OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA Phone (781) 932 0151 Fax (781) 932 0787
E-mail: info@orachrom.com www.orachrom.com

APPLICATION NOTE

Simulated-Monolith™, a line of stable Polymeric that Allows Universal Separations of Different Molecules Size through Rapid Convection.

To become practical, scientists resorted to "Simulation" for "moving bed chromatography".
Same is true for Monolithic Media for chromatography.
At this time of urgent need in downstream processing of vaccines, we need a product that can process not only at linear velocities that are higher than the soft gel media used by the major manufactures, but at the same time yield pure products that no longer needs sub-zero temperature to prevent enzyme impurities of spoiling them.

We have here made the case for a polymeric Simulated-Monolith™ that can not only be provided in any size columns, in ion exchanger to affinity, but as a quintessential requirement would no longer need "polishing" as a curse for leaching media for chromatography.
The need for it at this juncture is more than obvious to the vaccines manufacturers.

The following superimposed chromatograms shows several runs at high flow rates (>1,000 cm/hr) on a 5 cm long narrow bore column from 6.25 µg to 50 µg.
They are superimposable.

The column displays a back pressure of less than 4,000 psi, that also includes the instrument (3,200 psi) rated for 18,000 psi.
This is to compare with soft gel (130 cm/hr) that is still being used for the downstream processing of vaccines and most biopharmaceuticals.

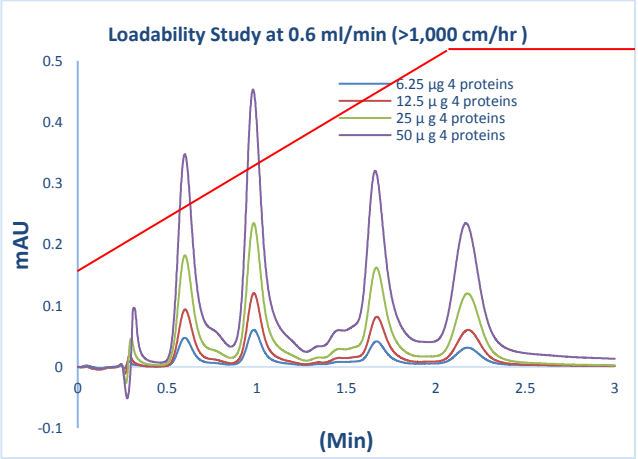

Table 1. Operating parameters.
lea

HPLC System.	Acquity UPLC *I Class Plus*
Column	**STYROS® 1R** 2.1 X 50 mm (0.173 ml volume)
Mobile phase.	A : 2% ACN in DI H2O, 0.1 % TFA B: 70:30 ACN: H2O, 0.1 % TFA
Flow rates	0.6 ml/min (>1,000 cm/hr of linear velocity)
Gradient	35 to 100 % B in 2 min, 100 % B in 3 min.
Temperature	30°C
Detection	220 nm
Injection volume	As indicated on the chromatograms
Pressure Drop	< 800 psi on the column
Sample:	5 mg/ml of 4 proteins in A.

At more than 7 times the speed of soft gel, the separation does show full separa
(13.7 kDa),
tion characteristic of monolith.
The pressure drop is only <800 psi on the column.
At 0.2 ml/min the back pressure drops to < 100 psi on the column and no sign of leaching verifiable by mass not gel electrophoresis.

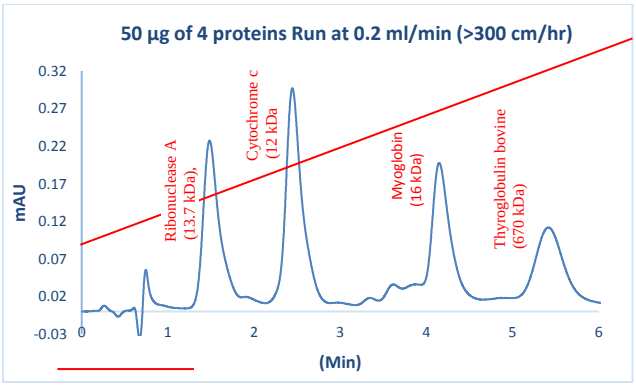

Table 2. Operating parameters.

HPLC System.	Acquity UPLC *I Class Plus*
Column	**STYROS® 1R** 2.1 X 50 mm (0.173 ml volume)
Mobile phase.	A : 2% ACN in DI H2O, 0.1 % TFA B: 70:30 ACN: H2O, 0.1 % TFA
Flow rates	0.2 ml/min (>300 cm/hr of linear velocity)
Gradient	35 to 100 % B in 6 min
Temperature	30°C
Detection	220 nm
Injection volume	10 µl
Pressure Drop	< 100 psi on the column
Sample:	5 mg/ml of proteins in A.

These Simulated-Monoliths™ operate like monolith but are not prone to the "wall effects" and "leaching", that monolithic media suffers from. They are stable and do not leach. Absolutely needed in the downstream processes of vaccines, among other biopharmaceuticals that heavily depends on high purity that even "Polishing" cannot provide. The 4 proteins used are Ribonuclease A (13.7 kDa), Cytochrome c (12 kDa), Myoglobin (16 kDa) and Thyroglobulin bovine (670 kDa) a mixture of proteins typically used in size exclusion that is a slow diffusive pore process that requires hours of elution time with media with specific pore size.
Simulated-Monoliths ™ offer a fast alternative in minutes with baseline separations.
Please also notice the minimal amount of waste generated in using narrow bore columns.