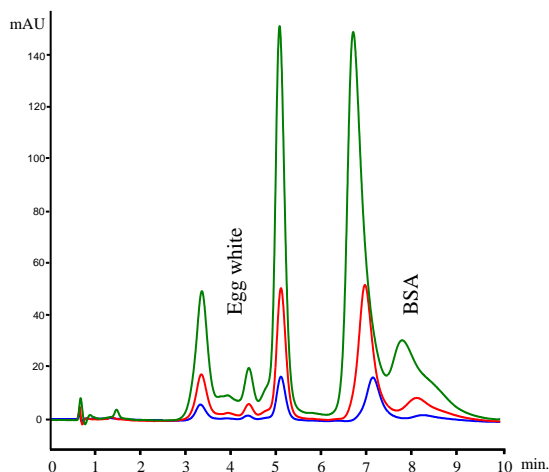


## APPLICATION NOTE

### STYROS™ HQ Simulated Monolith™ Polymeric: Loading Study.

The following superimposed chromatograms show the loading study for the separation of Egg white and Bovine Serum Albumin on a **STYROS™ HQ/XH Simulated Monolith™** column at 30°C.



Chromatogram 1

Loading Study of **STYROS™ HQ/XH** 4.6 x 50 mm at 1 ml/min (360 cm/hr)

Table 1. Operating parameters.

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment and quaternary pump.
<b>Column</b>	<b>STYROS™ HQ/XH</b> 4.6 X 50 mm (0.83 ml volume)
<b>Mobile phase.</b>	A: 20 mM Tris, pH=8.2 B: A + 1 M NaCl, pH= 8.2
<b>Flow rates</b>	1 ml/min (360 cm/hr of linear velocity)
<b>Gradient</b>	5 to 40 % B in 12 cv
<b>Temperature</b>	30°C
<b>Detection</b>	280 nm
<b>Injection volume</b>	10, 30 and 90 µl
<b>Pressure Drop</b>	3 bar (44 psi)
<b>Sample:</b>	Egg white and BSA (2.5 mg/ml each in buffer A)

The loading study is part of the evaluation of a column for consideration in the scale up.

It allows the column to be assessed as to its limits of loading during the scale up.

It is clear that every mixture would have its specificity that needs to be evaluated prior to the start of the regimen.

The high resolution does not result at the expense of high back pressure.

In fact the very low back pressure of the column makes it amenable to process scale pumps.

The sizes of the columns are not limited either.

Unlike Monoliths with high back pressures the Simulated Monolith™ columns can be made in all sizes including preparative columns with low back pressures.

The Simulated Monolith™ columns are ideal for Simulated Moving Bed Chromatography to generate continuous separation processes.

The high capacity of the resin allows the use of salt at the start of the gradient and an overall lower salt for the complete elution while retaining the shape of the peak.

No fronting or tailing is seen.

The benefits that Simulated Monolith™ columns can bring to the process can be summed up as follows:

- Absence of leachables
- High chemical stability
- High physical stability
- Availability in different sizes
- High resolution at low and high flow rates
- Low back pressures
- Tolerant to fast changes of buffer
- High capacity
- Possibility of CIP
- Extended lifetime
- High pressure tolerance
- Availability in most chemistry
- A first step towards process scale separations

A direct comparison with a similar size Monolith column of 4.6 x 50 mm provides a better picture.

	<b>Simulated Monolith™</b>	<b>Monolith</b>
<i>Maximum operating pressure</i>	3000 psi (21 MPa)	1200 psi (8.2 MPa)
<i>Maximum operating temperature</i>	70 °C	70 °C
<i>Recommended flow rate</i>	Up to 1,740 cm/hr	Up to 540 cm/hr
<i>Maximum flow rate</i>	1,800 cm/hr	720 cm/hr
<i>Typical back pressure at 1ml/min</i>	44 psi	290 psi
<i>Solvent compatibility</i>	All HPLC solvents	Most HPLC solvents
<i>pH range</i>	1-14	2-12
<i>Capacity (BSA)</i>	90 mg/ml	18 mg/ml
<i>Buffer changes</i>	No restrictions	Restricted

