OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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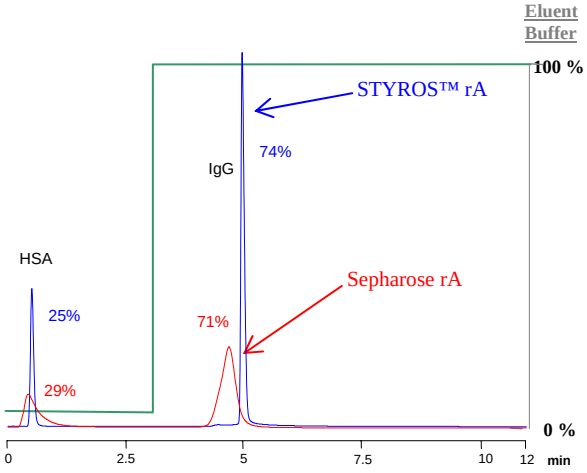
APPLICATION NOTE

Immobilized Protein rA on Polymeric Porous Hard Gel (STYROS™ rA): Comparison with Sepharose rA.

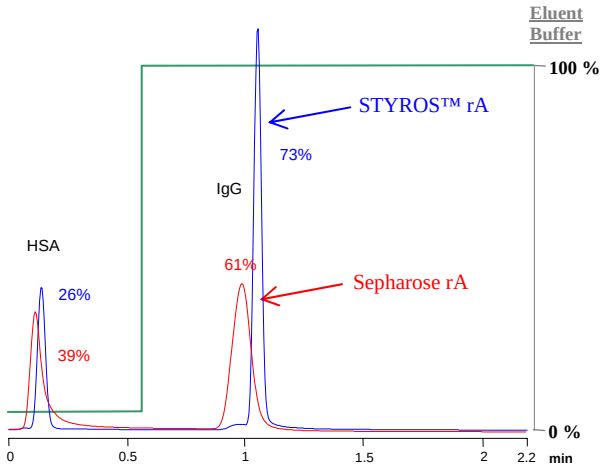
Affinity chromatography with immobilized Protein A is the principal method for the purification of antibodies. The method is highly selective and the recovery is high as well.
The ligand leakage of immobilized protein into the elution pool has been a major issue for the pharmaceutical industry to the point where a number of alternative affinity surfaces have been developed without any success. The concerns of ligand leakage of the matrix have not been addressed.
STYROS™ rA has been recently introduced as a hard gel gigaporous polymeric alternative to the soft gel Sepharose matrices. The primary focus in the development of STYROS™ has been the chemical and mechanical stability of the matrix resulting in a leakage free stationary phase. The gigaporous structure of the media allows full access of large biomolecules throughout the stationary phase
The present application note shows the chromatography advantages of the stationary phase compared with Sepharose rA in addition to its chemical and mechanical superiority.
A mixture of human serum albumin and IgG was separated using two similar size columns under similar conditions.
At low flow rates of 360 cm/hr (1ml/min in a 4.6mm ID column); the full amount (74%) of the IgG in the mixture was captured by STYROS™ rA whereas some part of the IgG (3%) was not adsorbed by Sepharose rA.



Capture of IgG from a mixture of human serum albumin and IgG, run at 1 ml/min (360 cm/hr) on a 4.6 x 33 mm STYROS™ rA column compared with a 4.6 x 33 mm Sepharose rA.

Increasing the flow rate to 1,800 cm/hr (5ml/min), a more substantial amount of IgG (12%) eludes capture in the case of Sepharose rA. There is also a drop of 1% with STYROS™ rA.

Operating at high velocities with STYROS™ saves 80 % in run time.
Compared with soft gel media, in addition to the time saved and the lack of contamination from ligand leakage, there is also a considerable recovery advantage.



Capture of IgG from a mixture of human serum albumin and IgG, run at 5 ml/min (1,800 cm/hr) on a 4.6 x 33 mm STYROS™ rA column compared with a 4.6 x 33 mm Sepharose rA.

Operating parameters for the chromatograms.

HPLC System	Agilent 1100, Standard Cell
Columns	STYROS™ rA 4.6 x 33 mm. Sepharose rA 4.6 x 33 mm
Binding buffer	50 mM Phosphate, 150 mM NaCl, pH 7
Eluent buffer:	0.1 M Citric Acid, pH 2.2
Detection:	280 nm
Flow rate:	1 and 5 ml/min
Temperature	30 °C
Injection volume	5 µ l
Sample:	HSA III + IgG in 20 mM Phosphate, pH=7

Due to the polymeric nature of STYROS™ and its stable, covalent coating, it is possible to clean the column with organic solvents, as well as acidic and basic solutions.

Inc.