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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT.7. Ion Exchange chromatography

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Definition & Principles

Ion Exchange chromatography

Ion-exchange chromatography is a type of chromatography that separates analytes based on charge. This technique enables the separation of similar types of molecules that would be difficult to separate by other techniques because the charge carried by the molecule of interest can be readily manipulated by changing buffer pH.

- Ion exchange chromatography is commonly used to separate charged biological molecules such as proteins, peptides, amino acids, or nucleotides mainly because of its high resolving power and capacity.

Principle

This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte and is governed by the principles of ionic chemical interactions that lead to reversible adsorption of the analyte on the stationary phase. The strength of the bonding between the sample and the solid support is governed by the number and types of functional groups on the sample and the resin.

The resins used to prepare stationary phase can be of two types:

i. Cation exchanger or acidic ion exchanger

ii. Anion Exchanger or basic ion exchanger

Cation exchanger:

Cation exchangers possess negatively charged groups and these will attract positively charged cations. These exchangers are also called acidic ion exchangers because their negative charges result from the ionisation of acidic groups. E.g. sodium polystyrene sulfonate, sodium zirconium cyclosilicate

Anion exchanger

Anion exchangers have positively charged groups that will attract negatively charged anions. The term basic ion exchangers is also used to describe these exchangers, as positive charges generally result from the association of protons with basic groups. e.g. Q (quaternary resins) , DEAE (diethylaminoethane).

Materials & Operations

Matrices used include polystyrene, cellulose and agarose. They can be either packed with strong or weak exchanger.

Strong exchanger and weak exchanger

The terms strong and weak refer to the acid/base properties of the column material and not how well it binds an analyte. Ion exchange resins come in two types: strong and weak.

The number of charges on a **strong ion exchanger** remains constant regardless of the buffer pH. Strong exchangers because they are totally ionised at all normal working pH values . These types of resins retain their selectivity and capacity over a wide pH range. Examples of strong ion exchangers are quaternary ammonium (Q), sulfonate (S), and sulfopropyl (SP) resins. Strong-cation exchange resins typically feature sulfonic acid functional groups while Strong anionic-exchange resins contain quaternary amines.

Weak ion exchangers, in contrast, display pH-dependent function and so deliver optimal performance over only a small pH range. When the pH of the buffer no longer matches the acid dissociation constant (pKa) of the resin functional group, these resins suffer significant capacity loss. Weak anion exchangers function poorly above a pH of 9 and weak cation exchangers begin to lose their ionization below pH 6. When working with weak ion exchange resins such as

diethylaminoethyl (DEAE) or carboxymethyl (CM) resins, it is important to work within the supplier-provided working pH range. weak cation-exchange resins have carboxylic acids while weak anion-exchange resins feature secondary or tertiary amines.

Strong ion exchangers are often preferred resins for many applications because their performance is unaffected by pH. However, weak ion exchangers can be powerful separation tools in cases where strong ion exchangers fail because the selectivities of weak and strong ion exchangers often differ.

Choice of exchanger

The choice of the ion exchanger depends upon the stability of the test analytes, their relative molecular mass and the specific requirements of the separation. Following criterions are used for selection of ion exchanger

Generally, if an analyte is most stable below its isoionic point (giving it a net positive charge) a cation exchanger should be used, whereas if it is most stable above its isoionic point (giving it a net negative charge) an anion exchanger should be used. For e.g. for separation of proteins, the choice of exchanger is dependent upon whether the protein is above or below its isoleectric pH.

•In a buffer with a pH greater than the pI of the protein of interest, the protein will carry a net negative charge; therefore, a positively charged **anion exchange** resin is chosen to capture this protein.

•In a buffer with a pH lower than the pI of the protein of interest, the protein will carry a positive net charge; thus a negatively-charged **cation exchange** resin is chosen.

•When an ion exchange chromatography column is loaded with a sample at a particular pH, all proteins that are appropriately charged will bind to the resin. For example, if an anion exchange resin is chosen, all proteins that are negatively charged at the loading buffer pH will bind to the positively charged column resin. A good rule of thumb for choosing a buffer pH is the following:

•**Anion exchanger** — 0.5–1.5 pH units **greater** than the pI of the protein of interest

•**Cation exchanger** — 0.5–1.5 pH units **less than** the pI of the protein of interest

Eluent pH

The pH of the buffer selected as eluent should be at least one pH unit above or below the isoionic point of the analytes. In general, cationic buffers such as Tris, pyridine and alkylamines are used in conjunction with anion exchangers, and anionic buffers such as acetate, barbiturate and phosphate are used with cation exchangers. If, however, gradient elution is to be used, the initial conditions chosen are such that the exchanger binds all the test analytes at the top of the column.

Elution

Gradient elution is far more common than isocratic elution. Continuous or stepwise pH and ionic strength gradients may be employed but continuous gradients tend to give better resolution with less peak tailing

Test your understanding

Cation exchangers have

a. negatively charged groups

b. Attract positively charged cations

c. sodium polystyrene sulfonate, sodium zirconium cyclosilicate

d. All of the above

Anion exchangers have

a. positively charged groups

b. will attract negatively charged anions

c. Q (quaternary resins) , DEAE (diethylaminoethane).

d. All of the above

What is the starting point for selection of a suitable Ion exchange (IEX)matrix for purification of a recombinant protein?

a. Test protein binding to an IEX matrix at a range of pHs and salt concentrations

b. Test protein binding to a selection of anion and cation exchange matrices

c. Pass your sample through a preparative column and elute with a salt gradient

d. Prediction of isoelectric point (pI) from the amino acid sequence

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.