OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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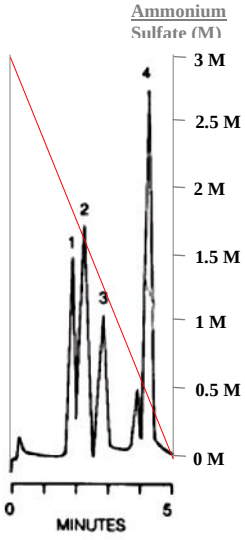
APPLICATION NOTE

SIMULATED MONOLITH™: Comparison With Non Covalently Coated Polymeric Hard Gel.

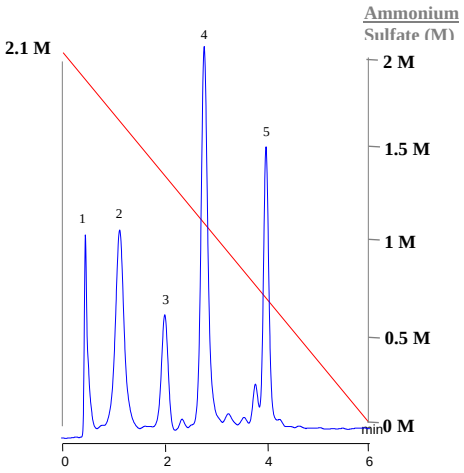
Polymeric hard gel media with large throughpores are commonly produced as hydrophobic particles that are subsequently coated to generate hydrophilic activated surfaces to be functionalized.
It is crucial for the coating process not to result in the obstruction of the pores or the alteration of their size.
STYROS™ media are covalently coated with a single hydrophilic layer to fully preserve the pore structure.
A typical separation of some standard proteins on similar phases (HIC-Phenyl), shows the outcome of the two coating processes.
STYROS™ **HIC-Phenyl/XH** is compared to its commercial counterpart in which the throughpores are for the most part obstructed.



Chromatogram 1
Protein Separation on **Commercial Porous HIC-Phenyl Column.**

Table 1. Operating parameters.

HPLC System	Agilent 1100, Standard Cell
Columns	4.6 x 100 mm. Commercial HIC-Phenyl, hard gel porous polymeric column.
Eluent A	0.1M Phosphate, pH = 7
Eluent B:	A + 3 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 5 minutes (15 C V) as suggested by the manufacturer
Sample	1. Myoglobin, 2. Ribonuclease A, 3. Lysozyme, 4. α-Chymotrypsinogen.



Chromatogram 2
Protein Separation on **STYROS**™ **HIC-Phenyl/XH**

Table 2. Operating parameters.

HPLC System	Agilent 1100, Standard Cell
Columns	4.6 x 100 mm. **STYROS**™ **HIC-Phenyl/XH**.
Eluent A	0.1M Phosphate, pH = 7
Eluent B:	A + 2.1 M SO4(NH4)2, pH = 7
Detection:	280 nm
Flow rate:	3 ml/min (1,100 cm/hr)
Temperature	20 °C
Gradient:	100 to 0%B in 6 minutes (11 C V)
Sample	1. Cytochrome c, 2. Myoglobin, 3. Ribonuclease A, 4. Lysozyme, 5. α-Chymotrypsinogen.

The optimum capacity chosen for the **STYROS**™ **HIC** media allows the use of lower salt to retain the proteins. As a result, a shallower gradient can be chosen for the baseline separation in terms of absolute salt concentration.
In addition, the pore structure of the **Simulated Monolith**™ bed provides unobstructed throughpores for a uniform flow path.
It is evident from the second chromatogram, that there are no long obstructed or diffusive pores involved in the separation process.
The present set of polymeric columns are not only a considerable improvement over soft gel columns with pressure and flow restrictions, they also far exceed the characteristics of other non covalently coated hard gel media with either obstructed throughpores or a wide range of throughpore distribution.