Ion Exchange Chromatography

Anion Exchange

Cation Exchange

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Ion Exchange Chromatography

Ion Exchange chromatography (IEC) is a technique based on the difference in the strength of the interaction between a sample ion and an oppositely charged functional group on the support. The sample ion competes for the functional group with a counter ion that has been added to the mobile phase as a salt. Elution is most often accomplished by increasing the salt concentration over time.

Ion exchange chromatography is the most common separation mode for protein purification schemes. Biomolecules generally have charged groups on their surfaces, which change with the pH of the solution.

Anion Exchange Chromatography is performed with either a strong anion-exchange column, containing a quaternary ammonium ion, or with a weak anion-exchanger, having either a tertiary or secondary amine functional group, such as DEAE (diethylaminoethyl). A counter ion, often Cl-, maintains electroneutrality.

Cation Exchange Chromatography is performed with either a strong cation exchanger, containing a bonded sulfonic acid group, such as sulfopropyl (SP), or with a weak cation exchanger, containing a weak acid such as carboxymethyl (CM). The advantage of strong vs. weak ion exchangers is that the first are charged over a wider pH range. Weak ion-exchangers often provide slightly different selectivity from strong exchangers.

Ion chromatography (IC) refers to ion exchange chromatography of inorganic anions and cations using an ion exchange column.

Ion pair chromatography (IPC) represents another means of separating ionic species. In IPC the retention of sample ions is increased by adding to the mobile phase an ionic detergent or salt (ion pair reagent) that can form an ion pair association with an oppositely charged sample ion. The ion pair shields the charge of the sample ion and increases its interaction with the (usually non-polar) stationary phase.

Ion pair chromatography is employed when traditional methods (such as reversed phase LC) do not give sufficient retention, or when the charged sample components interact with the packing material resulting in asymmetrical peaks.

Tosoh Bioscience offers a broad line of high efficiency columns for analysis and isolation of biomolecules by **ion exchange chromatography**. For Anion as well as Cation Exchange Chromatography, the product line contains *methacrylate*-based and *silica*-based columns for the analysis of proteins, peptides, DNA- and RNA- derived oligonucleotides and other nucleic acid fragments.

Polystyrene-based ion exchange columns are available for analyzing low MW sugars, amino acids, nucleic acid bases, and small drug candidates.

Hydrophilic polymer-based, exchange columns packed with non-porous resin particles are available for the separation of proteins, protein aggregates, charge isomers of monoclonal antibodies, PEGylated proteins, DNA fragments, nucleic acids, oligo DNA, and siRNA.

Specialty columns are available for **organic acids** by ion-exclusion (OApak-A and SCX) and **mono- and disaccharides** (Sugar AXI and Sugar AXG).

Common Characteristics of TSK-GEL Anion and Cation Exchangers

The methacrylate base resin, G5000PW (5PW), is a spherical 10µm particle with approximately 1000Å pores. TSK-GEL 5PW is derivatized with diethylaminoethyl (DEAE), sulfopropyl (SP) or carboxymethyl (CM) functionalities to provide a weak anion, a strong cation, and a weak cation exchanger, respectively. The polyamine chemistry employed in TSKgel SuperQ-5PW results in a high capacity strong anion exchanger with a smaller effective pore size than TSKgel DEAE-5PW.

Particles in TSKgel BioAssist Q columns contain very large pores (~4000Å) that are functionalized with polyamine groups to form a network structure. The capacity of BioAssist Q columns is high over a wide molecular weight range. TSK-GEL BioAssist S columns are packed with particles possessing 1300Å pores functionalized with sulfopropyl groups.

Methacrylate is also the backbone of non-porous resin (NPR) columns such as DEAE-NPR, DNA-NPR and SP-NPR, which are packed with 2.5µm particles. High column efficiency coupled with low sample capacity restricts the application of these columns to fast analysis and micro-scale preparative isolation. Due to the absence of large pores, protein recovery is generally very high on NPR columns.

TSK-GEL STAT Series non-porous resin columns contain large particles (5, 7 and 10µm). This relatively large particle size, combined with an innovative bonding chemistry, results in columns that enable fast equilibration and analysis of complex biomolecular samples.

Anion Exchange Columns (type, matrix)

- Strong anion, methacrylic
  - TSKgel BioAssist Q
  - TSKgel SuperQ-5PW
- Strong anion, silica
  - TSKgel QAE-2SW
- Strong anion, polymer
  - TSKgel Q-STAT
- Strong anion, polystyrene
  - TSKgel Sugar AXI
  - TSKgel Sugar AXG
  - TSKgel SAX
- Weak anion, methacrylic
  - TSKgel DEAE-5PW
  - TSKgel DEAE-NPR
  - TSKgel DNA-NPR
- Weak anion, silica
  - TSKgel DEAE-2SW
  - TSKgel DEAE-3SW
- Strong anion, polymer
  - TSKgel DNA-STAT

Cation Exchange Columns (type, matrix)

- Strong cation, methacrylic
  - TSKgel BioAssist S
  - TSKgel SP-5PW
  - TSKgel SP-NPR
- Strong cation, silica
  - TSKgel SP-2SW
- Strong cation, polymer
  - TSKgel SP-STAT
- Strong cation, polystyrene
  - TSKgel SC
- Weak cation, methacrylic
  - TSKgel CM-5PW
  - TSKgel OApak-A
- Weak cation, silica
  - TSKgel CM-2SW
  - TSKgel CM-3SW
- Weak cation, polymer
  - TSKgel CM-STAT