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PURIFICATION OF BIOMOLECULES LIQUID CHROMATOGRAPHY MODES

Biomolecules are purified by a succession of chromatography steps. Each step uses a different mode of separation, based on specific interactions between the biomolecules and the packing material.

The various modes are based on specific physical, chemical, or biological features of the target or sample, like size, charge, hydrophobicity, function or specific content of the molecule.

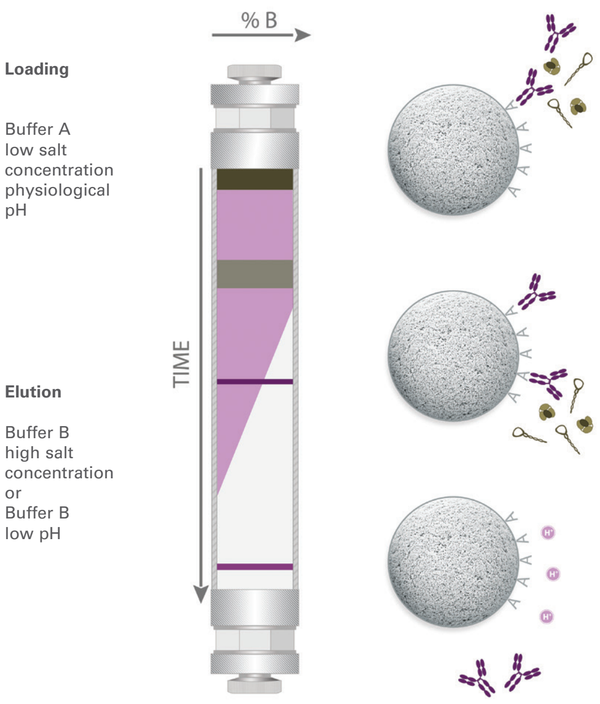
TOSOH BIOSCIENCE offers a comprehensive line of media and prepacked columns for all modes of liquid chromatography - affinity, ion-exchange, mixed-mode, hydrophobic, hydroxyapatite, size exclusion.

AFC Affinity Chromatography

AFC is based on the specific adsorption of a molecule to a ligand or macromolecule.

Affinity Chromatography media have ligands that are bonded to the packing material. A specific biological molecule is then reversibly adsorbed to the ligand. The adsorbed molecule is eluted through a change in pH or ionic strength.

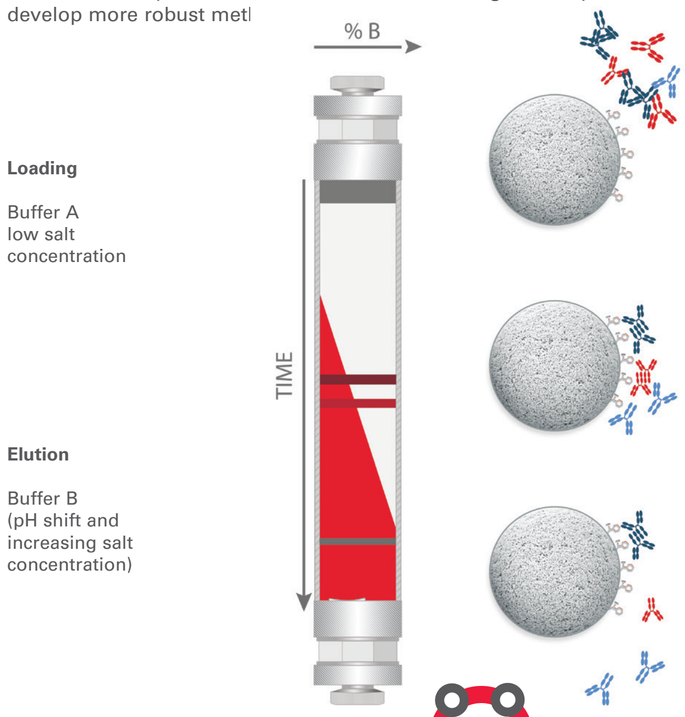
Two AFC resins are predominantly used for bioseparation:
- Protein A chromatography has become a widely used platform in monoclonal antibody (mAb) purification.
- Protein L binds a wider range of antibody classes than Protein A. Typical targets are antigen binding fragments (Fabs), single-chain variable fragments (scFvs), and domain antibodies (dAbs).

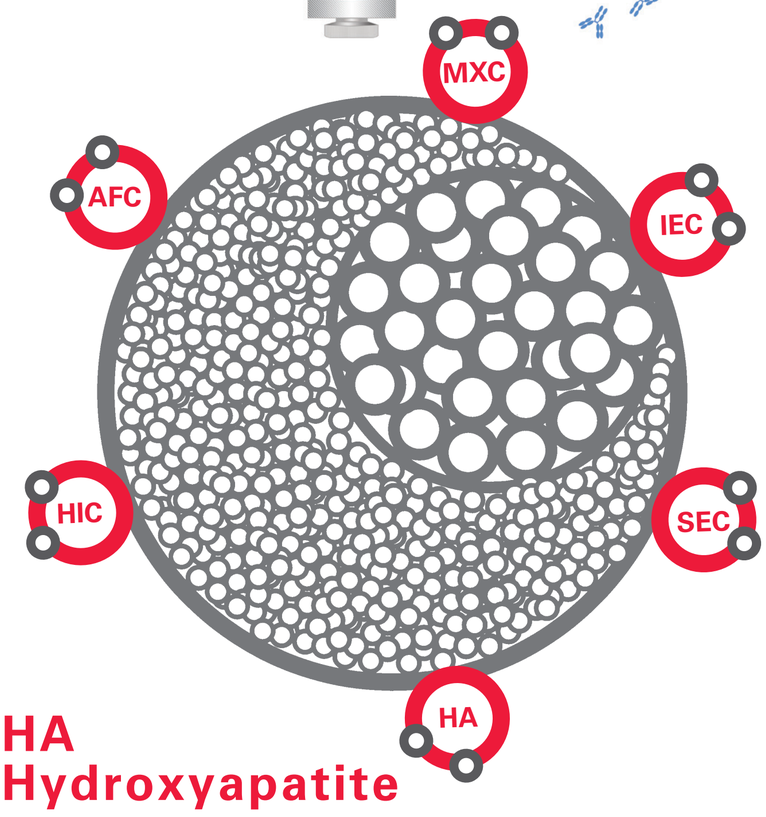


MXC Mixed Mode Chromatography

Mixed-Mode Chromatography resins have both ionic and hydrophobic groups in their composition. These unique ligands allow the separation of acidic, basic, and neutral proteins by one resin.

Method development requires more attention, as both ionic strength and pH have a non-linear impact on the binding and elution. Use of high-throughput method development tools combined with Design of Experiment help develop more robust meth



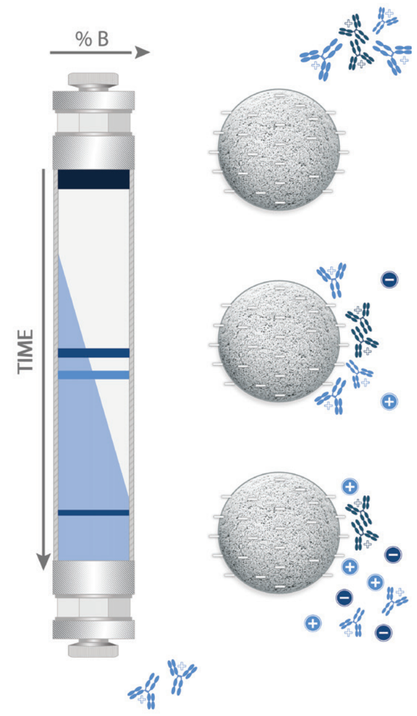


IEC Ion Exchange Chromatography

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

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.

IEC is a very powerful separation tool because it is highly selective and specific and has a high capacity. Modern resins offer salt-tolerance capabilities, allowing to remain at physiological conditions, avoiding destabilizion of the target molecules.

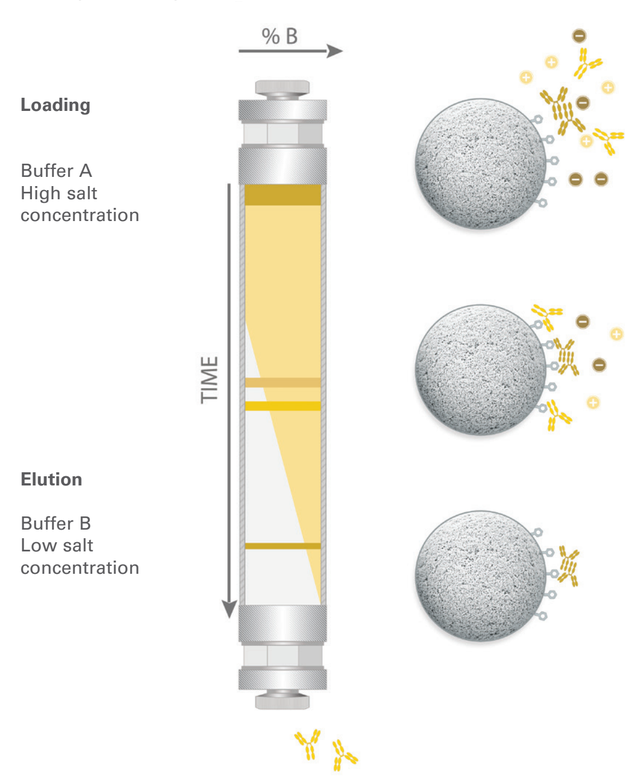


HIC Hydrophobic Interaction Chromatography

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. The strength of the hydrophobic interaction is influenced strongly by the kosmotrope or chaotrope nature of the salt components in the mobile phase.

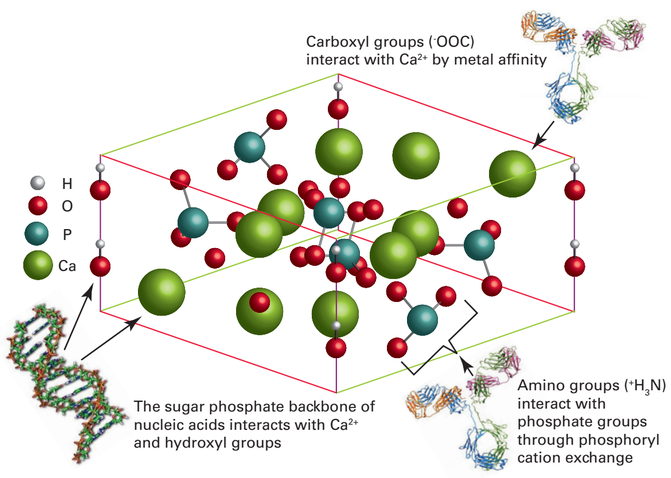
The biological activity of the eluted molecules is maintained.

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.



HA Hydroxyapatite

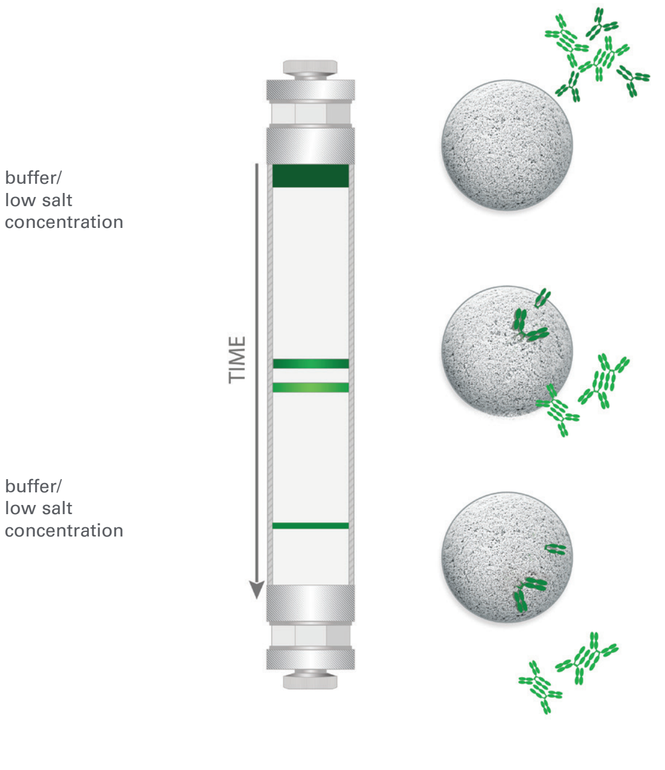
Chromatographic separations using hydroxyapatite involve non-specific interactions between molecules through weak anion exchange or calcium metal affinity, cation exchange with phosphate sites and hydrogen bonding with hydroxyl groups. Key applications for use of Ca^{++}Pure-HA include the purification of monoclonal and polyclonal antibodies, including IgG, IgA, IgM, antibody fragments, fusion- and phosphoproteins, and the separation of single-stranded and double-stranded DNA.



SEC Size Exclusion Chromatography

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. Smaller molecules can partially or completely enter the stationary enter the stationary phase and will therefore elute later.

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 100 %.



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