



About: Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) separates molecules based on their size, or more precisely, their hydrodynamic volume. It is based on the discrimination of individual sample components by the pores of the packing material. Large sample molecules cannot or can only partially penetrate the pores and elute from the column first, whereas smaller molecules can access all or a larger number of pores and elute later. SEC is the only mode of chromatography that does not involve interaction with a stationary phase by means of adsorption or partitioning of the solutes.

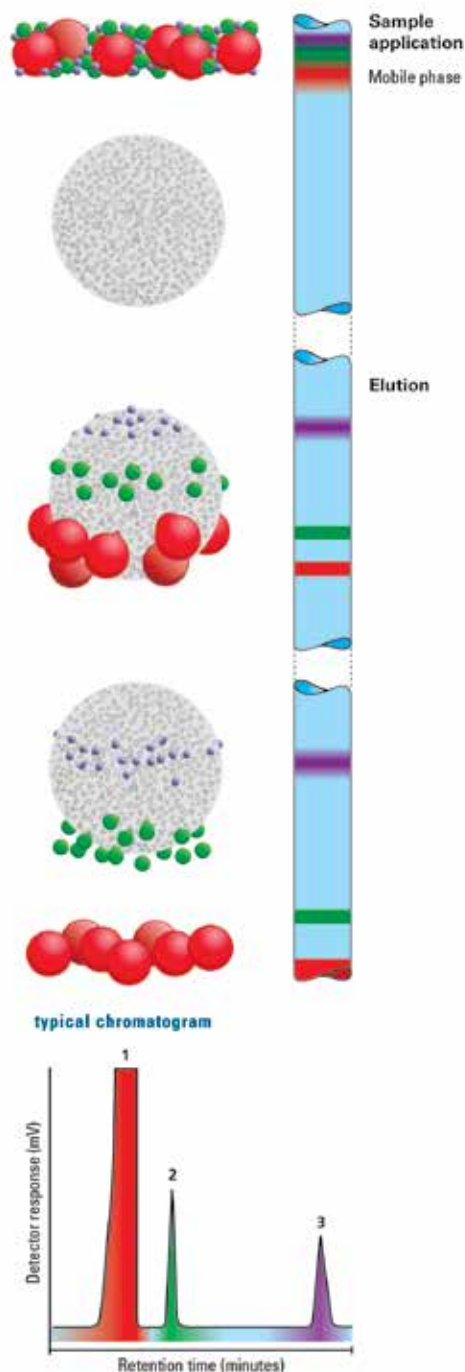
The terms SEC, GFC (gel filtration chromatography) and GPC (gel permeation chromatography) all refer to the same chromatographic technique. In GFC an aqueous mobile phase is used, while an organic mobile phase is employed in GPC. The general term SEC covers both uses.

SEC is the dominant mode of separation for natural and synthetic polymers:

- GFC is the term used for the size-based separation of water-soluble polymers, for example biopolymers or natural polymers.
- GPC is the term used for the size-based separation of polymers soluble in organic solvents.

Size exclusion chromatography columns are traditionally packed with porous polystyrene divinylbenzene (PS-DVB) or silica particles. PS-DVB columns are commonly used for the analysis of synthetic polymers in organic solvents, while silica-based columns are used for the separation of biopolymers.

Figure 1: Size Exclusion Chromatography



TSKgel Gel Filtration Chromatography Columns: GFC

The principal feature of GFC is its gentle non-interaction with the sample, enabling high retention of biomolecular enzymatic activity while separating multimers that are not easily distinguished by other chromatographic methods. SEC has limited peak capacity, however, requiring that the molar mass of the biomolecules differ by at least two-fold. GFC is popular among biochemists for the isolation of protein fractions or for the removal of aggregates in a final polishing step in biotechnology production. GFC is also frequently used for desalting a (protein) sample solution, often to prepare the sample for elution by another chromatographic mode.

- TSKgel columns for GFC analysis consist of the TSKgel SW and PW series column lines. The main criterion in choosing between these TSKgel columns is the molar mass of the sample and its solubility. The fact that the TSKgel SW columns are based on silica and the TSKgel PW columns are derived from a hydrophilic polymer network has less impact on the separation than the particle and pore size differences between the column lines.
- Due to higher resolving power, the TSKgel SW series columns are suitable for the separation of the monodisperse biopolymers such as proteins and nucleic acids. The TSKgel SW mAb columns within the TSKgel SW series are designed specifically for the analysis of monoclonal antibodies. TSKgel PW series columns are commonly used for the separation of synthetic water-soluble polymers because they exhibit a much larger separation range, better linearity of calibration curves, and less adsorption than the TSKgel SW columns. While a TSKgel SW column is typically the first column to try for biopolymers, TSKgel PW columns have demonstrated good results for smaller peptides (<1,000 Da), protein aggregates, DNA fragments, and viruses.

TSKgel Gel Permeation Chromatography Columns: GPC

GPC plays an important role in the characterization of polar organic-soluble and organic-soluble polymers in consumer, chemical, and petrochemical industries. GPC is often used to determine the relative molar mass of polymer samples as well as the distribution of molar masses.

- TSKgel Alpha and SuperAW columns were developed for the GPC analysis of polymers of intermediate polarity. The TSKgel Alpha columns are compatible with a wide range of solvents. TSKgel SuperAW columns are based on the same chemistry as TSKgel Alpha columns but have smaller particle sizes and shorter, narrower column dimensions for high throughput applications.
- For the GPC analysis of organic-soluble polymers, Tosoh developed TSKgel H series columns. Each line of columns within the TSKgel H series differs in degree of inertness and operating temperature range.

Table 1: All TSKgel SEC columns share these features and benefits

Features	Benefits
Rigid hydrophilic and hydrophobic packings	Minimal swelling and excellent physical strength; Low adsorption resulting in high mass recovery
Four series of SEC columns with different ranges of solvent compatibility	Suitable for both types of size exclusion, aqueous (GFC) and non-aqueous (GPC)
Easy scale up	Analytical and preparative pre-packed SEC columns

