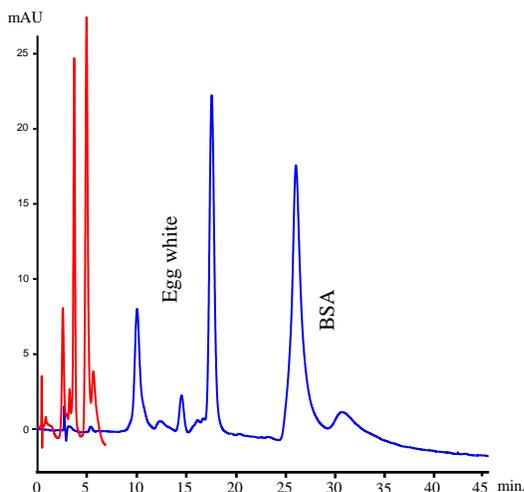


APPLICATION NOTE

STYROS™ HQ Simulated Monolith™ Polymeric: Separation of 2 Protein Mixtures .

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



Chromatogram 1

Separation on **STYROS™ HQ/XH** and **STYROS™ HQ/NB**

Both columns give a realistic picture of the steps ahead during the scale up of the process.

Furthermore the sizes of the columns are not limited.

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

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

The overall 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 operating back pressures
- Tolerant to 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 clear picture.

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	STYROS™ HQ/XH 4 X 300 mm (3.77 ml volume) and STYROS™ HQ/NB 2.1 X 150 mm (0.52 ml volume).
Mobile phase.	A: 20 mM Tris, pH=8.2 B: A + 1 M NaCl, pH= 8.2
Flow rates	1 ml/min (478 cm/hr and 1,733 cm/hr of linear velocity)
Gradient	5 to 40 % B in 12 and 13.5 cv
Temperature	30°C
Detection	280 nm
Injection volume	30 and 10 µl
Pressure Drop	22 and 41 bar (320 and 595 psi)
Sample:	(2.5 mg/ml each in buffer A)

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

The high resolution of the separations can be achieved either on a normal bore column of 4 mm diameter (in 46 minutes) or on a narrow bore column of 2.1 mm diameter (in 7 minutes) using similar gradient depths.

The sample amounts are also reduced to 1/3 and the time is reduced by a factor of 6 to 7 using a narrow bore column.

The fast resolution and high flow rates do not result at the expense of high back pressures.

It is important to note that the high back pressure of a Monolith column is prohibitive in the use of more than a single one at a time.

It is therefore not possible to use them in tandem in order to gain added resolving power

