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# GENERAL PRINCIPLES OF LIQUID CHROMATOGRAPHY

### Size Exclusion Chromatography

#### **TSKgel<sup>®</sup> Columns for SEC**

TSKgel UP-SW Series

TSKgel Super mAb TSKgel SW-Series TSKgel SWxL-Series TSKgel SuperSW-Series TSKgel UltraSW TSKgel PW-Series TSKgel PWxL-Series TSKgel SuperMultiporePW TSKgel SuperOligoPW TSKgel Alpha-Series TSKgel SuperAW-Series TSKgel H-Series TSKgel HxL-Series TSKgel Super H-Series TSKgel Super HZ-Series TSKgel SuperMultipore TSKgel High/Ultra-High Temperature GPC

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

### Ion Exchange Chromatography

#### **TSKgel® Columns for IEC**

➤ Anionic Exchange Columns TSKgel Q-STAT TSKgel DNA-STAT TSKgel DNA-NPR TSKgel DEAE-3SW

TSKgel DEAE-2SW TSKgel DEAE-5PW TSKgel DEAE-NPR TSKgel BioAssist Q TSKgel SAX TSKgel Sugar AXG/AXI

TSKgel SuperQ-5PW

**➤** 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 Chromatography (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.

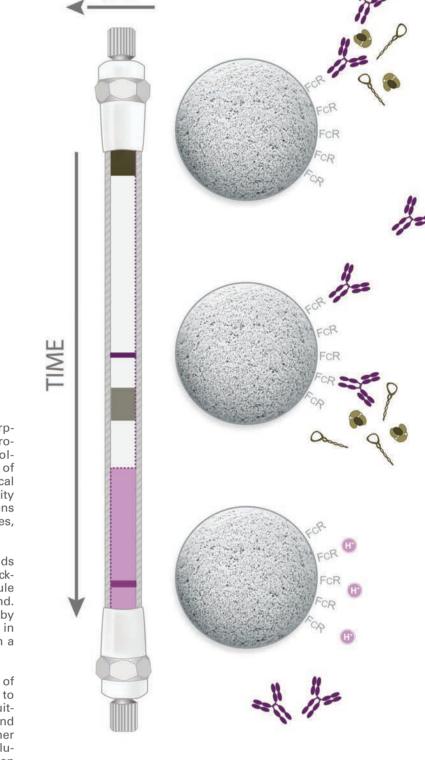
Molecules with a higher charge density bind more strongly to the packing. The bound sample may be selectively removed from the stationary phase by changing the pH or salt concentration of the mobile phase. The higher the charge of the molecule and the stronger the binding to the stationary phase, the greater is the change in the salt concentration required. In IEC it is possible to load samples in a very dilute solution and to 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

#### TSKgel® Columns for AFC

- **→** Antibody Affinity TSKgel FcR-IIIA-NPR TSKgel Protein A-5PW
- **→** Group Specific Columns 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

Antibody Affinity columns are used for specific analyses in antibody therapeutics development, such as fast screening of cell lines for antibody titer or activity.

### **Hydrophilic Interaction** Chromatography

### TSKgel® Columns for HILIC

TSKgel Amide-80 TSKgel NH<sub>2</sub>-100

**Hydrophilic Interaction** 

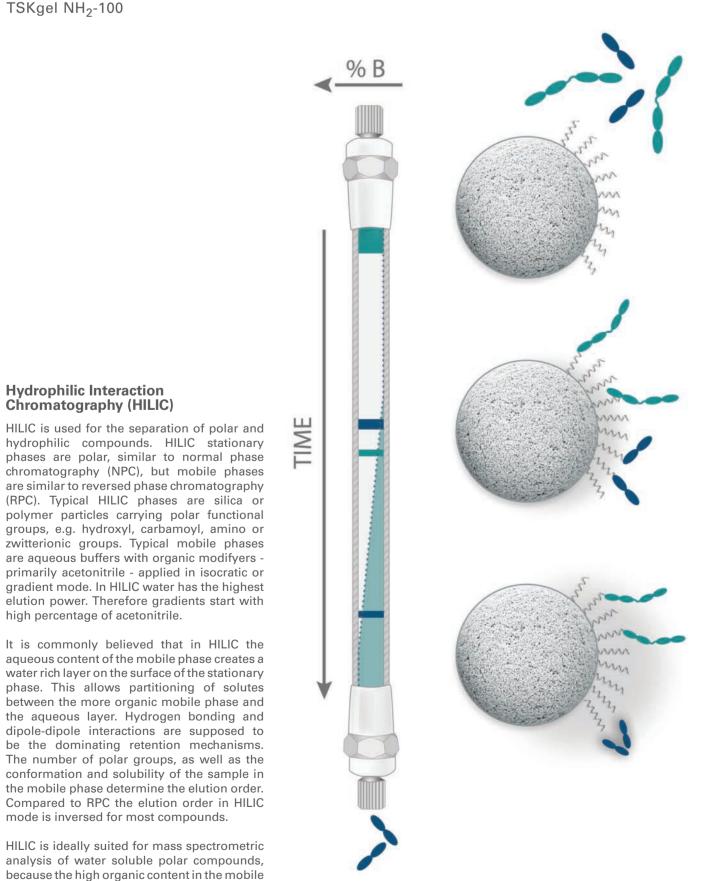
**Chromatography (HILIC)** 

high percentage of acetonitrile.

mode is inversed for most compounds.

phase increases MS detection sensitivity.

at least 10 to 20 %.



### **Reversed Phase** Chromatography

### **TSKgel**<sup>®</sup> Columns for RPC

**➤** Silica based Columns TSKgel Super Series TSKgel ODS-140HTP TSKgel ODS-120A/T TSKgel ODS-100V/Z TSKgel ODS-80T<sub>S</sub>/T<sub>M</sub> TSKgel Octyl-80T<sub>S</sub> TSKgel CN-80T<sub>S</sub> TSKgel Oligo DNA TSKgel TMS-250 TSKgel Protein C<sub>4</sub>-300

**➤ Polymer based Columns** TSKgel Octadecyl TSKgel Phenyl-5PW-RP

### Reversed Phase Chromatography

In this technique, one uses hydrophobic interactions between the sample and the ligand on the chromatographic support to obtain separation. For proteins, mobil 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

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 is mainly 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 fragments.

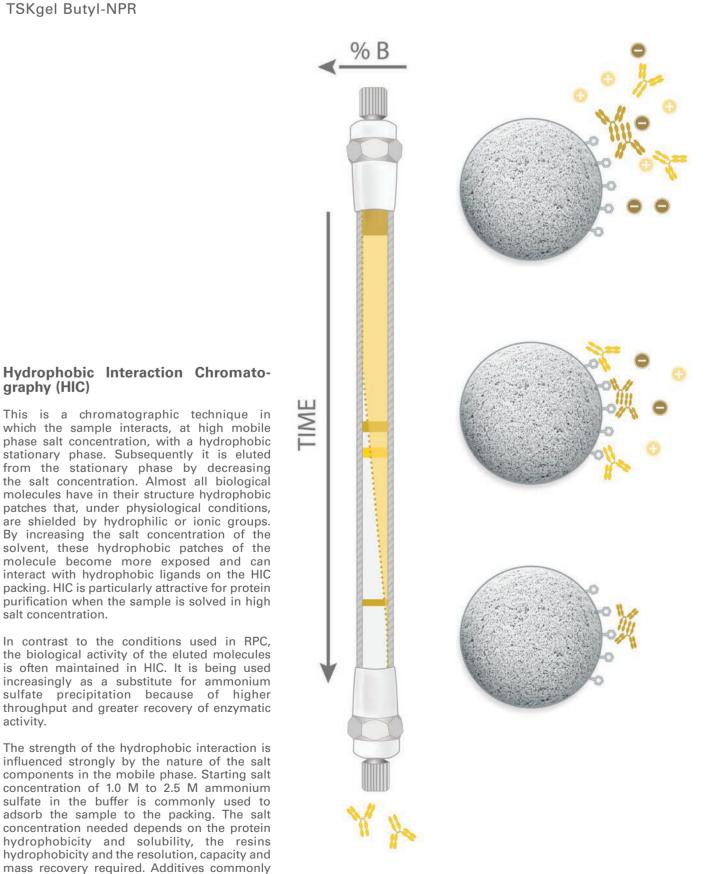
# **Hydrophobic Interaction** Chromatography

### **TSKgel® Columns for HIC**

used are methanol, ethanol, isopropanol, acetone, SDS, urea and guanidinium hydro-

chloride.

TSKgel Phenyl-5PW TSKgel Ether-5PW



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 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 TOYOPEARL and TSKgel media and pre-packed TSKgel columns for all common modes of liquid chromatographyincluding ion-exchange, hydrophobic and hydrophilic interaction, reversed phase, size exclusion and affinity.

## **TOSOH BIOSCIENCE**