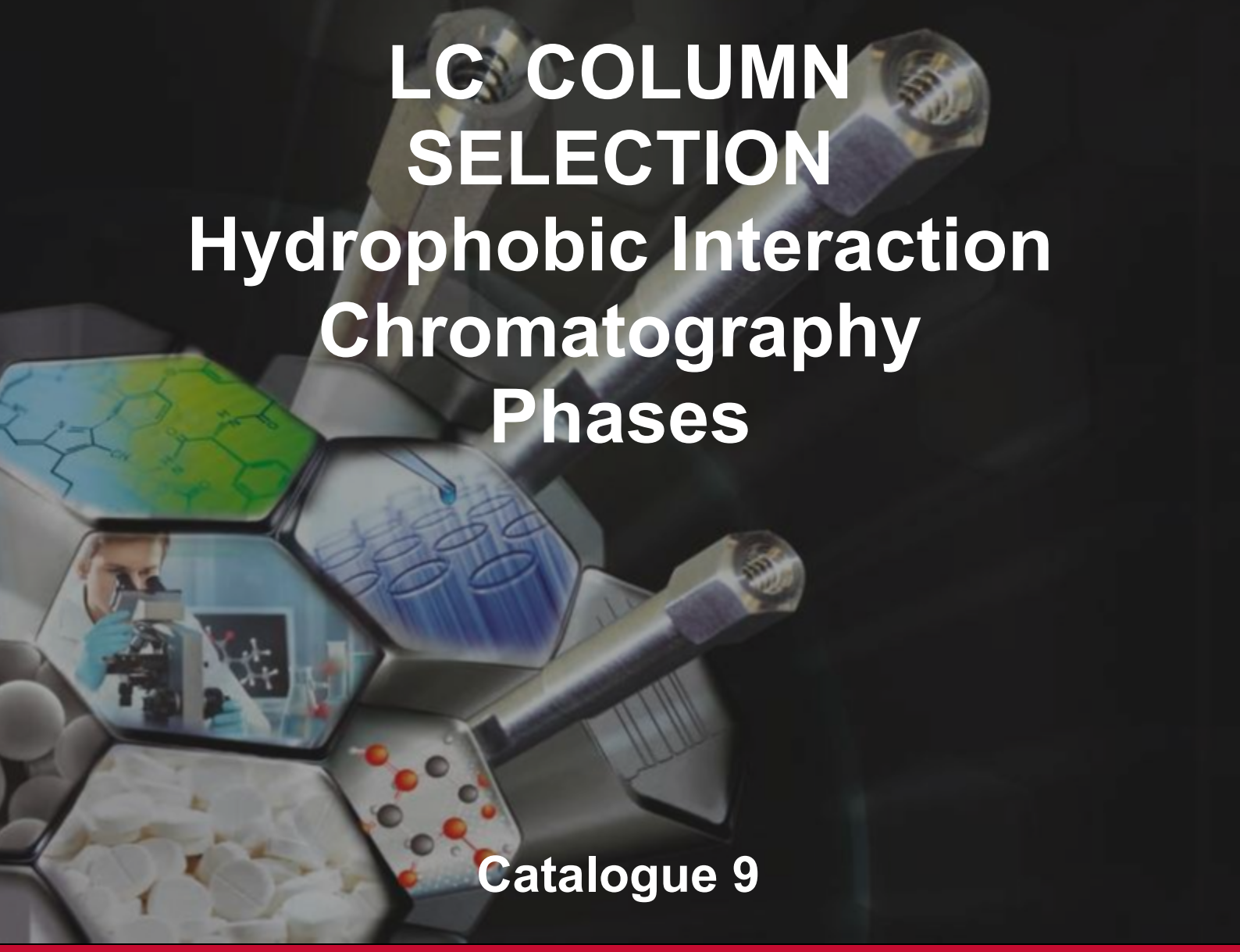
HICHROM

Chromatography Columns and Supplies

LC COLUMN SELECTION Hydrophobic Interaction Chromatography Phases



Catalogue 9

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HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC) PHASES

Introduction

Hydrophobic Interaction Chromatography (HIC) is a powerful technique for the separation and purification of proteins and peptides. Separations are based on the interaction between hydrophobic groups on a protein and a hydrophobic ligand on the solid support. Although the separation mechanism of HIC has similarities with that of standard reversed-phase HPLC, the density of the bonded phase ligands on the surface of the HIC packing material is much lower. HIC therefore involves weaker interactions and weaker eluents can be used. Samples are adsorbed to the HIC resin at relatively high salt concentrations and eluted by applying a linear or stepwise decreasing salt gradient. The mild conditions used in HIC typically maintain tertiary protein structure and thus biological activity (ie. no denaturation).

Selectivity

An optimum HIC separation will combine high dynamic binding capacity (DBC), adequate selectivity, good mass recovery and retention of biological activity. Proteins show varying degrees of hydrophobicity depending on their amino acid composition, structure and size. Separation can be optimised by varying the nature of the HIC phase or by varying the eluent. Very hydrophilic proteins are generally purified using highly hydrophobic stationary phases, whereas very hydrophobic proteins are separated using the least hydrophobic phases.

Method Development

In addition to the hydrophobicity of the phase ligand, several parameters affect HIC separations. These include salt type, pH, buffer concentration, temperature and gradient. Ammonium sulphate (1 or 2M) or sodium chloride (3M) salts are most commonly used for HIC applications. The pH of the salt solution will influence retention; pH 7.0 is a good starting point. Figure 1 shows the influence of pH for various salts on the DBCs of lysozyme.

Applications

Hydrophobic interaction chromatography is suitable for the separation and purification of a wide range of biomolecules. In addition to proteins, antibody fragments, RNAs, antibiotics etc. can be analysed by HIC. HIC can also be used for protein desalting. Figure 2 illustrates the separation of 16S and 23S ribosomal RNA on a TSKgel Phenyl-5PW column.

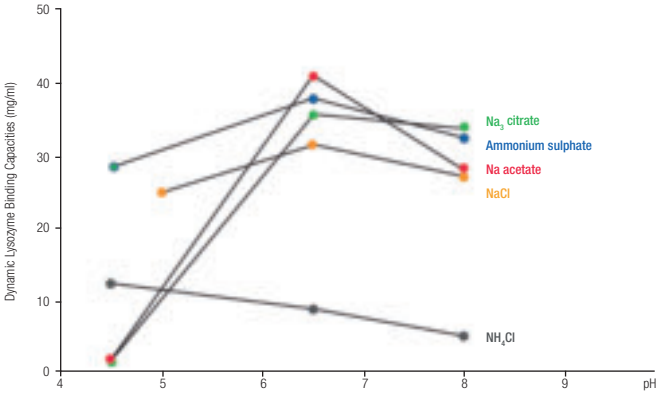
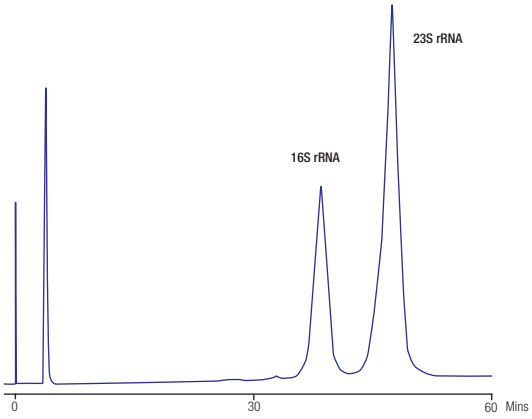


Figure 1. Influence of pH



Column: TSKgel Phenyl-5PW (75 x 7.5mm)
Eluent: Linear gradient from 2mol/l to 0mol/l $(NH_4)_2SO_4$ in 0.1mol/l phosphate buffer, pH 7.0
Flow rate: 0.5ml/min
Detection: UV, 280nm

Figure 2. Separation of RNAs on TSKgel Phenyl-5PW

HIC Phases

Phase	Manufacturer	Base material	Bonding	Particle Size (µm)	Pore Size (Å)	Page
COSMOSIL HIC	Nacalai Tesque	Silica	Diol	5	300	91, 94
HIC PH-814	Shodex	Polyhydroxymethacrylate	Phenyl	10	2,000	214
MCI GEL CQH Series	Mitsubishi Chemicals	Polyhydroxymethacrylate	Ether, Butyl, Phenyl	10	600	172
PolyPROPYL A	PolyLC	Silica	Propylaspartamide	3, 5, 12	300, 1000, 1500	196, 199
PolyETHYL A			Ethylaspartamide			199
PolyMETHYL A			Methylaspartamide			199
ProPac HIC-10	Thermo Scientific	Silica	Amide/ethyl	5	300	240
TSKgel	Tosoh Bioscience	Methacrylate	Ether, Phenyl	10, 13, 20	1,000	249
			Butyl-NPR	2.5	-	249