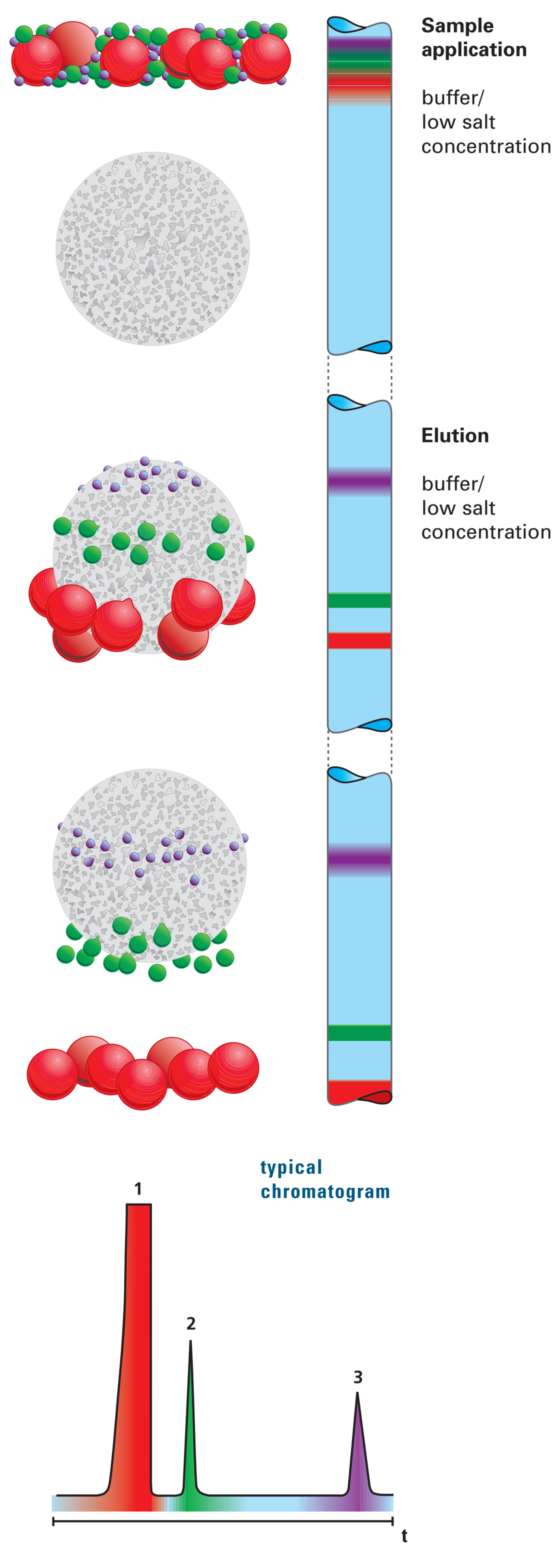
General Principles of Liquid Chromatography

Size Exclusion Chromatography

TSK-GEL® Columns for SEC

TSKgel SW-Series
TSKgel SW_{XL}-Series
TSKgel SuperSW-Series
TSKgel PW-Series
TSKgel PW_{XL}-Series
TSKgel Alpha-Series
TSKgel Super AW-Series
TSKgel H-Series



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 (hydrodynamic volume), based on the flow of the sample through a porous packing. Large biomolecules that cannot penetrate the pores of the packing material elute first. 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 stationary phase. Because these smaller molecules have to flow through both, the interparticle space, as well as through the pore volume, they will elute from the column after the excluded sample components.

SEC 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 %.

Ion Exchange Chromatography

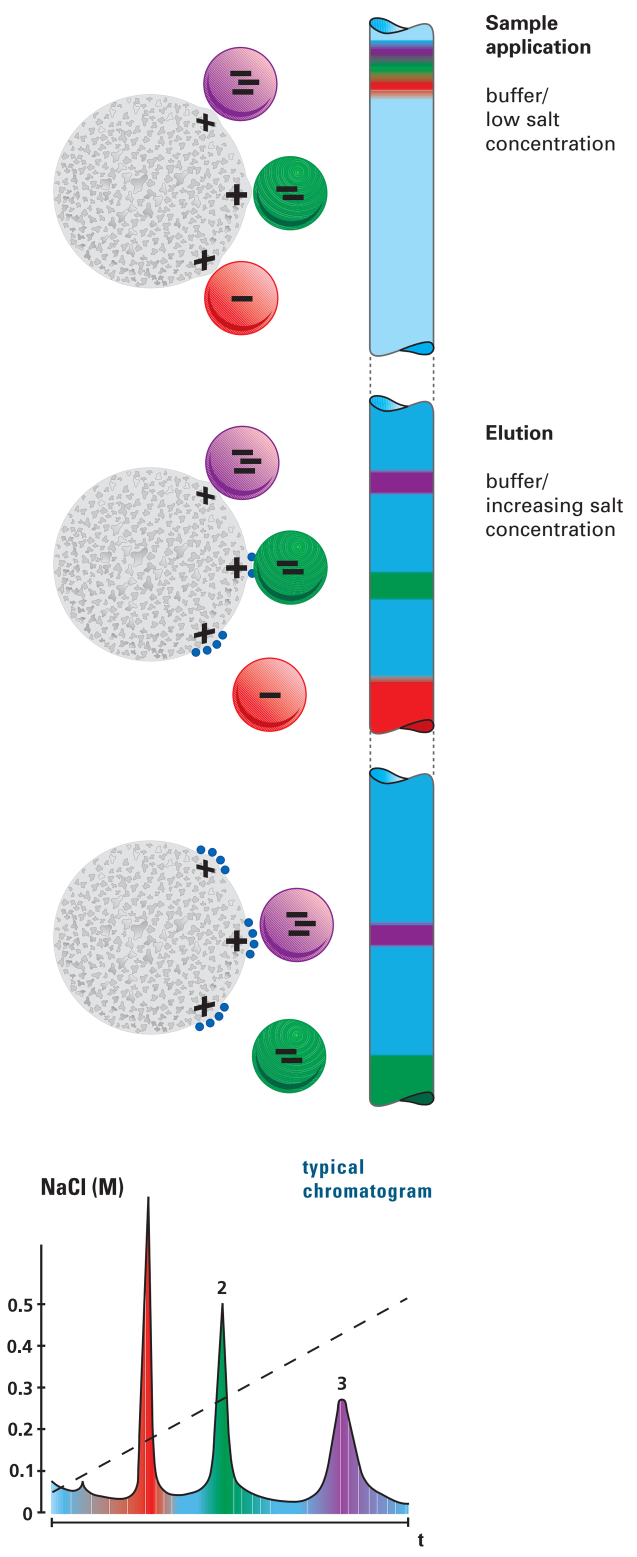
TSK-GEL® Columns for IEC

Anionic Exchange Columns

TSKgel Q-STAT
TSKgel DNA-STAT
TSKgel DEAE-3SW
TSKgel DEAE-2SW
TSKgel DEAE-5PW
TSKgel DEAE-NPR
TSKgel BioAssist Q
TSKgel SAX

Cationic Exchange Columns

TSKgel SP-STAT
TSKgel CM-STAT
TSKgel CM-2SW
TSKgel CM-3SW
TSKgel CM-5PW
TSKgel SP-2SW
TSKgel SP-5PW
TSKgel SP-NPR
TSKgel BioAssist S
TSKgel SCX



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 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. In IEC it is possible to load samples in a very dilute solution and elute rapidly with a step gradient, thus producing a concentrated sample. IEC is a very powerful separation tool because it is highly selective and specific and has a high capacity. Although the technique is used for a variety of samples, it is particularly effective for proteins because they are amphoteric. It is estimated that 70 % of all separation methods for proteins involve IEC.

Affinity Chromatography

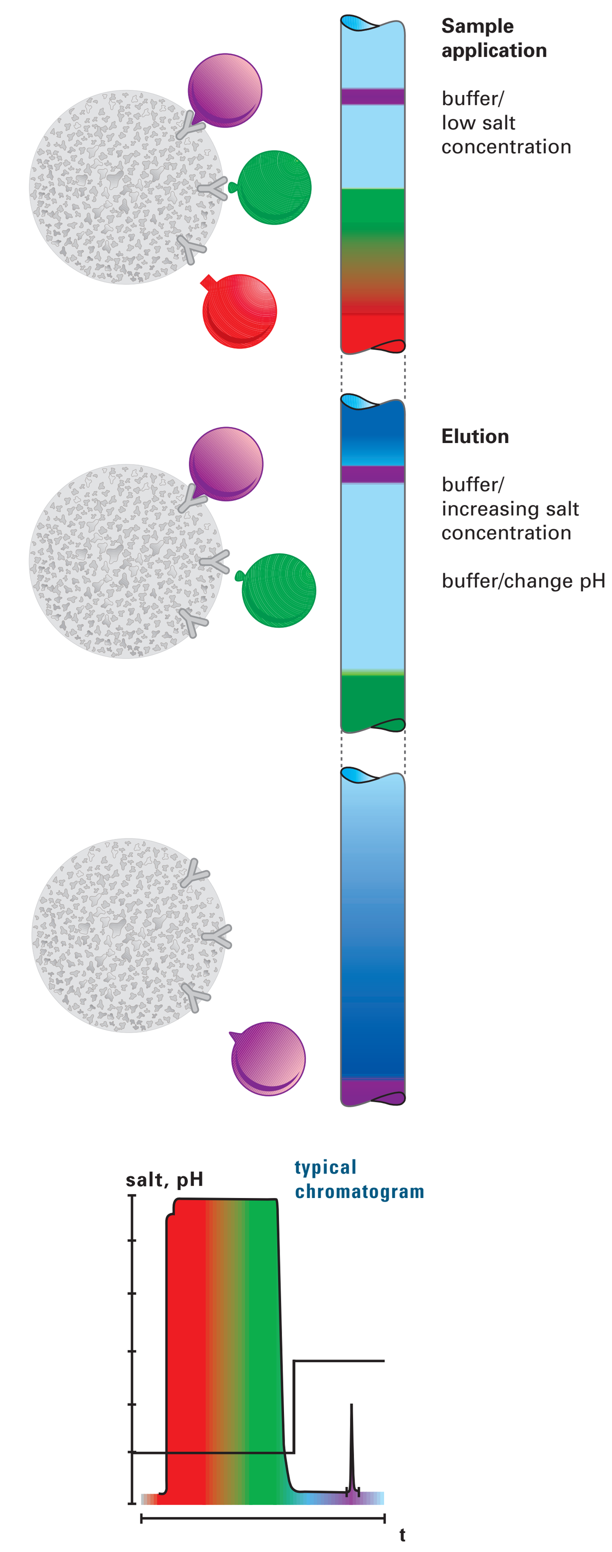
TSK-GEL® Columns for Affinity

Group Specific Columns

TSKgel ABA-5PW
TSKgel Boronate-5PW
TSKgel Chelate-5PW

Activated Columns

TSKgel Tresyl-5PW



Affinity Chromatography (AFC)

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.

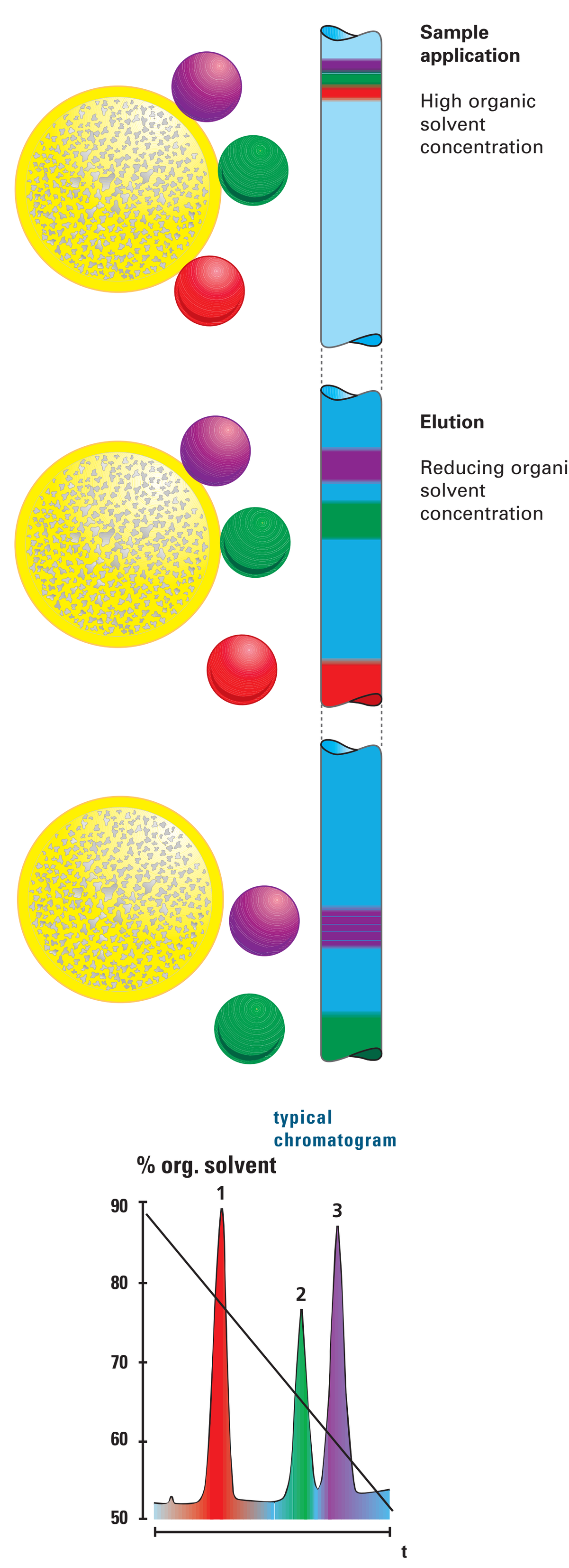
Affinity Chromatography media have ligands that are bonded via a spacer arm to the packing material. A specific biological molecule is then reversibly adsorbed to the ligand. The adsorbed molecule is eluted either by competitive displacement or by a change in the conformation of the molecule through a change in pH or ionic strength.

Because of the intrinsic high selectivity of Affinity Chromatography, 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.

Hydrophilic Interaction Chromatography

TSK-GEL® Columns for HILIC

TSKgel Amide-80
TSKgel NH_2-100



Hydrophilic Interaction Chromatography (HILIC)

HILIC is used for the separation of polar and hydrophilic compounds. HILIC stationary phases are polar, similar to normal phase chromatography (NPC), but mobile phases are similar to reversed phase chromatography (RPC). Typical HILIC phases are silica or polymer particles carrying polar functional groups, e.g. hydroxyl, carbamoyl, amino or zwitterionic groups. Typical mobile phases are aqueous buffers with organic modifyers - primarily acetonitrile - applied in isocratic or gradient mode. In HILIC water has the highest elution power. Therefore gradients start with high percentage of acetonitrile.

It is commonly believed that in HILIC the aqueous content of the mobile phase creates a water rich layer on the surface of the stationary phase. This allows for partitioning of solutes between the more organic mobile phase and the aqueous layer. Hydrogen bonding and dipole-dipole interactions are supposed to be the dominating retention mechanisms. The number of polar groups, as well as the conformation and solubility of the sample in the mobile phase determine the elution order. Compared to RPC the elution order in HILIC mode is inversed for most compounds.

HILIC is ideally suited for mass spectrometric analysis of water soluble polar compounds, because the high organic content in the mobile phase increases MS detection sensitivity.

Reversed Phase Chromatography

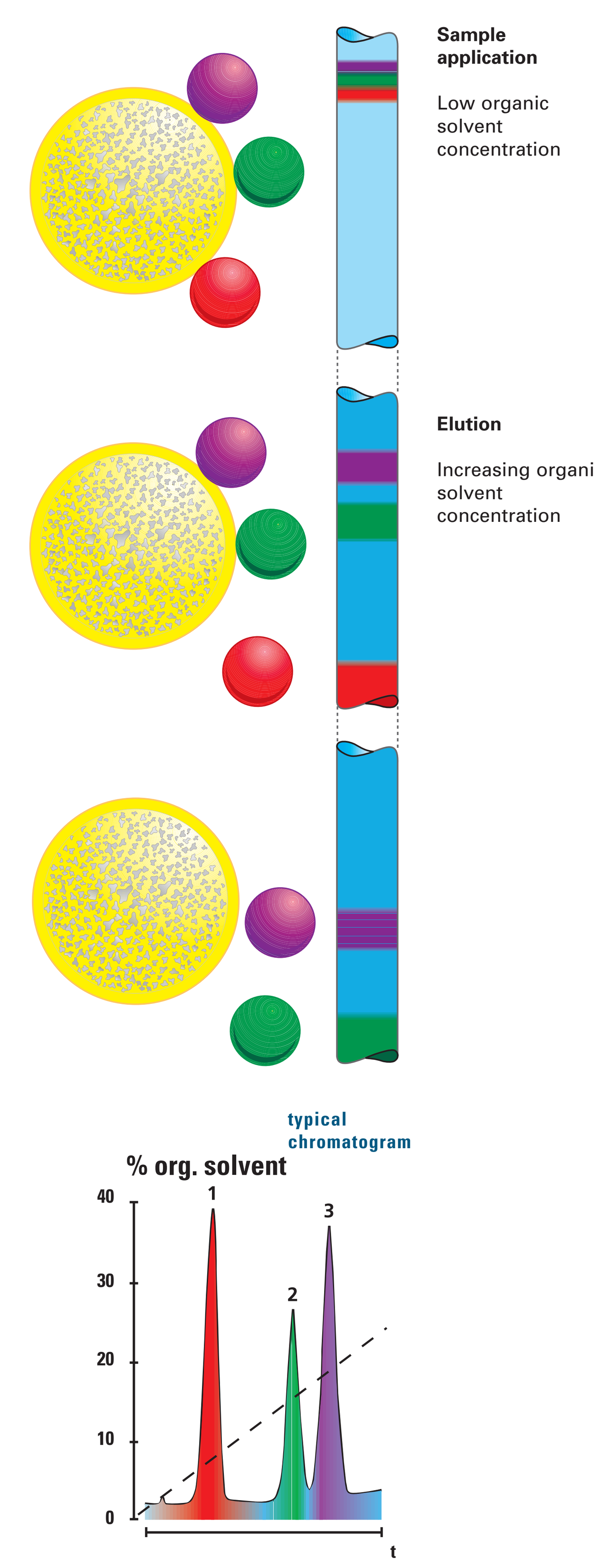
TSK-GEL® Columns for RPC

Silica based Columns

TSKgel Super Series
TSKgel ODS-140HTP
TSKgel ODS-120A/T
TSKgel ODS-100V/Z
TSKgel ODS-$80T_S/T_M$
TSKgel Octyl
TSKgel CN
TSKgel Oligo DNA
TSKgel TMS

Polymer based Columns

TSKgel Octadecyl
TSKgel Phenyl-5PW-RP



Reversed Phase Chromatography (RPC)

In this technique, one uses hydrophobic interactions between the sample and the ligand on the chromatographic support to obtain separation. For proteins mobile phase additives, such as trifluoroacetic acid, increase hydrophobicity by forming ion pairs that strongly adsorb to the stationary phase. Adsorption is so strong that a gradient of increasing concentration of organic solvent such as acetonitrile or 2-propanol, is required for elution.

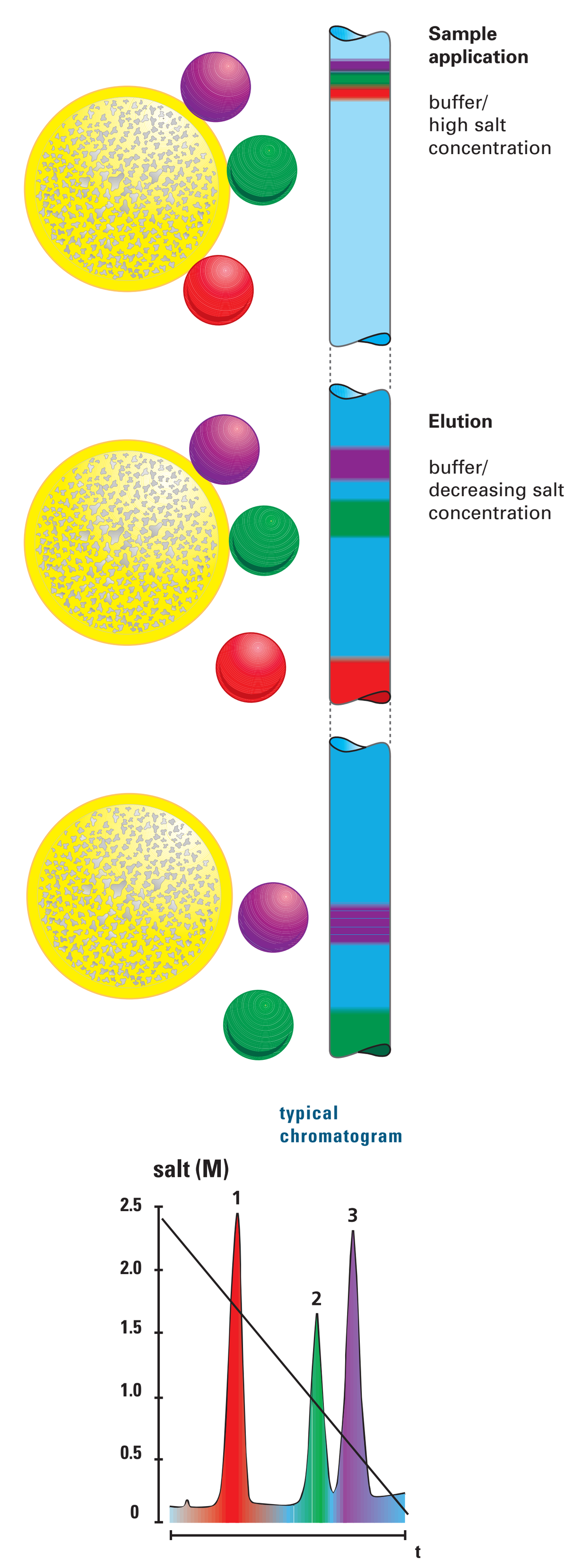
Because of the high ligand density of RPC media and the drastic elution conditions required, the enzymatic and immunologic activity of proteins is generally not maintained after RPC separation. RPC mainly is used for separating small molecules and peptides and is not commonly used for proteins.

The advantage of RPC is that this technique is perhaps the most efficient of all HPLC separation modes. RPC has a high peak capacity and is particularly effective for separating small molecules, peptides, nucleotides, and restriction fragments.

Hydrophobic Interaction Chromatography

TSK-GEL® Columns for HIC

TSKgel Phenyl-5PW
TSKgel Ether-5PW
TSKgel Butyl-NPR



Hydrophobic Interaction Chromatography (HIC)

This is a chromatographic technique in which the sample interacts, at high mobile phase salt concentration, with a 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 patches that, under physiological conditions, are shielded by hydrophilic or ionic groups. By increasing the salt concentration of the solvent, these hydrophobic patches of the molecule become more exposed and can interact with hydrophobic ligands on the HIC packing. HIC is particularly attractive for protein purification when the sample is solved in high salt concentration.

In contrast to the conditions used in RPC, the biological activity of the eluted molecules is often maintained in HIC. It is being used increasingly as a substitute for ammonium sulfate precipitation because of higher throughput and greater recovery of enzymatic 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 1.0 M to 2.5 M 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.

TOSOH BIOSCIENCE´S BIOSEPARATION COLUMNS

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 inter-actions between the sample biomolecules and the packing material.

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

TOSOH BIOSCIENCE offers a comprehensive line of media and pre-packed columns for all common modes of liquid chromatography including ion-exchange, hydrophobic- and hydrophilic-interaction, reversed-phase, size-exclusion and affinity. TSK-GEL® is available as bulk polymeric resin or in silica or polymeric-based pre-packed columns.

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