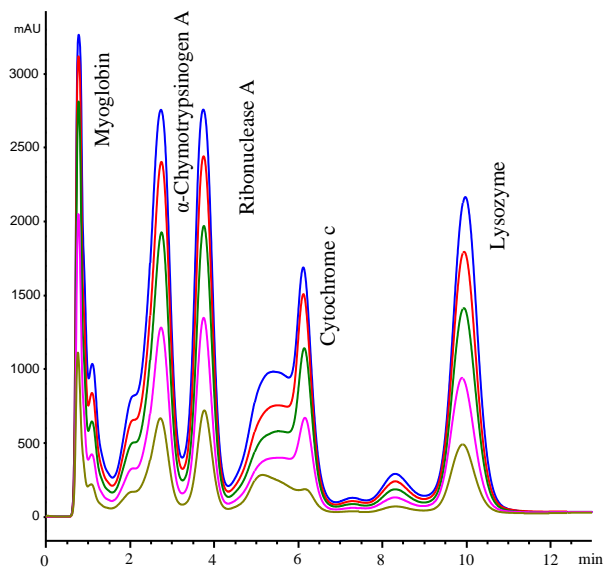


APPLICATION NOTE

STYROS™ SP Simulated Monolith™ Strong Cation Exchanger Compared to CM Simulated Monolith™ Weak Cation Exchanger.

It is critical in the downstream process of biopharmaceutical to have access to resins with high capacity, high speed, very high resolution, high salt tolerance, low back pressure as well as non-leaching media that can be used many times over to justify not only its cost but also the time saved by allowing CIP procedures in a fast and efficient way.

STYROS™ Simulated Monolith™ cation exchangers are offered in both high capacity strong cation exchanger (SP) and lower capacity weak cation exchanger (CM). These are based on hard gel polymeric with over 100 mg/ml and 50 mg/ml of Lysozyme capacity.

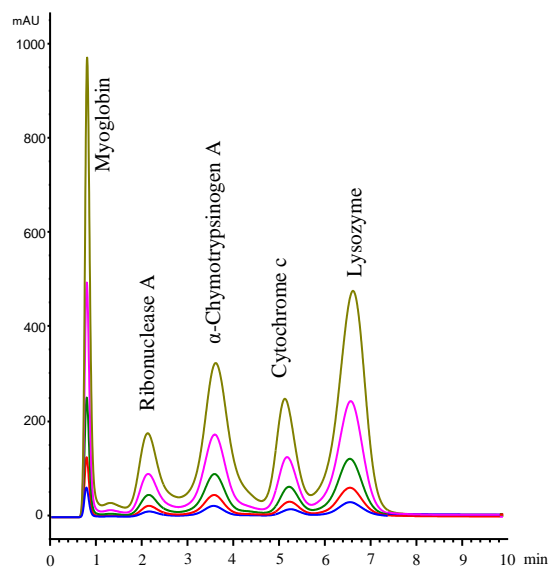


Chromatogram 1

Loadability study at 720 cm/hr on a 4.6 x 150 mm STYROS™ SP/XH.

Table 1. Operating Parameters.

HPLC System.	HP 1100 with thermostatted column compartment.
Columns	STYROS™ SP/XH 4.6 x 150 mm
Mobile Phase	A: 20 mM Phosphate, pH = 6.8 B: A + 1 M NaCl, pH = 6.8
Flow rate	2 ml/min (720 cm/hr)
Gradient	12 to 55 % B in 10.5 cv.
Temperature	30°C
Detection	214 nm
Injection volume	20, 40, 60, 80 and 100 µl
Pressure Drop	32 bar (464 psi)
Samples (1:3:3:3:3 mg/ml each)	Myoglobin, α-Chymotrypsinogen A, Ribonuclease A, Cytochrome C from Bovine, Lysozyme.



Chromatogram 2

Loadability Study at 720 cm/hr on a 4.6x150mm STYROS™ CM/XH

Table 2. Operating Parameters.

HPLC System.	HP 1100 with thermostatted column compartment.
Columns	STYROS™ CM/XH 4.6 x 150 mm
Mobile Phase	A: 20 mM Phosphate, pH = 6.8 B: A + 1 M NaCl, pH = 6.8
Flow rate	2 ml/min (720 cm/hr)
Gradient	5 to 30 % B in 8 cv.
Temperature	30°C
Detection	280 nm
Injection volume	From 2.5 µl to 40 µl
Pressure Drop	32 bar (464 psi)
Samples (5:10:10:5:10 mg/ml each)	Myoglobin, Ribonuclease A, α-Chymotrypsinogen A, Cytochrome C from Equine, Lysozyme.

Although there is no sign of “peak saturation” it is important to note the effects of ionic strength as well as high capacity on peak retention reversal as well as the discriminatory property of the strong cation exchanger on α-Chymotrypsinogen and Cytochrome c.

