OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: ***info@orachrom.com*** ***www.orachrom.com***

APPLICATION NOTE

SIMULATED MONOLITH™: Fast Separations with HIC (Hydrophobic Interaction Chromatography)

The presence of high salt, up to 2 to 3 molar, in the starting buffer during HIC separations, considerably increases the eluent's viscosity giving rise to high back pressures.
It is important for the media to have the optimum capacity in order to require lower salt concentration.
The resolving power of the media is also essential as it allows the use of shorter columns.
The following chromatograms depict the baseline separation of 5 proteins using 2.1M salt at the start of the gradient. The column length is only 33 mm making it possible to run high flow rates and achieve fast separations.

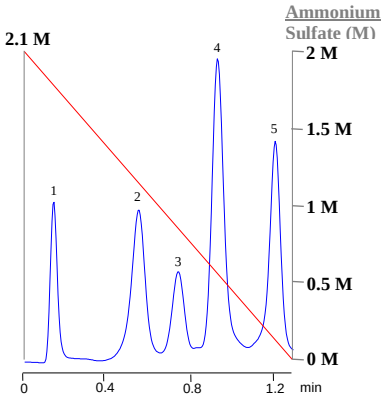

Figure 1. Protein Separation on **STYROS™ HIC Phenyl/XH.**

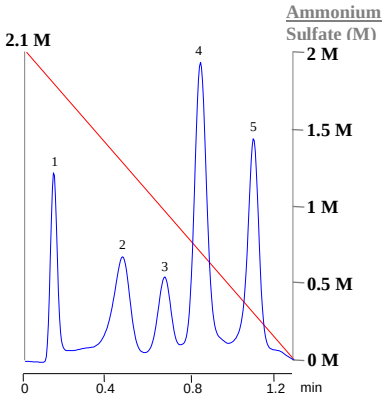

Figure 2. Protein Separation on **STYROS™ HIC-Butyl/XH.**

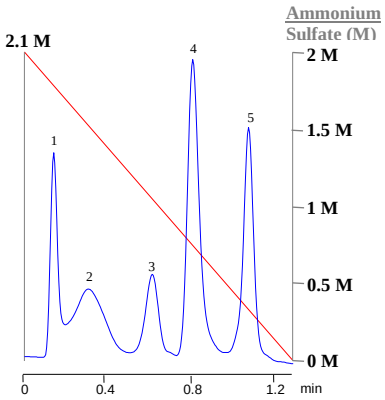

Figure 5. Protein Separation on **STYROS™ HIC-Ether/XH.**

Table 1. Operating parameters for the chromatograms.

HPLC System	Agilent 1100, Standard Cell
Columns	4.6 x 33 mm. Phases as indicated.
Eluent A	0.1M Phosphate, pH = 7
Eluent B:	A + 2.1 M SO4(NH4)2, pH = 7
Detection:	280 nm
Flow rate:	5 ml/min (1,800 cm/hr)
Temperature	20 °C
Gradient:	100 to 0%B in 1.3 minutes (12 C V)
Injection volume	5 µ l
Sample 1:	1. Cytochrome c, 2. Myoglobin, 3. Ribonuclease A, 4. Lysozyme, 5. α-Chymotrypsinogen.

A typical soft gel column with pressure limit can only run at 140 cm/hr of linear velocity.
The unique macroporous structure of the Simulated Monolith™ bed provides a maximum surface contact as well as a uniform flow path, making it possible to run high speed, high-resolution separations without any mass transfer restrictions.
HIC has been used successfully in cases where the target protein already exists in high salt medium or when the protein readily denatures or other methods of separations such as ion exchange or gel filtration are not effective.
Another use of HIC is in the separation of Monoclonal antibodies (MAbs) for research, diagnostic or therapeutic applications. MAbs are among the most hydrophobic proteins in ascites and cell culture supernatant. Such characteristic results in high retention, providing better separation and purification.
Unlike Monolith, Simulated Monolith™ columns are provided in different formats giving the end user a far greater selection.