ION-EXCHANGE CHROMATOGRAPHY

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Principle

- This form of chromatography relies on the attraction between oppositely charged stationary phase, known as an ion exchanger, and analyte.
- It is frequently chosen for the separation and purification of proteins, peptides, nucleic acids, polynucleotides and other charged molecules, mainly because of its high resolving power and high capacity.
- There are two types of ion exchanger, namely cation and anion exchangers.
- 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.
- 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.

Materials and applications

- Matrices used include:
  - polystyrene,
  - cellulose and
  - agarose
- Functional ionic groups include sulphonate ($–SO^{–3}$) and quaternary ammonium ($–N^{+}R_3$), both of which are strong exchangers because they are totally ionised at all normal working pH values.
- Carboxylate ($–COO^{-}$) and diethylammonium ($–HN^{+}(CH_2CH_3)_2$), both of which are termed weak exchangers because they are ionised over only a narrow range of pH values.
- Most HPLC ion exchangers are stable up to 60° C and separations are often carried out at this temperature, owing to the fact that the raised temperature decreases the viscosity of the mobile phase and thereby increases the efficiency of the separation.

Table 11.3 **Examples of commonly used ion exchangers**

Type	Functional groups	Functional group name	Matrices
Weakly acidic (cation exchanger)	$-COO^-$	Carboxy	Agarose
	$-CH_2COO^-$	Carboxymethyl	Cellulose
			Dextran
			Polyacrylate
Strongly acidic (cation exchanger)	$-SO_3^-$	Sulpho	Cellulose
	$-CH_2SO_3^-$	Sulphomethyl	Dextran
	$-CH_2CH_2CH_2SO_3^-$	Sulphopropyl	Polystyrene
			Polyacrylate
Weakly basic (anion exchanger)	$-CH_2CH_2N^+H_3$	Aminoethyl	Agarose
	$-CH_2CH_2N^+H(CH_2CH_3)_2$	Diethylaminoethyl	Cellulose
			Dextran
			Polystyrene
			Polyacrylate
Strongly basic (anion exchanger)	$-CH_2N^+(CH_3)_3$	Trimethylaminomethyl	Cellulose
	$-CH_2CH_2N^+(CH_2CH_3)_3$	Triethylaminoethyl	Dextran
	$-CH_2N^+(CH_3)_2(CH_2CH_2OH)$	Dimethyl-2-hydroxyethyl-aminomethyl	Polystyrene

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.
- Many biological analytes, especially proteins, are stable within only a fairly narrow pH range so the exchanger selected must operate within this range.
- 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.
- Weak electrolytes requiring a very low or high pH for ionisation can be separated only on strong exchangers, as they only operate over a wide pH range.
- In contrast, for strong electrolytes, weak exchangers are advantageous for a number of reasons, including a reduced tendency to cause protein denaturation, their inability to bind weakly charged impurities and their enhanced elution characteristics.

Eluent pH and Elution

- 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.
- The precise initial buffer pH and ionic strength should be such as just to allow the binding of the analytes to the exchanger.
- 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.
- Generally with an anion exchanger, the pH gradient decreases and the ionic strength increases, whereas for cation exchangers both the pH and ionic gradients increase during the elution.