OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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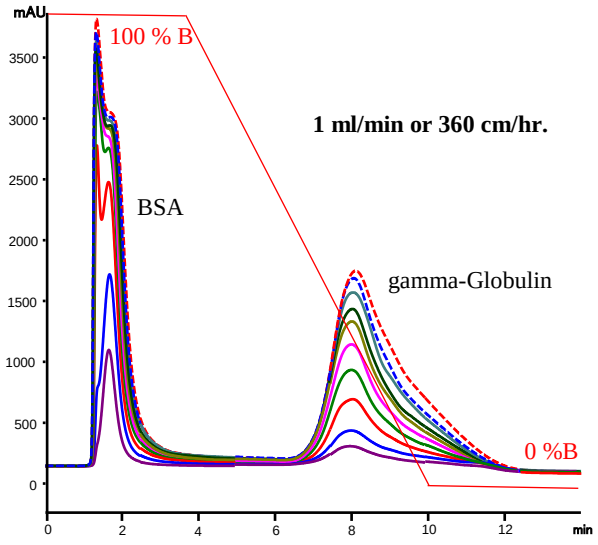
APPLICATION NOTE

Hydrophobic Interaction Chromatography: Separation of gamma-Globulin (bovine blood) from Bovine Serum Albumin. Loadability Study.

Contaminants and impurities are still challenging the production of biopharmaceuticals. The least of which are opportunistic microorganisms.
It is all too common to see a drug fail in clinical trials despite well thought design and execution without any obvious reasons other than the purity of the end product.
To address such challenges, one needs to use non-leaching media during downstream processing to eliminate cross contamination. This would certainly address one major variable that is the cause of impurity and contamination.
The panoply of STYROS® media developed at OraChrom were developed with that in mind.
Made of polystyrene cross-linked with divinylbenzene they offer the stability of hard gel media with high dynamic capacities.
Their Simulated-Monolith™ feature makes the pore size requirements obsolete.
They readily lend themselves to continuous processing such as Simulated Moving Bed, opening the capabilities of large productions.
More importantly their use results in drastic reduction of industrial footprint making it possible to respond to any accelerated production in a short period of time and at large scale.
In the present application we have used STYROS® HIC-Butyl to separate Bovine Serum Albumin from gamma-globulin from bovine as well.
This Loadability study shows the efficiency of the media in separating the two components from 5 µl to 100 µl sample injected.
It starts with a gradient of 1.2 M Ammonium Sulfate, 0.1 M Phosphate at pH=7 for 4 minutes and then sharply decreases to
0 M Ammonium Sulfate, 0.1 M Phosphate at pH=7.
This is a Normal Bore column of 4.6 mm ID run at 1 ml/min which corresponds to 360 cm/hr of linear velocity for the empty column.
The pressure drop on the column is less than 20 bars at the start of the gradient with 1.2 M Ammonium Sulfate and decreases during the gradient as the salt concentration decreases.

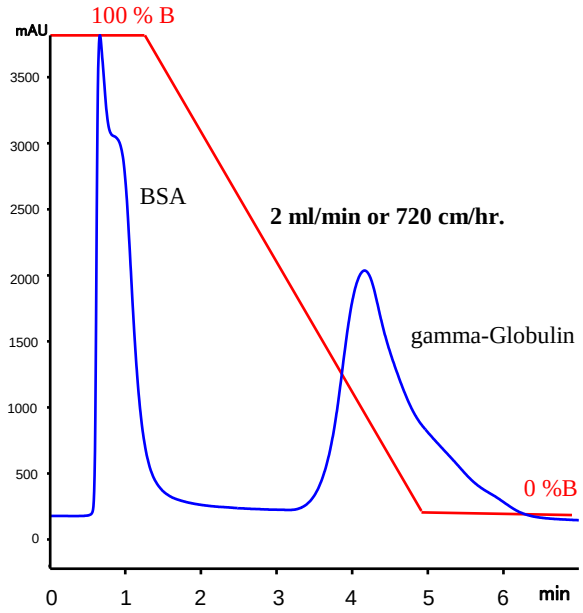


The column is run at volumetric flow rates of 1 and 2 ml/min. The linear velocities are 360 and 720 cm/hr. respectively.

Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS® HIC-Butyl/XH** 4.6 X 100 mm (v=1.66 ml)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 1.2 M SO4(NH4)2, pH=7
Flow rate	1 and 2 ml/min (360 and 720 cm/hr. on an empty column)
Gradient	As shown in chromatograms
Temperature	30°C
Detection	214 nm
Injection volumes	5 to 100 µl
Sample:	10 mg/ml of each component in buffer A.

The linear flow rate can be increased to 2 or 3 ml/min without major increase in pressure drop (20 and 40 bars respectively) as well as performance.



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