography is slightly different from other techniques of liquid chromatography. Samples are dissolved in an proper solvent, in the case of GPC these tend to be organic solvents and after filtering the solution it is inserted onto a column. In the column the separation of multi-component mixture takes place. With the help of a pump, the constant supply of fresh eluent to the column is accomplished. Detector is used because most analytes are not visible to the naked eye. To gain additional information about the polymer sample multiple detectors are used. The availability of a detector makes the fractionation accurate and convenient.

Figure 2: Instrument of gel permeation chromatography

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3b. Gel/stationary phase:

[5][6]A gel is a semi-solid substance that can have properties ranging from soft and weak to hard and tough.[7]Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state. [8]A gel has been defined phenomenologically as a soft, solid or solid-like material consisting of two or more components, one is liquid, present in substantial quantity. In GPC gel is used as stationary phase. In order to apply the gel to a given separation the pore size of a gel must be carefully controlled.Other desirable properties of the gel forming agent are low affinity for the substance to be separated, and absence of ionizing group. [9][10]Generally PLgel and Styragel(crosslinked), [11]LH-20(hydroxyl propylated sephadex), BioGel(crosslinked polyacrylamide),HW-20 and [12] HW-40(hyrdroxylated methacrylic polymer), [13] agarose gel and are often used in separation.

3c.Column:

In GPC column is filled with a microporous packing material. The column is filled with gel. Inside the column separation of sampletakes place, a hollow tube tightly paced with extremely small porous beads, polymer or silica have well defined pores size. Primarily for different molecular weight ranges, columns are packed with different sized particles with different sized pores. To improve the resolution, column are usually employed in combination of two or three columns. Before the main line guard columns are used. The guard column protects the main column by stopping insoluble particles or contaminants that could block the main column set.

Any of the following kinds may be used:

Analytical column-7.5-8mm diameters.

Preparative columns-22-25mm

Usual column length-25, 30, 50, and 60cm.

Narrow-bore columns-2-3mm diameter have been introduced.

3d. Eluent/Mobile phase:

The eluent should be permit high detector response from the polymer, should be a good solvent for the polymer and should wet the packing surface. The common eluent for polymers that diss the remainder of the system.

3e.Pump:

At constant, accurate and reproducible flow, the pump takes the solvent and delivers It to the remainder of the system. The pump has got to be ready to run an equivalent flow regardless of viscosity, in order that results are often compared from one analysis to a different. For uniform delivery of relatively small liquid volumes there are two sorts of pumps available they are: piston and peristaltic pumps. The pressure Delivered by the pump to be smooth in order that there are not any pulses within the flow. The solvent Is not wasted, when the inner volume of the pump is little. Pumps are very expensive in the equipment because they need to try to made of chrome steel, titanium and ceramics, which Do not react with the solvents utilized in GPC. They ought to withstand very high pressures. For uniform delivery of relatively small liquid volumes there are two sorts of pumps Available they are: piston and peristaltic pumps.

3f. Detectors:

To detect their presence as they elute from a column, chromatography uses the chemical and physical properties of sample molecules and mobile phase and so different detectors have been developed that make use of the different characteristics of compounds. Detectors can be divided based on measure concentration alone, such as UV, differential refractive index (DRI), and evaporative light scattering (ELS) detectors, and those whose response is proportional to concentration and other properties of the polymer molecules, such as viscometers or static light scattering detectors. The most common gel permeation chromatography detector is based on the principle of refractive index. In GPC, the concentration by weight of polymer in the eluting solvent may be monitored continuously with a detector. The first is concentration sensitive detectors which includes UV absorption, differential refractometer (DRI) or refractive index (RI) detectors, infrared (IR) absorption and density detectors. [14] The second category is molecular weight sensitive detectors, which include low angle light scattering detectors (LALLS) and multi angle light scattering(MALLS).[15] The determination of most copolymer compositions is done using UV and RI detectors, although other combinations can be used.

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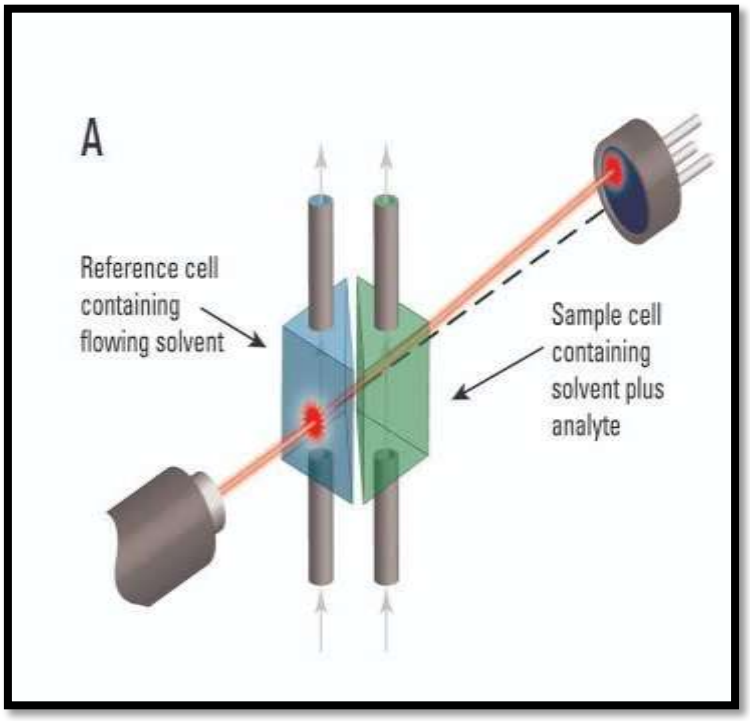

Figure 3.Differential refractive index:

This detector estimates the difference in refractive index between the solvent and the eluting polymer solution. It can be used with almost any polymer solvent combination.

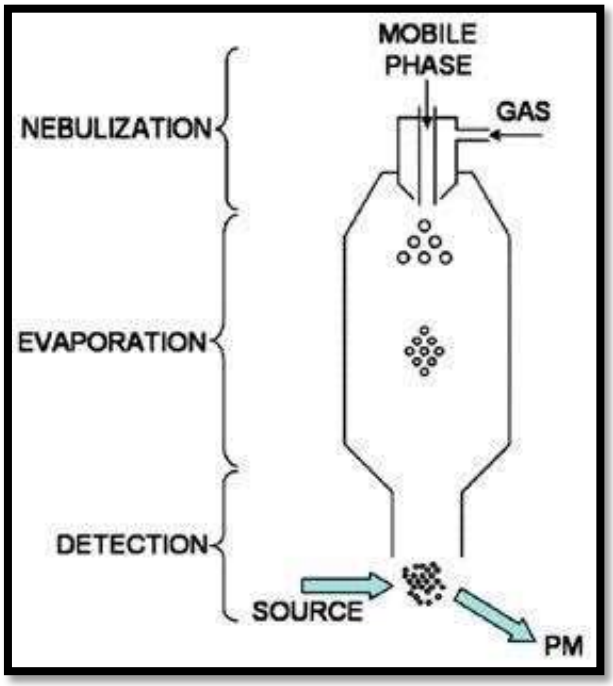

Figure 4: Evaporative light scattering:

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It is the universal detector and functions by nebulizing column effluent into droplets, which are the evaporated in a heated gas stream and solvent remains in vapour. It is scattered by Mie scattering phenomenon and this intensity of scattered light is detected by a photomultiplier tube. The carrier gas flow causes nebulization of solutes and temperature causes evaporation in drift tube to form small particles of non-volatile solute.

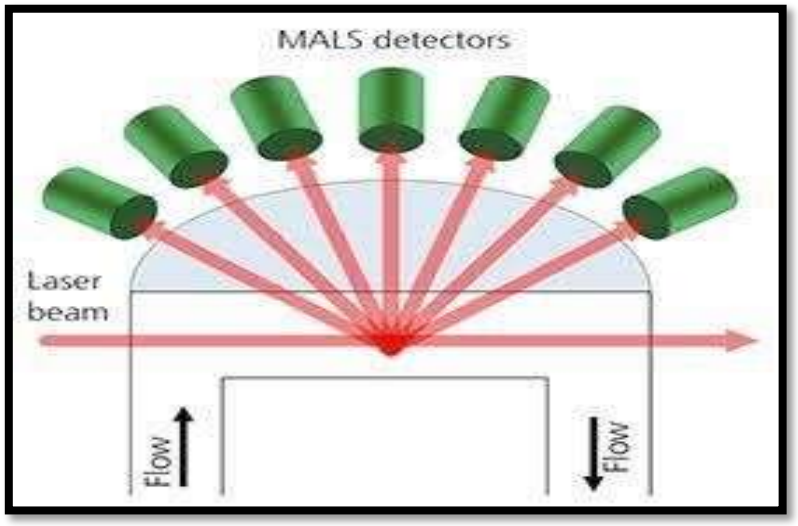

figure 5: Multi angle light scattering:

The light scattering as an inherent limitation in determining the size of very small polymer molecules in solution. Molecular weight of a small polymer can be estimated by measuring the scattered-light intensity at 90 to the incident angle.

Steps in Gel Permeation Chromatography: It involves three major steps

A. Preparation of column for gel filtration

It involves:

1. Swelling of the gel
2. Packing the column semi-permeable, porous polymer gel beads with a well-defined range of pore sizes.
3. Washing: After packing, several column volumes of buffer solution is passed through the column to remove any air bubbles and to test the column homogeneity.

B. Loading the sample onto the column using a syringe

C. Eluting the sample and detection of components

IV. APPLICATIONS:

1 Gel permeation chromatography truly measures molecular volume and shape. GPC is used to determine the relative molecular weight of polymer samples.

2.It also determines the distribution of molecular weights.

Biochemical applications:

Sec can determine the quaternary structure of purified proteins. It can also be used for measurement of hydrodynamic volume with folded and unfolded versions of the same proteins. Eg: hydrodynamic radius of a typical proteins domain can be 14 A and 36 A for the folded and unfolded forms respectively.

Surfactants study:

Non-ionic surfactants contain water-soluble group, and fatty acids. Various synthetic polymers contain hydrophobic segment e.g.: Tween, Igepal, Brij, Pluronic, Triton etc. the analysis of such surfactants is possible using SEC analysis and differential refractive index detector.

Polyesters analysis

Aromatic polyester analysis require the high temperature solvent.due to its crystalline structure it uses viscous m cresol or o chlorophenol as the mobile phase which causes hydrolytic degradation of the polymer

Polycarbonates property study

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Polycarbonates are non-crystalline thermoplastic and are linear aromatic polyesters of carbonic acids the properties of polycarbonate like strength clarity and heat deflection temperatures. Can be studied by sec

Polyamides property study:

Polyamides are used as adsorbent for isolation of various polyphenol compounds from plants and as packaging material for different pharmaceutical products. SEC is employed during determinations of molecular weight distribution of polyamides to study different key physical parameters like strength, toughness, abrasion resistance, and retention of physical and mechanical properties.

Natural rubber property study

The natural rubber is widely used as pharmaceutical aid. Eg new drug delivery system rubber latex. Contraceptives packaging material etc hence the quality control check of natural rubber is essential to ensure its suitability for different applications sec is used to distinguish natural rubberfrom other conventional polymers.

5.Gel permeation chromatography can be used on humic acids or fulvic acids and in water and is usually applied to the analysis of fatty samples like fish.

6.It determine the quaternary structure of purified proteins.

V. ADVANTAGES:

It has many advantages. It has a well-defined separation time. Most samples can be analysed in an hour or less. It provides narrow bands. There is a lower chance for analyte loss to occur, since the analytes do not interact chemically or physically with the column. molecular weights and mass distributions typically were not analysed, as these processes were quite labor-intensive. It is quick and relatively easy estimation of molecular weights and distribution for polymer samples.

VI. DISADVANTAGES:

Filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors. Although useful for protecting the instrument, there is the possibility of the pre-filtration of the sample removing higher molecular weight sample before it can be loaded on the column. Another possibility to overcome these issues is the separation by field-flow fractionation (FFF). Another disadvantage of GPC for polymers is that filtrations must be performed before using the instrument to prevent dust and other particulates from ruining the columns and interfering with the detectors.

VII. CONCLUSION:

Gel permeation chromatography is a powerful analytic technique. The preference for GPC is because of its relatively low cost, simplicity and ability to provide accurate, reliable information about the molecular weight distribution of polymer.It is used to determine the relative molecular weight of a polymer. Most samples can analyse in an hour.

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