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Techniques and Methods of Ion Chromatography

Jahnavi Nayak*

Department of Integrative and Functional Biology, CSIR Institute of Genomics & Integrative Biology, Mall Road, Delhi, India

Editorial

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***For Correspondence:**
Jahnavi Nayak, Department of Integrative and Functional Biology, CSIR Institute of Genomics & Integrative Biology, Mall Road, Delhi, India
E-mail: Jahnavi437@gmail.com

ABOUT THE STUDY

Ion chromatography (also known as ion-exchange chromatography) separates ions and polar molecules based on their affinity for an ion exchanger. It is effective on almost any charged molecule, including large proteins, small nucleotides and amino acids. Ion chromatography on the other hand must be performed in conditions one unit away from a protein's isoelectric point. Ion chromatography is classified into two types anion-exchange and cation-exchange. When the molecule of interest is positively charged, cation-exchange chromatography is used. Because the pH for chromatography is less than the pI (a/k/a pH (I)) the molecule is positively charged. The stationary phase in this type of chromatography is negatively charged and positively charged molecules are loaded to be attracted to it. Anion-exchange chromatography is when the stationary phase is positively charged and negatively charged molecules are loaded to be attracted to it (meaning that the pH for chromatography is greater than the pI).

It is frequently employed in protein purification water analysis and quality control. Water-soluble and charged molecules like proteins, amino acids and peptides bind to oppositely charged moieties by forming ionic bonds with the insoluble stationary phase [1]. The equilibrated stationary phase is made up of an ionizable functional group to which the targeted molecules of a mixture to be separated and quantified can bind while passing through the column—a cationic stationary phase is used to separate anions and an anionic stationary phase is used to separate

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cations. Cation exchange chromatography is used to separate cations while anion exchange chromatography is used to separate anions. The bound molecules can then be eluted and collected using an eluent containing anions and cations by passing more ions through the column or changing the pH of the column [2]. One of the primary benefits of using ion chromatography is that there is only one interaction involved during the separation process, as opposed to other separation techniques thus ion chromatography may have higher matrix tolerance. The predictability of elution patterns is another advantage of ion exchange (based on the presence of the ionizable group). When cation exchange chromatography is used, for example, cations are the last to elute. Meanwhile, the negatively charged molecules will be the first to elute. However, there are some drawbacks to using ion-exchange chromatography, such as constant evolution with the technique which results in inconsistency from column to column [3]. One significant limitation of this purification technique is that it is only applicable to ionizable groups.

Ion-exchange chromatography separates molecules based on their charged groups. Based on coulombic (ionic) interactions, ion-exchange chromatography retains analyte molecules on the column [4]. The ion exchange chromatography matrix is made up of positively and negatively charged ions. Essentially, molecules on the stationary phase matrix interact electrostatically with opposite charges. The stationary phase is made up of an immobile matrix containing charged ionizable functional groups or ligands. Ionic functional groups (R-X) on the stationary phase surface interact with analyte ions of opposite charge [5]. These inert charges couple with exchangeable counter ions in the solution to achieve electro neutrality. Purifiable ionizable molecules compete for binding to the immobilized charges on the stationary phase with these exchangeable counter ions. Based on their charge, these ionizable molecules are retained or eluted. Molecules that do not bind or bind weakly to the stationary phase are washed away first [6]. Changes in conditions are required for the elution of molecules bound to the stationary phase.

The concentration of the exchangeable counter ions that compete with the molecules for binding can be increased or the pH can be altered. A change in pH affects the charge on the specific molecules and, as a result, changes binding. The molecules then begin to elute based on the changes in their charges caused by the adjustments. Such modifications can then be used to release the protein of interest [7]. Furthermore, the concentration of counter ions can be gradually increased to separate ionised molecules. Gradient elution is the name given to this type of elution. Step elution, on the other hand, can be used to vary the concentration of counterions in a single step [8]. This chromatography type is further divided into cation exchange chromatography and anion-exchange chromatography.

Equilibration is required before starting ion-exchange chromatography. The stationary phase must be equilibrated to certain requirements that vary depending on the experiment. The charged ions in the stationary phase will be attached to their opposite charged exchangeable ions once equilibrated. Ions can be exchanged, such as Cl^- or Na^+. Following that, a buffer to which the desired protein can bind should be chosen [9]. The column must be washed after equilibration. The washing phase will aid in the removal of any impurities that do not bind to the matrix while

the protein of interest remains bound. To aid in the binding of the desired proteins, this sample buffer must have the same pH as the equilibration buffer. Elution is carried out to elute the desired proteins that are bound to the matrix after the sample has been loaded onto the column and the column has been washed with the buffer to elute out all non-desired proteins. Bound proteins are eluted using a linearly increasing salt concentration gradient. As the ionic strength of the buffer increases, the salt ions compete with the desired proteins for binding to charged groups on the medium's surface. As a result, the desired proteins will be eluted from the column.

Proteins with a low net charge will be eluted first as the salt concentration rises, resulting in an increase in ionic strength. Proteins with a high net charge will require a higher ionic strength to be eluted from the column. Ion exchange chromatography can be done in bulk on thin layers of medium like glass or plastic plates coated with a layer of the desired stationary phase or in chromatography columns [10]. Thin layer and column chromatography are similar in that they both operate under the same governing principles there is constant and frequent exchange of molecules as the mobile phase travels along the stationary phase.

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