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Review Article

**A REVIEW ON SIZE EXCLUSION CHROMATOGRAPHY
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Abstract:

Size Exclusion Chromatography (SEC), also known as gel filtration or gel permeation chromatography, is a powerful analytical technique that separates molecules according to their hydrodynamic size without chemical or ionic interactions. This manuscript provides a concise review of SEC principles, instrumentation, and recent advancements, emphasizing its role in the characterization of proteins, polymers, nanoparticles, and biotherapeutics. SEC's non-destructive mechanism preserves sample integrity, making it essential for macromolecular analysis. Developments in multi-angle light scattering (MALS), ultra-high performance SEC (UHP-SEC), and bio-inert column technology have further improved precision and resolution. The review also highlights practical aspects such as column selection, mobile phase optimization, and detector configurations. Despite limitations in resolving similarly sized species, SEC remains indispensable in pharmaceutical and polymer research.

Keywords: Size Exclusion Chromatography, Gel Filtration, SEC-MALS, Polymer Analysis, Protein Purification, Biopharmaceuticals.

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1. INTRODUCTION

Size Exclusion Chromatography (SEC) is an analytical method used to separate molecules based on their size in solution. Unlike other chromatographic techniques that depend on chemical interactions, SEC functions purely on an entropic mechanism—larger molecules elute first as they cannot enter the porous stationary phase, whereas smaller ones are retained longer. Developed in the 1950s by Lathe and Ruthven, SEC has evolved into a fundamental tool in polymer chemistry, protein analysis, and biotechnology.

2. Principle and Mechanism

In SEC, separation is governed by differential access of solute molecules to the pores of a stationary phase composed of porous beads such as agarose, dextran, or polyacrylamide. Larger molecules elute earlier as they are excluded from the pores, while smaller molecules penetrate and are delayed. The separation range depends on the pore size distribution of the stationary phase. The elution volume (V_e) is related to molecular size through the distribution coefficient K_{av} , enabling molecular weight estimation when calibrated with standards.

3. Instrumentation and Components

A standard SEC system includes a mobile phase delivery pump, injector, column, and detector. Columns are packed with porous beads selected based on the molecular weight range of interest. Detectors such as Refractive Index (RI), UV-Visible, Multi-Angle Light Scattering (MALS), and viscometers are used to monitor elution. Modern SEC instruments integrate multiple detectors (SEC-MALS) for absolute molecular weight and size determination without calibration standards.

4. Mobile Phase and Column Considerations

The mobile phase composition is crucial for maintaining sample stability and minimizing secondary interactions. Aqueous buffers with neutral pH and physiological salt concentrations are preferred for biomolecules, while organic solvents such as tetrahydrofuran are used for synthetic polymers. Columns may be made from cross-linked dextran (Sephadex), agarose (Sephacrose), or silica for high-performance applications. Optimizing pH, ionic strength, and flow rate ensures reproducible and accurate separations.

5. Applications of SEC

SEC finds extensive use in protein purification, aggregate analysis, and molecular weight determination of polymers. In the biopharmaceutical industry, it is critical for assessing product purity and aggregation in monoclonal antibodies. In polymer science, SEC

(also termed GPC for organic systems) is the standard method for molecular weight distribution analysis. Recent advances extend its applications to nanoparticles, liposomes, and viral vector characterization.

6. Advantages and Limitations

Advantages include gentle, non-destructive separation, minimal sample preparation, and high reproducibility. However, SEC cannot differentiate molecules with similar hydrodynamic radii and may exhibit secondary interactions with column materials. Accurate calibration and column maintenance are essential for consistent performance.

7. Recent Advances and Future Trends

Recent progress in SEC technology includes the development of sub-2 μm particles, bio-inert hardware, and UHPLC-compatible systems that improve speed and resolution. Coupling SEC with detectors like native mass spectrometry and fluorescence enhances molecular characterization. Emerging trends include nano-flow SEC for micro-sample analysis and integrated SEC-MALS-MS workflows for biotherapeutic characterization.

8. CONCLUSION:

Size Exclusion Chromatography remains a cornerstone analytical method for studying macromolecular structure and purity. Advancements in column chemistry, detector integration, and instrumentation have greatly expanded its capabilities. As biopharmaceutical and nanotechnology research progresses, SEC will continue to play a vital role in ensuring the quality and characterization of large, complex molecules.

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