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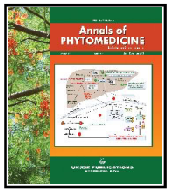

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A review of advanced approaches to gel permeation chromatography

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Abstract

The technology of gel permeation chromatography is extensively employed in the analysis and separation of polymers according to their molecular weight. Utilizing multi-detection methods like UV absorbance and light scattering, this improved GPC approach offers more precise details regarding the molecular weight distribution, chemical structure, and composition of the polymer. Researchers can gain a thorough understanding of complex polymer materials, including the analysis of additives, contaminants, and copolymers, by integrating various detection techniques. Furthermore, reliable molecular weight measurements for polymers with varying chemical structures and solvent analysis are made possible by this sophisticated GPC approach. In research and industrial contexts, where accurate polymer characterization is essential for product development and quality control, this sophisticated GPC approach is useful.

1. Introduction

Gel permeation chromatography (GPC) is a widely used method for molecular weight-based polymer separation and analysis. It is a powerful tool in polymer science and plays a crucial role in various industries, such as plastics, materials, and pharmaceuticals. GPC allows researchers to obtain important information about polymer samples, such as their molecular weight distribution, chemical composition, and structural properties (Almdal *et al.,* 1993; Yang Song *et al.,* 2020).

The stationary phase in a typical GPC approach is a column filled with porous gel beads. After dissolving the polymer sample in an appropriate solvent, it is injected into the column. Bigger molecules elute more quickly than smaller ones because both types remain outside through the pores. As the sample passes through the column and more readily *via* the pores of the gel beads. By keeping an eye on the amount or length of elution, one can determine the molecular weight of the polymer (Dai *et al.,* 1998). Traditional GPC might not fully capture the complexity of the material, but it does offer useful information on the average molecular weight of a polymer sample. Advanced GPC techniques combine several detection methods, like light scattering and UV absorbance, to provide a more thorough polymer characterization.

In GPC, UV absorbance detection is frequently employed to measure the polymer sample's concentration as it elutes from the column. Researchers can determine the concentration and degree of polymerization, as well as the amount of polymer present, by measuring the UV absorbance at a particular wavelength. However, UV absorbance detection does not reveal the molecular weight of the polymer only its concentration. This is the application of light scattering detection. By examining the intensity of scattered light as the polymer passes through the detector, light scattering can be used to determine a polymer's molecular weight directly (Raghavi *et al.,* 2023; Akella Anuradha *et al*., 2023). Larger polymer molecules scatter more light than smaller ones, which is how light scattering detection operates. By measuring the intensity of scattered light at different angles, researchers can calculate the molecular weight of a polymer in terms of both its weight-average molecular weight (Mw) and number-average molecular weight (Mw). Understanding the polymer's molecular weight distribution is essential for comprehending its physical and chemical properties, and this knowledge makes that determination more precise.

Furthermore, advanced GPC techniques enable analysis in a range of solvents, allowing scientists to examine polymers with varying chemical structures or solubility properties (Dhritimoni and Sumithra, 2023). This adaptability is especially helpful when examining polymers that have polar or non-polar functional groups or when researching how solvents affect the characteristics of the polymer. For polymer multi-detection approaches combined with modern GPC techniques offer a potent tool. They make it possible to precisely characterize polymers, which contributes to a better knowledge of their characteristics, actions, and effectiveness. Researchers can optimize products and develop new materials by having access to extensive information on structural features, chemical makeup, and molecular weight distribution (Moore, 1964).

2. Material and Methods

Molecules in a solution are separated according to their size using gel permeation chromatography (GPC), sometimes referred to as size exclusion chromatography SEC (Skoog, 2006). Here is an advanced overview.

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2.1 Principle

GPC uses a column that has porous beads inside of it. Smaller molecules can pass through the pores and elute later, while larger molecules cannot pass through and elute early.

2.2 Column selection

Select a column whose pore size falls within the desired range of molecular sizes. Fewer pores for molecules that are smaller, and *vice versa*.

2.3 Mobile phase

Selecting the right solvent is essential. It ought to work well with the beads and sample. Chloroform and tetrahydrofuran are common solvents (Dhritimoni and Sumithra, 2023).

2.4 Calibration standards

To calibrate the column, use a set of standards with established molecular weights. Based on the elution durations of unknown molecules, aid in estimating their size.

2.5 Sample preparation

Ensure that the sample dissolves properly in the mobile phase to avoid interactions that can skew the results.

2.6 Detector selection

Use sophisticated detectors, such as refractive index (RI) or multi-angle light scattering (MALS), to determine the molecular weight precisely. This holds particular significance for intricate samples (Khademhosseini and Demirci, 2016).

2.7 Temperature control

To ensure reproducibility, keep the temperature consistent. A column oven with temperature control might be a part of some systems.

2.8 Data analysis

The chromatograms are analyzed with sophisticated software. This incorporates sophisticated modeling for precise molecular weight measurement and deconvolution algorithms for complicated samples.

2.9 Triple detection system

For increased precision, some setups use triple detection (MALS, RI, and UV), particularly when working with polymers or biopolymers.

2.10 Column regeneration

Develop methods for column regeneration to ensure longevity and consistent performance. Remember, the effectiveness of GPC depends on the careful optimization of these parameters based on the specific characteristics of the molecules you are analyzing (Seiffert, 2015; Kiran *et al.,* 2019).

2.11 Instrumentation

Advanced methods in gel permeation chromatography (GPC) instrumentation involve the incorporation of state-of-the-art technologies to enhance resolution, sensitivity, and data accuracy.

2.12 High-resolution columns

Using columns with better packing materials and particle size distribution will increase separation efficiency and resolution.

2.13 Multi detector system

Combining several detectors, including light scattering (MALS), refractive index, UV-Vis, and viscosity detectors, to get detailed data on the molecule weight, size, and structure.

2.14 Advanced pump system

Utilizing cutting-edge pump technologies to provide accurate and consistent flow rates helps to increase the repeatability and precision of elution profiles (Almdal *et al.,* 1993).

2.15 Online coupling with mass spectroscopy

When mass spectrometry and GPC are combined, it is possible to determine molecular weight and structural details at the same time, giving macromolecules a more thorough characterization.

2.16 Temperature control systems

Improved temperature control capabilities will enable researchers to better understand how temperature affects molecular interactions and the behavior of polymers. Advanced sample preparation and autosamplers for higher throughput, lower error rates, and enhanced overall effectiveness.

2.17 Advanced data analysis software

Application of complex software with cutting-edge algorithms to precisely identify peaks, determine molecular weight, and evaluate data in-depth.

2.18 Hyphenated techniques

Integration with additional chromatographic methods, such as gas chromatography (GC) or liquid chromatography (LC), offers additional data and a more thorough examination of complicated materials.

2.19 Inert material construction

Instrumentation constructed from inert materials to minimize sample surface interactions and prevent adsorption, ensuring accurate measurement of molecular weights.

2.20 Real-time monitoring

Real-time monitoring features that provide immediate feedback on chromatographic runs and allow for optimization and correction while the analysis is being conducted. These advancements collectively contribute to the evolution of gel permeation chromatography, enabling researchers to obtain more precise and detailed information about the molecular characteristics of polymers and macromolecules.

3. Gel permeation chromatography used in the analysis

The molecular weight and dispersity were determined using GPC techniques. These results aid in determining the degree of regulation and molecular weights. Reached throughout the polymerization process. Using a PSS security GPC system with an RI detector and a flow rate of 1.0 ml/min, GPC was performed in THF at 30°C. To use HHRH Guard-17360 and GMHH-N-18055 of a column to a refractive index detector (VE 3580 RI detector, Viscotec), GPC analyses for PGA, saturated fatty acid grafted PGAs, and unsaturated fatty acid grafted PGAs were carried out in THF using a viscotec GPC max VE 2002. Calibration was performed using polystyrene standards. For PGA (M), which was made from glycerol and dimethyl adipate, and PEG and oleate grafted PGAs, poly (methyl methacrylate) standards

were used to calibrate the thermostatically controlled column at 25°C for the GPC measurements. Salts like (LiBr) are added to the mobile phase to inhibit aggregation by reducing polymer chain interactions like hydrogen bonding. Each sample had a concentration of 3 mg/ml, and each sample had a flow rate of 1 ml/min (Muhammad Humayun *et al.,* 2019). GPC is used to examine variations in the molecular weight of the PS polymer. Frass sample preparation is comparable to the THF extraction test mentioned earlier (4.2.7). THF is used to dissolve a sample of PS feedstock. PS feedstock (0.2-1.0 g) and the Frass tests (0.5-1.0 g) from larvae fed PS are commonly used. In a glass vial holding 50 ml and covered with a Teflon-coated septum, THF extraction is done overnight. After filtering through a 0.22 mm PVDF sterile syringe filter (Thermo Fisher Scientific Inc., Dublin, Ireland), the THF solution is transferred into a pre-weighed 50 ml glass vial and either vacuum-dried overnight or combined with a magnetic stirrer and gradually heated to 60°C. A highly helpful method for the purification, separation, and characterization of both natural and synthetic polymers is size exclusion chromatography, also known as gel permeation chromatography or GPC. Using this method, porous inert material columns are filled with the analyte, which is typically a polymer, after it has been dissolved in an appropriate solvent. Instead of using the analyte's molecular weight to determine separation, the columns use its hydrodynamic size. Typically, the columns of GPC systems are equipped with a light scattering unit, UV detector, or RI detector. By attaching advanced detection devices to the GPC, we may obtain vital information about polymers, including their degree of branching, average molecular mass, and molecular weight dispersion. Apart from polymer separation, GPC facilitates the separation of nucleic acids, polysaccharides, enzymes, and hormones. GPC can be used to quantitatively assess the tissue deposition of polymer nanoparticles after *in vivo* exposure in terms of nanotoxicity. Understanding the exposure of a nanomaterial to an organ makes it easier to determine possible risks, choose appropriate toxicity assays, and interpret findings from other toxicity studies (Abdul and Joshua, 2012). Theoretical gel permeation chromatography curves selected for model polydisperse polymers, average molecular weights, and a predefined level of random trifunctional branching have been devised. The generated molecular weight averages and branching characteristics were compared to those obtained directly from the model (the stockmeyer type of distribution) after Drott's method was applied to gel permeation chromatography traces on a computer. We discuss Drott's basic conjecture, according to which there can be no change in the number of branch points divided by the molecular weight (Cervenka and Bates, 1970).

Gel permeation chromatography in the petrochemical laboratory is demonstrated through experimental optimization of the separation, detection, and results calculation for fuel oil samples. One common petrochemical test for residue fuel, the conradson carbon residue (CCR) determination, is used to compare the results of a chromatographic reaction based on the separation and detection of fuel oil GPC. It was verified that there was a linear relationship between the two test results. In the range of 7.5–18.5% CCR, the chromatographic method's coincidence with the CCR determination is better than 34 ± 1.5%. A comparison between UV and refractive methods for fuel oil classification is given (Elsa and Tyge, 1985). A technique for identifying a technique called high-performance gel-permeation chromatography (HPGPC) has been developed to analyze the proteins in human pancreatic juice, bile, and tissue homogenate. With a diol-type silica gel column (35 x 8 mm i.d., 5 nm average pore diameter), a temperature of 8°C was used. The eluent employed in this experiment was an acidic phosphate buffer with high concentrations of sodium chloride, glycerol, acetyl ether (brij-58), nonionic detergent of polyoxymethylene (20), and 2-propanol. The UV wavelength used to identify proteins was 210 nm. An analysis took 3.5 min. Using a spectrophotometric bicinchoninic acid (BCA) method at 8°C for the column temperature, using this HPLC approach, excellent correlation coefficients were found. It was discovered that there was a strong correlation between this HPLC method and a photometric approach using the red molybdate-pyrogallol complex, but only for tissue homogenate. It is anticipated that this HPGPC protein assay method will find broad application in biochemical and clinical research due to its simplicity, convenience, speed, reproducibility, and reliability (Kou Hayakawa, 2001; Baira Venkatesham *et al*., 2021)

3.1 Advantages

Gel permeation chromatography (GPC) advanced techniques have many benefits, such as increased sensitivity, increased size range, and higher resolution. Better separation of complex polymer mixtures and more precise molecular weight determination are possible with high-performance GPC columns that use cutting-edge packing materials. Furthermore, sophisticated detectors like viscometer and multi-angle light scattering (MALS) help to characterize macromolecules with more accuracy (Vimal and Sumithra, 2023; Aaisha Ansari *et al.,* 2020). These developments make GPC an effective tool in polymer science and related fields by enabling researchers to get comprehensive data about the structure and composition of polymers.

3.2 Disadvantages

Advanced gel permeation chromatography (GPC) techniques have certain drawbacks despite their benefits. Because high-resolution GPC techniques separate complicated mixtures in great detail, they can be time-consuming and require longer analysis time. Advanced GPC requires expensive detectors and complex equipment, which prevents some laboratories from using it. Furthermore, interpreting the data might be difficult, particularly when working with mixtures or highly polydispersity samples that have overlapping peaks. Researchers must carefully weigh the advantages and potential disadvantages of advanced GPC methods in light of their unique analytical requirements and available resources (Subhamalar, *et al.,* 2023).

4. Applications

Advanced gel permeation chromatography (GPC) methods find diverse applications in various scientific fields, particularly in polymer science and related areas (Helmut, 1969). Some notable applications include:

4.1 Polymer characterization

Researchers can accurately characterize polymers by precisely determining the molecular weight distribution through the use of advanced GPC. Understanding the structure and properties of polymers requires knowledge of this.

4.2 Quality control in polymer production

GPC is used in polymer manufacture to ensure quality control. The performance of final goods is impacted by the consistency that is ensured in polymer batches by precise molecular weight measurement.

4.3 Biopolymer analysis

Biopolymers such as proteins, nucleic acids, and polysaccharides are studied using GPC. It facilitates the determination of their purity, size distribution, and structural alterations under various circumstances (Trathnigg, 1995; Justin Cole *et al.,* 2017).

4.4 Pharmaceuticals

GPC is used in pharmaceutical research to analyze and characterize biodegradable materials, and polymers utilized in formulations, and drug delivery systems.

4.5 Environmental analysis

GPC is used in environmental research to analyze complicated mixtures and find the molecular weight distribution of pollutants, like natural organic materials in water.

4.6 Food Science

Food macromolecules including proteins, lipids, and polysaccharides are analyzed and characterized using GPC, which helps to explain the composition and quality of food.

4.7 Material science

GPC is a tool that researchers use to analyze how the molecular weight distribution of different materials such as adhesives, coatings, and biomaterials affect the stability and performance of the material.

4.8 Nanoparticle characterization

By using GPC to characterize nanoparticles, scientists can learn more about their size distribution, stability, and interactions with various environments.

4.9 Biomedical research

GPC is used to analyze biomolecules, which helps in the creation of biomaterials, medication delivery systems, and diagnostics.

4.10 Fuel and lubricant analysis

The stability and performance of fuels and lubricants can be understood by analyzing and characterizing the polymers in them using GPC. The advanced features of GPC, such as improved resolution and extended size range, make it a versatile tool across various scientific disciplines for detailed molecular analysis and characterization (Pasch, 2000; Pradeep Singh *et al.,* 2020; Raghavi *et al.,* 2023).

5. Conclusion

Advanced gel permeation chromatography (GPC) methods offer significant advantages in terms of improved resolution, extended size range, and enhanced sensitivity. These advancements, achieved through innovations in column materials, detectors like multi-angle light scattering (MALS), and viscometers, provide researchers with more precise information about polymer structures and compositions. However, it is essential to consider the associated disadvantages, such as increased analysis time, cost of sophisticated instrumentation, and potential challenges in result interpretation, especially with complex mixtures. Researchers should weigh these factors based on their specific analytical needs and resource constraints when choosing advanced GPC methods for polymer characterization.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article..

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