

APPLICATION NOTE

Polymeric Gigaporous Strong Cation Exchangers: Bead Size Distribution versus Pore Size Distribution.

One of the advantages offered by stationary phases with **fully pervious convective pores** is the involvement of the inner bead volume in the separation process.

The resolution of the separation with non-porous media with rigid spherical bead is directly related to the bead size distribution that controls the size of the interstitial channels of the packed bed. Porous beads with convective pore sizes of 100 nm and more offer an added path to the eluent's flow and as a result change the mass transfer characteristics of the stationary phase.

The bead size distribution no longer accounts as a primary parameter in the separation and resolution of the components of a protein mixture when using porous beads with throughpores.

This assertion can be verified by using **STYROS™ SP/XP** columns as an example, with an average bead size of 15 to 20 μm and comparing it with a mono-sized 15 μm stationary phase with similar functionality.

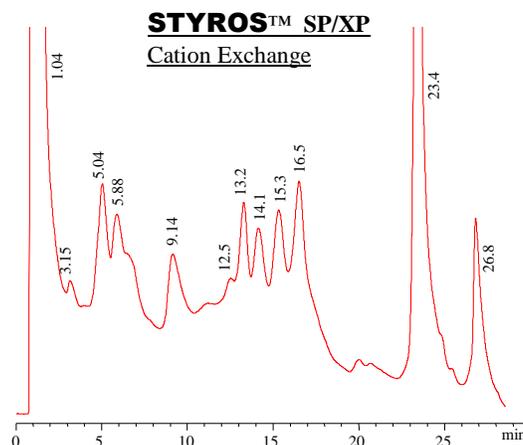
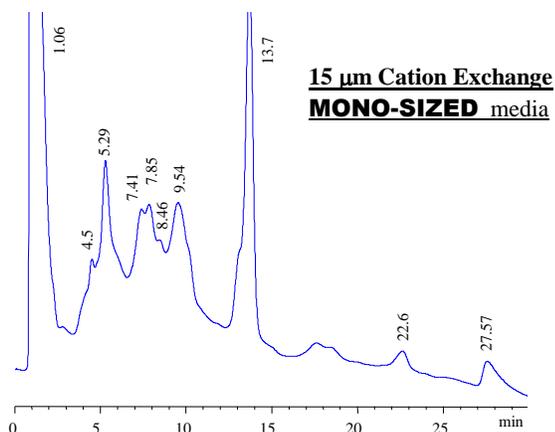
The throughpores on the **STYROS™** media are narrowly sized between 1,000-2,000 Å, whereas the pores on the mono-sized media range in size from 200 to 10,000 Å.

Using similar conditions, a sample of naturally occurring proteins from snake venom was separated on both columns and compared with one another.

The following table summarizes the conditions:

Table 1. Operating Parameters.

HPLC System.	HP 1100
Columns	STYROS™ SP/XP 100x4.6mm and 15 μm MONO-SIZED media 100X4.6 mm
Mobile Phase	A: 20 mM MES, pH = 6.3 B: A + 1 M NaCl
Flow rate	1 ml/min (300 cm/hr)
Gradient	0 to 27 % B in 12 cv, to 85% B in 18 cv.
Temperature	30°C
Detection	280 nm
Injection volume	100 μl
Samples	5 mg/ml Snake Venom (<i>Crotalus atrox</i>) in buffer A.



Clearly the beads with the narrow pore size distribution achieve a significantly better resolution than the mono-sized beads with wide pore size distribution.

Pore size distribution should be the primary criterion in choosing a porous media for a specific separation, along with media capacity, protein recovery, carry-over between runs, absence of leaching during use, column lifetime, and stability to a wide range of pH and chemical cleaning agents.