

Principle of Gel Filtration Chromatography

The principle of gel filtration chromatography, also known as size exclusion chromatography (SEC), is based on the differential size-based separation of molecules in a mixture. Gel filtration chromatography is commonly used to separate biomolecules, such as proteins and nucleic acids, based on their molecular size and shape. Here is an outline of the principle:

Column packing: A gel filtration column is prepared by packing a stationary phase of porous beads or gel matrix into a column. The beads are typically made of cross-linked agarose or dextran, and they contain pores or channels of a specific size range.

Sample application: The sample to be analyzed is applied to the top of the column. The sample consists of a mixture of molecules of different sizes, including the target molecule(s) and other contaminants.

Elution process: When a suitable mobile phase, also known as the running buffer, is introduced into the column, it flows through the gel matrix. As the mobile phase passes through the column, molecules in the sample mixture interact with the gel matrix based on their size.

Separation based on size: Larger molecules cannot enter the pores of the gel matrix and, therefore, pass through the column more quickly because they take the path of least resistance. Smaller molecules, on the other hand, can enter the pores of the gel matrix and, thus, experience a greater degree of interaction with the gel. Consequently, smaller molecules move more slowly through the column, taking a longer path. As a result, molecules are separated based on their size, with larger molecules eluting first and smaller molecules eluting later.

Elution profile and collection: As the mobile phase continues to flow through the column, eluted molecules are detected using appropriate detectors, such as UV absorbance, refractive index, or fluorescence detectors. The elution profile is a plot of the detector response (e.g., absorbance) against the elution volume or time. Fractions containing the separated molecules of interest are collected for further analysis or downstream applications.

Gel filtration chromatography is a versatile technique used for various purposes, including sample purification, determination of molecular size, removal of contaminants, and buffer exchange. The choice of the gel matrix and column parameters, such as the pore size and column dimensions, can

be optimized based on the specific requirements of the experiment and the size range of the target molecules.