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Commentary

A Brief Notes on Chromatography and Separation Techniques

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COMMENTARY

Chromatography is the most widely used technique that involves the separation, identification, of component mixture for both qualitative and quantitative analysis. Proteins can be separated based on size, shape, and total charge, hydrophobic groups present on the surface. Based on the hydrophobic groups present on the surface there are different types of chromatographic techniques: Column chromatography, Ion-exchange chromatography, gel-permeation (molecular sieve) chromatography, affinity chromatography, paper chromatography, thin-layer chromatography, gas chromatography, dye-ligand chromatography, hydrophobic interaction chromatography, pseudoaffinity chromatography, high-pressure liquid chromatography (HPLC). The purpose of applying chromatography is a method of quantitative analysis of separation based on two phases: a stationary phase and a mobile phase. The type of interaction between the stationary phase and mobile phase and the substances contained in the mixture is the most effective component in separating molecules from each other.

Ion exchange chromatography is based on electrostatic interaction between two charged protein groups and a solid support matrix .matrix has an ion load opposite to that of the protein to be separated .proteins are separated by changing ph concentration of ion salts or ionic strength of buffer solution .positively charged ion exchange matrices are called anion-exchange matrices and they will absorb negatively charged proteins in case if the matrices bound with negatively charged groups are called cation exchange matrices and absorb positively charged proteins. The principle involved in Gel-permeation (molecular sieve) chromatography method is to use dextran-containing materials to separate macromolecules based on their differences in molecular sizes. This process is used to determine the molecular weights of proteins and to decrease the salt concentrations of protein solutions. In a gel permeation column, the stationary phase consists of inert molecules with small pores the solution contains molecules that are flowing with a flow rate through the column. Molecules larger than pores cannot permeate into gel particles and are retained within a particular area. Larger molecules pass through the pores and move rapidly inside the column.

The affinity chromatography technique is most widely used for the purification of enzymes hormones, antibiotics, nucleic acids, and specific proteins. A ligand that can make a complex with specific protein binds with the filling material of the column. By using this High-pressure liquid chromatography we can perform the structural and functional analysis of many molecules within a short time. The high-pressure liquid chromatography technique yields perfect results in the separation and identification of carbohydrates, lipids, amino acids, nucleic acids, proteins, steroids, and other biologically active molecules in the HPLC mobile phase passes through columns under 10-400 atmospheric pressure, and with a high flow rate.

Chromatographic techniques were used to separate substances based on color and pigments. With time its application area was extended considerably nowadays chromatography is a widely accepted effective separation technique. Chromatography is one of the useful separations and determination methods. column chromatography is a protein purification method realized based on the characteristic features of proteins. Besides these methods HPLC has many features in its higher sensitivity, rapid turnover rate, its use as a quantitative method can purify amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, antibiotics, and steroids.

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CONFLICT OF INTEREST

The author has declared that no competing interests exist..

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