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APPLICATION NOTE

STYROS® HQ Simulated-Monolith™ Polymeric Compared with Commercial POROUS HQ.

In this Application Note we have compared two polymeric anion exchangers of different size, with an average of 30 µm and 60 µm, and established that despite having half the size of the 60 µm, the media with 30 µm size displays lower backpressure and expectedly, better resolution.
We have therefore concluded the only cause is the presence of fines from the larger size beads slowly being released and obstructing the pores of the frit and finally resulting in loss of characteristics of the column, namely its dynamic and static capacities, and in need of replacement.

The following superimposed chromatograms show the separation of Egg White in buffer A injected under similar conditions in the two columns with similar gradients.
To be noted that both columns were packed with identical procedure, using frits of similar porosity (2 µm).

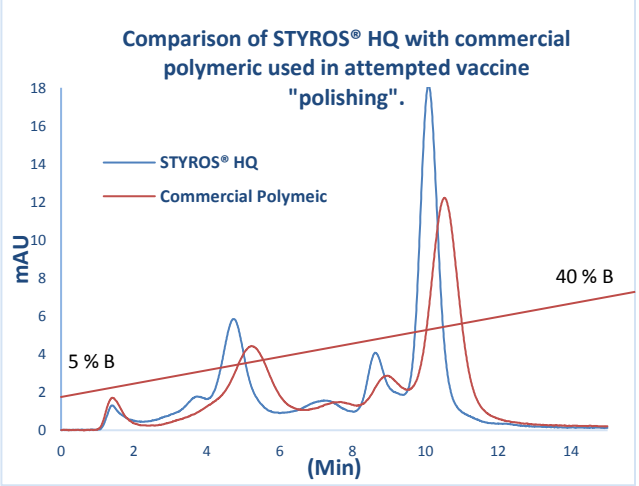

Table 1. Operating parameters.

HPLC System.	Agilent 1260 with thermostatted column compartment and quaternary pump.
Columns	**STYROS® HQ** 2.1 X 100 mm (0.346 ml volume) **POROUS HQ** 2.1 X 100 mm (0.346 ml volume)
Mobile phase.	A: 20 mM Bis-Tris, pH=6 B: A + 1 M NaCl, pH= 6
Flow rates	0.2 ml/min (347 cm/hr of linear velocity)
Gradient	5 to 40 % B in 15 minutes.
Temperature	30°C
Detection	280 nm
Injection volume	2 µl of a solution of Egg white 1:9 in buffer A
Pressure Drop	19 bar for STYROS® HQ. 21 bar for POROUS HQ System back pressure 13.5 bars.
Sample:	Egg white in buffer A 1: 9 ratios.

To have a better understanding of the deleterious effects of impurities usually resulting from leaching and unstable media for chromatography, it is important to understand the phenomenon of leaching.
As mentioned earlier, fines are released during the process of purification when impurities come in contact with the unstable solid phase extricating the loose and leachable part of it.
These loose impurities are now encapsulated and no longer have hooks to accroach to any "polishing" phase.
Gel electrophoresis cannot identify it as it is outside of its range.
Main reasons for a polymeric porous particle to leach:

1- The polymerization was unsuccessful
2- The process of reverting the hydrophobic media to hydrophilic was most likely done via a passive chemistry such as "shrink wrapping".

To Surmont these deficiencies and move beyond it one needs a stable polymeric phase such as Simulated-Monolith™.
A product with the characteristic of monolith without its limitations.

- Absence of leachable
- 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
- **And most of all the possibility to finally automate the bioprocess purifications using SMB, a well-established, well documented process for automation.**