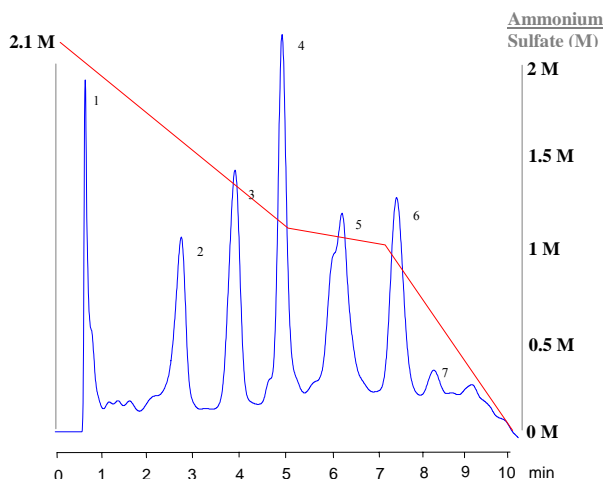


## APPLICATION NOTE

### Hydrophobic Interaction Chromatography: Comparison of STYROST<sup>TM</sup> HIC-Phenyl with TSKgel Phenyl-5PW from TOSOH.

A mixture of 7 proteins were separated on a STYROST<sup>TM</sup> HIC-Phenyl 4.6 x 100 mm column (volume 1.7 ml) at linear flow rates of 720 cm/hr (2 ml/min volumetric flow) and compared with the performance of a TOSOH TSKgel Phenyl-5PW 7.5 mm x 7.5 cm, 10 µm particles column (volume 3.3 ml) run by the manufacturer at a linear flow rate of 136 cm/hr (1 ml/min volumetric flow) using 5 proteins.

Both resins are porous; STYROST<sup>TM</sup> is a Simulated Monolith<sup>TM</sup> column made of hard gel polymeric that can withstand high back pressures and therefore can be run at high flow rates, whereas TSKgel is a soft gel resin with pressure and flow rates limitations.

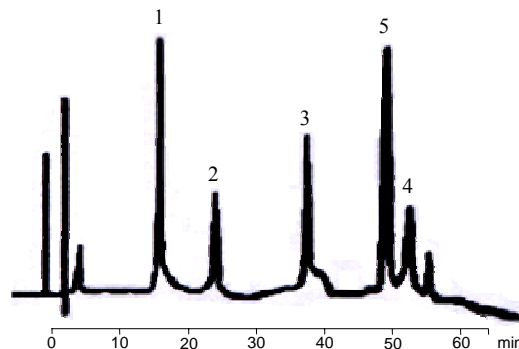


**Chromatogram 1**

Separation of 7 proteins on STYROST<sup>TM</sup> HIC-Phenyl/XH (Linear Flow Rate: 720 cm/hr)

**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	STYROST <sup>TM</sup> HIC-Phenyl/XH 4.6 X 100 mm
<b>Mobile phase.</b>	A: 0.1 M Phosphate, pH=7 B: A + 2.1 M SO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> , pH=7
<b>Flow rate</b>	2 ml/min (720 cm/hr )
<b>Gradient</b>	100 to 50 % B in 5 min, to 45% B in 7 min, to 0% B in 10 min (12 cv)
<b>Temperature</b>	30°C
<b>Detection</b>	280 nm
<b>Injection volume</b>	10 µl
<b>Sample:</b>	1- Cytochrome c, 0.1 mg/ml, 2- Myoglobin 2.5 mg/ml, 3- Ribonuclease A, 5 mg/ml, 4- Lysozyme 2 mg/ml, 5- Ovalbumin 5 mg/ml, 6- -Chymotrypsin 2.5 mg/ml, 7- α-Chymotrypsinogen A 0.5 mg/ml in buffer A.



**Chromatogram 2**

Separation of 5 proteins on TSKgel Phenyl-5PW (Linear Flow Rate: 136 cm/hr)

**Table 2. Operating parameters.**

<b>Columns</b>	TSKgel Phenyl-5PW, (7.5 cm X 7.5 mm)
<b>Mobile phase.</b>	A: 0.1 M Phosphate, pH=7 B: A + 1.8 M SO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> , pH=7
<b>Flow rate</b>	1 ml/min (136 cm/hr )
<b>Gradient</b>	100 to 0 % B in 60 min (18 cv)
<b>Temperature</b>	25°C
<b>Detection</b>	280 nm
<b>Injection</b>	100 µl (50-100 µg)
<b>Sample:</b>	1-Myoglobin, 2-Ribonuclease, 3-Lysozyme, 4-α-Chymotrypsin, 5-α-Chymotrypsinogen.

The clear advantage of hard gel polymeric STYROST<sup>TM</sup> media are made of is obvious from these chromatograms.

The porosity in the case of STYROST<sup>TM</sup> enhances the resolving power whereas it is a detriment in the case of TSKgel.

The chemical and mechanical stability of hard gel are also advantages soft gels polymeric do not have.

