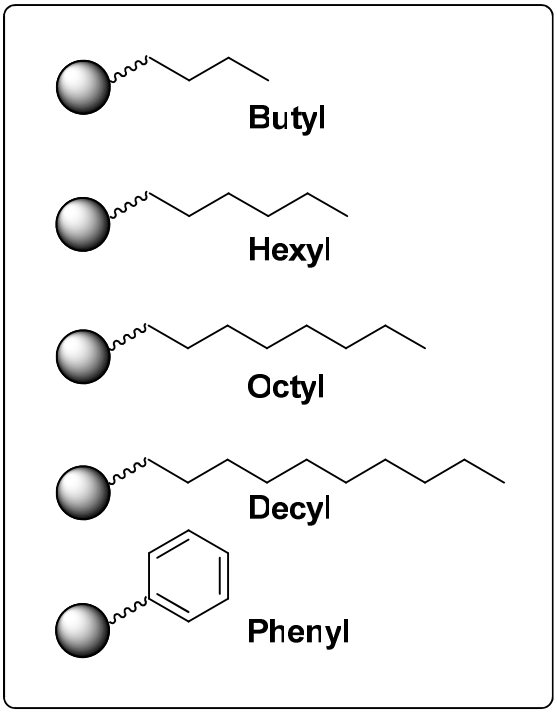
Hydrophobic Interaction Chromatography Media

PHENYL AGAROSE A6XL (Product Code 0405)
BUTYL AGAROSE A6XL (Product Code 0415)
HEXYL AGAROSE A6XL (Product Code 0425)
OCTYL AGAROSE A6XL (Product Code 0435)
DECYL AGAROSE A6XL (Product Code 0445)



Introduction

Hydrophobic Interaction Chromatography (HIC) is used to separate proteins on the basis of relative hydrophobicity. Interactions between hydrophobic groups in water are promoted by the presence of water structuring salts, and at high ionic strengths, hydrophobic residues on the surface of a protein associate strongly with other hydrophobic species (the ‘’salting-out’’ effect). At very high ionic strengths protein precipitation occurs. However, at intermediate ionic strengths, proteins can be adsorbed from solution onto hydrophobic surfaces. This adsorption is reversible, and elution is achieved by simply lowering the ionic strength. Consequently, HIC is particularly useful for the purification from high ionic strength biological extracts since binding is performed in the presence of salt and elution in the absence of salt. The technique may be applied to the purification of most soluble proteins.

The strength of adsorption is determined by a number of factors including the inherent hydrophobicity of the protein, the hydrophobicity of the adsorbent, temperature, and the concentration and type of electrolyte used to promote salting-out. Water structuring salts such as ammonium sulphate are normally used for promoting protein adsorption. Both anions ans cations can be sorted according to those that most favour the interaction, the ‘’Hofmeister’’or ‘’lytropic’’ series.

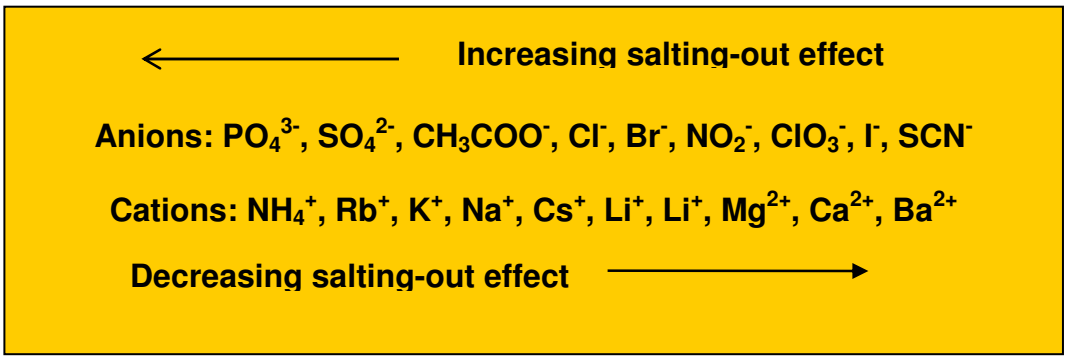


Figure 1 The Hofmeister Series

Since proteins have very different hydrophobic properties, and there is a limit to the amount of salt that can be added, it follows that a series of adsorbents with increasing hydrophobicity are required. This is achieved by increasing the size of the immobilized alkyl/aryl group. Relatively hydrophobic proteins require mildly hydrophobic ligands such as butyl groups (otherwise elution problems are encountered), whereas very hydrophilic proteins require relatively hydrophobic ligands such as decyl groups (otherwise excessive salt concentrations may be required for binding).

HIC offers a high degree of purification comparable to those of ion-exchange chromatography and is often used in combination with other chromatographic separation methods. The application of HIC includes purification of cytosolic proteins, antibodies, membrane proteins and recombinant proteins. For elution of very hydrophobic proteins, the use of detergents is required. It is suitable for removal of detergents, separation of nucleic acids and removal of endotoxins from protein solutions.
ProMetic BioSciences offers a comprehensive range of HIC media comprising five ligands of increasing hydrophobicity immobilized onto cross-linked agarose beads. Determination of the most suitable adsorbent for a given separation is facilitated by use of the PIKSI^a-H Kit which is specifically designed for rapid screening of the HIC Chromatography media. Larger packs of individual adsorbents are available for method development and for process scale applications.

Properties of HIC Adsorbents

Highest quality cross-linked agarose beads with very low non-specific binding properties are used to produce the range of the HIC adsorbents. This range comprises butyl, hexyl, octyl, decyl and phenyl groups immobilized on 6% cross-linked agarose gels by a neutral and highly stable epoxide linkage to provide high protein binding capacities. All HIC adsorbents are produced to stringent quality control standards and protein binding capacities are accurately defined to ensure batch-to-batch consistency and reproducible separations. The adsorbents are compatible with most biological buffers and commonly encountered buffer additives including chaotropes, detergents, chelating agents, metal ions and thiols.

Support matrix	**6% cross-linked agarose**
Bead size	**45-165µm**
Exclusion limits:	**6 x 10^6 Daltons**
Ligand concentration*	**Phenyl Agarose 6XL: 50-60 µmol.g^{-1} moist gel**
Temperature Range	**4-120ºC**
pH range	**continuous:3.0-13.0**
periodic:	**2.0-14.0**
Recommended pressure	**7 psi. 0.5 bar**
Chemical stability:	**Compatible with detergents,chaotropes, metal ions thiols, water-miscible organic solvents**
Regeneration: Sanitization	**1 M NaOH; 8 M urea Autoclave 30 mins. at 12ûC, pH 7.0**
Sterilization: Recommended	**Soak in 1 M NaOH, Ethanol/0.1 M NaCl (25:75 v/v) preservative:**
Storage:	**2 - 30ºC in preservative**

* Ligand concentrations are only provided for Phenyl Agarose 6XL adsorbent

Protein binding capacities of HIC media in the presence of high salt.

	mg BSAbound/ g moist gel
Butyl Agarose 6XL	**15-25**
Hexyl Agarose 6XL	**20-30**
Octyl Agarose 6XL	**30-40**
Decyl Agarose 6XL	**40-50**
Phenyl Agarose 6XL	**47-63**

Data determined by incubating 0.1g adsorbent with bovine serum albumin (BSA) in 25 mM phosphate/l M ammonium sulphate, pH 6.8 (10 mg/ml: 1.1ml) 1hr. 20ûC. Supernatants were assayed by absorbance at 280nm.