

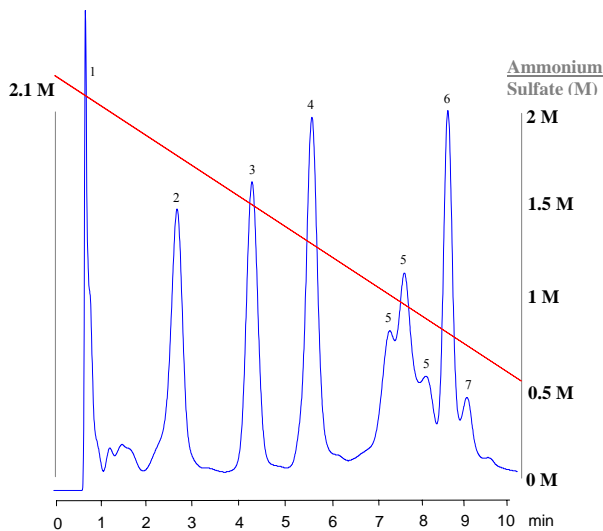
## APPLICATION NOTE

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

A mixture of 7 proteins were separated on a STYROST<sup>TM</sup> HIC-Butyl 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 Butyl-NPR 4.6 mm x 3.5 cm, 2.5 µm particles column (volume 0.6 ml) run by the manufacturer at a linear flow rate of 360 cm/hr (1 ml/min volumetric flow) using 5 proteins.

STYROST<sup>TM</sup> is a Simulated Monolith<sup>TM</sup> fully porous resin whereas the TSKgel NPR is a non porous resin made of 2.5 µm particles operating at 1000 to 2000 psi at flow rates of 1 ml/min with a 4.6 mm ID and a column length of 3.5 cm.

The following 2 chromatograms highlight the difference between the two stationary phases.

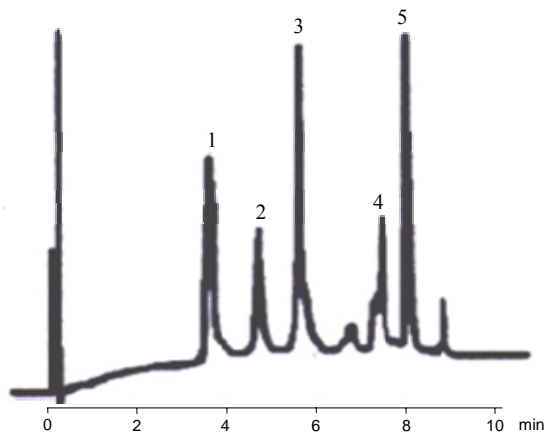


**Chromatogram 1**

Separation of 7 proteins on STYROST<sup>TM</sup> HIC-Butyl/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-Butyl/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 30 % B in 10 min (12.5 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- α-Chymotrypsin 2.5 mg/ml, 7- α-Chymotrypsinogen A 0.5 mg/ml in buffer A.



**Chromatogram 2**

Separation of 5 proteins on TSKgel Butyl-NPR (Linear Flow Rate: 360 cm/hr)

**Table 2. Operating parameters.**

<b>Columns</b>	TSKgel Butyl-NPR, (4.6 cm X 3.5 cm)
<b>Mobile phase.</b>	A: 0.1 M Phosphate, pH=7 B: A + 2.3 M SO <sub>4</sub> (NH <sub>4</sub> ) <sub>2</sub> , pH=7
<b>Flow rate</b>	1 ml/min (360 cm/hr )
<b>Gradient</b>	100 to 0 % B in 12 min ( 21cv)
<b>Temperature</b>	25°C
<b>Detection</b>	280 nm
<b>Sample: 20 µl (1.5-4.0 µg)</b>	1-Myoglobin, 2-Ribonuclease, 3-Lysozyme, 4-α-Chymotrypsin, 5-α-Chymotrypsinogen.

Although the TSKgel Butyl-NPR has a higher pressure tolerance than the soft gel series, the size of the particles (2.5µm) generates high back pressure that limits the flow and therefore slows down the chromatographic run as well as the re-equilibration and reconditioning of the column.

In comparison, the STYROST<sup>TM</sup> column with 3 times the length can be run at twice the flow rate with less than half the back pressure and provide substantially higher resolution. The components of a commercial sample of Ovalbumin have also been separated in the corresponding chromatogram.

Neither the decrease in size, nor the absence of pores has provided the TSKgel Butyl-NPR any advantage that would justify the high back pressure as well as the restrictions in column length.

