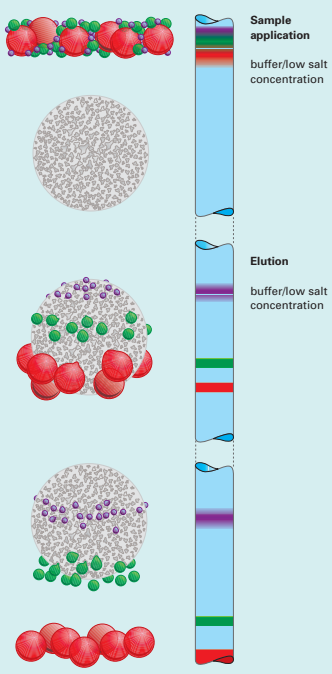
TOSOH BIOSCIENCE

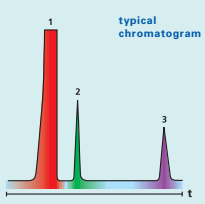
General Principles of Chromatography

Size Exclusion Chromatography

Toyopearl® Resins for SEC

Toyopearl HW 40
Toyopearl HW 50
Toyopearl HW 55
Toyopearl HW 65
Toyopearl HW 75





Size Exclusion Chromatography (SEC)

is the general name for the chromatographic mode also referred to as gel permeation chromatography (GPC) for non-aqueous elution systems or gel filtration chromatography (GFC) for aqueous systems. SEC is a method in which components of a mixture are separated according to their molecular size, based on the flow of the sample through a porous packing. Large biomolecules that cannot penetrate the pores of the packing material elute first from the column. These large biomolecules are said to be excluded from the packing; they flow with the mobile phase in the interparticle space of the packed column. Smaller molecules can partially or completely enter the packing particles. Because these smaller molecules have to flow through the interparticle space, as well as through the pore volume, they will elute from the column after the excluded sample components.

Size exclusion chromatography is a very simple method for separating biomolecules, because it is not necessary to change the composition of the mobile phase during elution. However, the separation capacity of this method is limited. For a baseline separation it is necessary that the molecular weights of the biomolecules differ at least 10 to 20 percent.

Adsorption Chromatography

Toyopearl® Resins for HIC

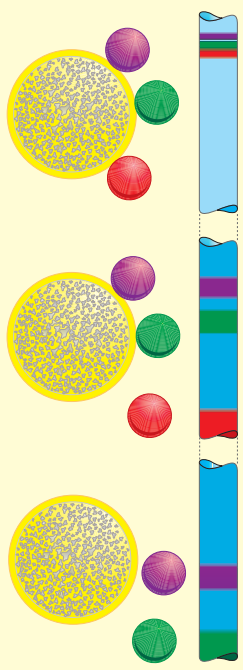
Toyopearl Hexyl-650
Toyopearl Butyl-650
Toyopearl Phenyl-650
Toyopearl Ether-650

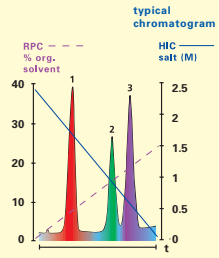
TSK-GEL® 5-PW Bulk Resins for HIC

TSK-GEL® Phenyl-5PW
TSK-GEL® Ether-5PW

Amberchrom Resins for RPC

Amberchrom CG-71
Amberchrom CG-161
Amberchrom CG-300
Amberchrom CG-1000





Reversed Phase Chromatography (RPC)

In this technique, one uses hydrophobic interactions between the biological molecule and the ligand on the chromatographic support to obtain separation. RPC stationary phases differ from HIC packings in that they contain a higher density of hydrophobic ligand. Mobile phase additives, such as trifluoroacetic acid, increase protein hydrophobicity by forming ion pairs that strongly adsorb to the stationary phase. Protein binding is so strong that a gradient of increasing concentration of organic solvent such as acetonitrile or 2-propanol, is required for elution.

Because of the high ligand density of the RPC medium and the drastic elution conditions required, the enzymatic and immunologic activity of proteins is generally not maintained after RPC separation. RPC mainly is used for separating peptides and is not commonly used for proteins.

The advantage of RPC is that this technique is perhaps the most efficient of all biopolymer separation techniques. Although the range of elution conditions is not as broad as for Ion Exchange Chromatography, RPC has a high peak capacity. Reversed Phase Chromatography is particularly effective for separating peptides, nucleotides, and restriction fragments.

Hydrophobic Interaction Chromatography (HIC)

This is a chromatographic technique in which the sample interacts, at high mobile phase salt concentration, with a weakly hydrophobic stationary phase. Subsequently it is eluted from the stationary phase by decreasing the salt concentration. Almost all biological molecules have in their structure hydrophobic portions that, under physiological conditions, are shielded by hydrophilic or ionic groups. By increasing the salt concentration of the solvent, these hydrophobic portions of the molecule become more exposed and can interact with hydrophobic ligands on the HIC packing. Hydrophobic Interaction Chromatography is particularly attractive for protein purification when the sample is already in high salt concentration. In contrast to the conditions used in Reversed Phase Chromatography, in which the sample is eluted with buffer and organic solvent, the biological activity of the eluted molecules in HIC is often maintained. HIC is being used increasingly as a substitute for ammonium sulfate precipitation because of higher throughput and greater recovery of enzyme activity.

The strength of the hydrophobic interaction is influenced strongly by the nature of the salt components in the mobile phase. Starting salt concentration of 1.0M to 2.5M ammonium sulfate in the buffer is commonly used to adsorb the sample to the packing. The salt concentration needed depends on the protein hydrophobicity and solubility, the resins hydrophobicity and the resolution, capacity and mass recovery required. Additives commonly used are methanol, ethanol, isopropanol, acetone, SDS, urea and guanidinium hydrochloride.

Ion Exchange Chromatography

Toyopearl® Resins for IEC

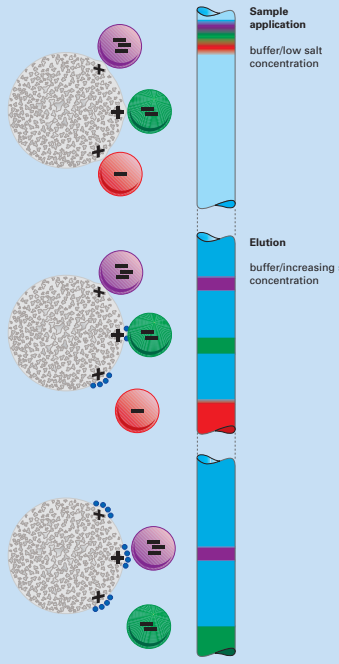
Anion Exchange Resins
Toyopearl DEAE-650
Toyopearl Super Q-650
Toyopearl QAE-550

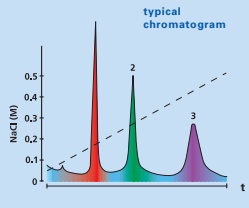
Cation Exchange Resins
Toyopearl CM-650
Toyopearl SP-650
Toyopearl SP-550
Toyopearl MegaCap

TSK-GEL® 5PW Bulk Resins for IEC

Anion Exchange Resins
TSK-GEL® SuperQ-5PW
TSK-GEL® DEAE-5PW

Cation Exchange Resins
TSK-GEL® SP-5PW





Ion Exchange Chromotography (IEC)

Biomolecules generally have charged groups on their surfaces, which change with the pH of the solution. This is the basis for Ion Exchange Chromatography (IEC), in which the molecule reversibly binds to an oppositely charged group on the ion exchange functional groups of the packing material.

Molecules with a higher charge density bind more strongly to the packing. The bound sample may be selectively removed from the stationary phase by changing the pH or salt concentration of the mobile phase. The higher the charge of the molecule and the stronger the binding to the stationary phase, the greater the change in the salt concentration required. It is possible in Ion Exchange Chromatography to load samples in the column in a very dilute solution and elute rapidly with a step gradient, thus producing a concentrated sample.

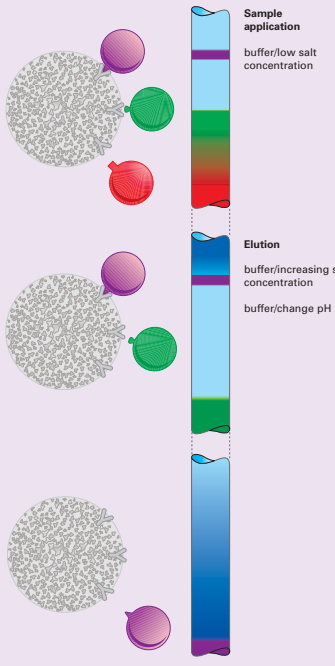
Ion exchange chromatography is a very powerful separation tool because it is highly selective and specific and has a high binding capacity. Although the technique is used for a wide variety of samples, it is particularly effective for proteins because they are amphoteric. It is estimated that 70 percent of all purification protocols for proteins involve an ion exchange step and that 70 percent of those ion exchange steps are based on anion exchangers, since most proteins have acidic ionization points.

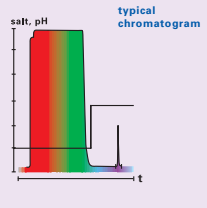
Affinity Chromatography

Toyopearl® Resins for AFC

Toyopearl® Reactive Resins
Toyopearl AF-Amino-650
Toyopearl AF-Carboxy-650
Toyopearl AF-Epoxy-650
Toyopearl AF-Formyl-650
Toyopearl AF-Tresyl-650

Toyopearl® Resins Ready to Use with Group Specific Ligands
Toyopearl AF-Blue-HC650
Toyopearl AF-Chelate-650
Toyopearl AF-Heparin-650
Toyopearl AF-Red-650





Affinity Chromatography (AFC)

is based on the specific adsorption of a molecule to a ligand or macromolecule. Almost all biological molecules can be purified on the basis of specific interaction between their chemical or biological structure and a suitable affinity ligand. Typical molecular pairs are antigens and antibodies, enzymes and coenzymes, and sugars with lectins.

Affinity Chromatography media have ligands that are bonded via a spacer arm to the packing material. A specific biological molecule is then reversibly adsorbed to the ligand. The adsorbed molecule is eluted either by competitive displacement or by a change in the conformation of the molecule through a change in pH or ionic strength. Because of the intrinsic high selectivity of AFC, it is, in contrast to other chromatographic methods, most suitable for specific separation problems and provides high purification yields. Another advantage of AFC is the simplicity of the elution technique, which involves a single-step gradient.

Bioseparation

Means purification of molecules retaining the biological function.

Depending on the characteristics of the target biomolecules, different chromatographic methods can be considered for purification. In most cases, it is necessary to use two or more chromatographic methods to purify a molecule to the desired purity.

When choosing the chromatographic separation mode, one must consider the sample solvent as well as the characteristics of the biomolecule. Since most biological molecules are stable only under certain conditions, they require chromatographic materials that don't denature the biomolecule during separation or purification steps.

Hence, the separation media or packing should be biocompatible, the material should allow for a wide range of chromatographic conditions, and should allow the separation to take place in a relatively short time.

The analysis, isolation, and purification of biomolecules can be accomplished by a number of chromatographic modes. Each mode is based on specific physical, chemical, or biological interactions between the sample biomolecules and the packing material.

The various modes of chromatography involve separations that are based on size, charge, hydrophobicity, function or specific content of the biomolecules. The general principles of the most commonly used modes are outlined here.

Explanations to these Products

TOSOH BIOSCIENCE offers a comprehensive line of media and pre-packed columns for all common modes of liquid chromatography including ion-exchange, hydrophobic-interaction, reversed-phase, size-exclusion and affinity.

TSK-GEL® is available as bulk polymeric resin or in silica or polymeric-based prepacked columns.

Toyopearl® chromatography resins are based on a semi-rigid, hydrophilic, macroporous backbone and are stable over the pH 2-13 range.

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