

Principle of Ion exchange chromatography

The principle of ion exchange chromatography (IEC) is based on the separation of charged molecules in a mixture by their interactions with charged stationary phases. IEC is commonly used to separate and purify biomolecules, such as proteins and nucleic acids, based on their differences in charge. Here is an outline of the principle:

Stationary phase: An ion exchange chromatography column is packed with a stationary phase consisting of porous beads or resin that carries a fixed charge. The stationary phase can be either a cation exchange (positively charged) or an anion exchange (negatively charged), depending on the type of molecules being separated.

Mobile phase: The mobile phase, also known as the eluent or running buffer, is a solution containing ions of opposite charge to the stationary phase. The choice of the eluent depends on the nature of the molecules being separated and the desired separation conditions. For cation exchange, the eluent contains anions, while for anion exchange, the eluent contains cations.

Sample application: The sample containing a mixture of charged molecules is applied to the top of the column. The molecules in the sample can be positively charged (in the case of cation exchange) or negatively charged (in the case of anion exchange).

Elution process: When the eluent is introduced into the column, it flows through the stationary phase. The charged molecules in the sample interact with the charged sites on the stationary phase. The strength of these interactions depends on the charge of the molecules and the charge of the stationary phase.

Separation based on charge: The charged molecules in the sample bind to the oppositely charged sites on the stationary phase. Molecules that have a stronger affinity for the stationary phase (greater charge or stronger interaction) will bind more tightly and, therefore, elute more slowly. In contrast, molecules with a weaker affinity for the stationary phase will elute more quickly.

Elution profile and collection: As the eluent continues to flow through the column, the bound molecules are released from the stationary phase. The eluted molecules are detected using appropriate detectors, such as UV absorbance or conductivity, and their elution profile is recorded.

Fractions containing the separated molecules of interest are collected for further analysis or downstream applications.

Ion exchange chromatography allows for the separation and purification of charged molecules based on their differences in charge. The choice of the stationary phase, eluent, and column conditions can be optimized to achieve the desired separation. IEC is a powerful technique used in various fields, including protein purification, analysis of protein charge variants, separation of nucleic acids, and purification of synthetic organic compounds.