

## Chromatography and their Separation Techniques

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### Introduction

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. The purpose of applying chromatography is a method of quantitative analysis of separation based on two phases: 1. stationary phase and 2. mobile phase. The type of interaction between stationary phase and mobile phase and the substances contained in the mixture is the most effective component on separation molecules from each other. Ion exchange chromatography is based on electrostatic interaction between two charged protein groups and a solid support matrix. The matrix has an ion load opposite to that of the protein to be separated. Proteins are separated by changing the 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. Negatively charged matrices are called cation exchange matrices and absorb positively charged proteins. The principle involved in this method is to use dextran containing materials to separate macromolecules based on their differences in molecular sizes. This process is basically 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 containing molecules is flowing with a flow rate through the column. Molecules larger than pores cannot permeate into gel particles and are retained within the particular area. Larger molecules pass through the pores and move rapidly inside the column. This technique is most widely used for the purification of enzymes, hormones, antibiotics, nucleic acids, and specific proteins. A ligand which can make a complex with specific protein binds with the filling material of the column. By using this chromatography, we can perform the structural and functional analysis of many molecules within a short time. This technique yields perfect results in separation and identification of amino acids, carbohydrates, lipids, nucleic acids, proteins, steroids, and other biologically active molecules. In HPLC, the mobile phase passes through columns under 10-400 atmospheric pressure and with a high flow rate. In this technique, the use of small particles and application of high pressure pump chromatographic techniques were used to separate substances based on the colour and pigments. With time, its application area was extended considerably. Nowadays, chromatography is widely accepted as an effective separation technique. Chromatography is one of the useful separation and determination methods. Column chromatography is a protein purification method realised 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. There are a few fluid chromatography strategies applied in food investigation, in particular paper chromatography, thin layer chromatography (TLC) (both of these strategies might be alluded to as planar chromatography), and size exclusion chromatography, all of which include a fluid versatile stage and either a strong or a fluid fixed stage. Nonetheless, the actual type of the fixed stage is very divergent for each situation. Detachment of the solutes depends on their physicochemical connections with the two stages.

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### Conflict of Interest Statement

Authors declare they have no conflict of interest with this manuscript.

