OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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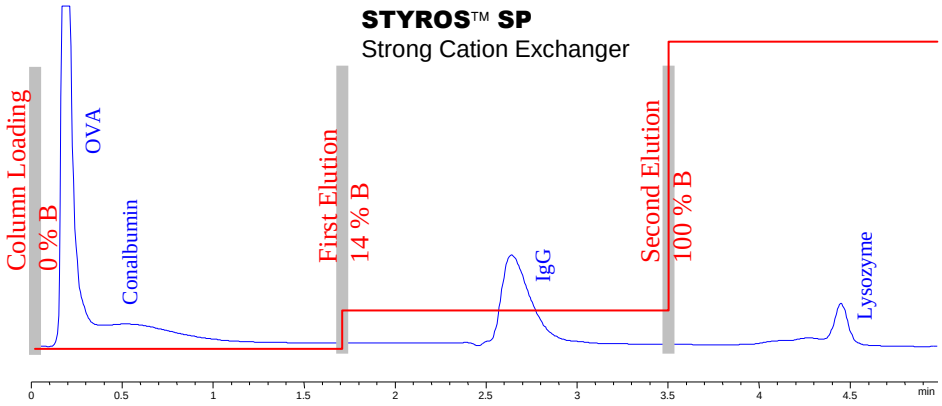
APPLICATION NOTE

Separation of IgG from Albumins in Commercial Production.

The optimum use of the stationary phase's capacity is critical for an industrial process to be economical.
To highlight this point we have considered the separation of Immunoglobulins from egg white proteins.
Since the majority of the proteins is made of egg albumin with an overall negative charge at neutral to acidic pH's, one can use the **STYROS**™ **SP** cation exchange media to capture the mass of the targeted IgG in the early stages of the purification and separate it from the egg albumin.
STYROS™ **SP** offers high dynamic capacity, therefore reducing the number of runs. It has large throughpores to accommodate the bulky biopolymers. The strong binding it offers is key in running it at high flow rates and still getting full separations.
The following elution profiles shows the separation of IgG from egg white proteins at a linear velocity of 1,800 cm/hr: the column is loaded in the first step with the mixture to be separated. OVA and Conalbumin are not retained at pH 7 of the phosphate buffer. The stronger retained proteins, in this case IgG and Lysozyme, also work as displacers and as a result get full access to the media's capacity. IgG is then eluted in the First Elution step with 14 % buffer B. The column is then washed with 100 % buffer B to remove Lysozyme.
The use of step gradient, combined with the high capacity and the fast run, provide the ideal characteristic for **STYROS**™ media to be used in continuous mode separations such as Simulated Moving Bed Liquid Chromatography.

The conditions are summarized in the following table.

HPLC System.	HP 1100
Columns	**STYROS**™ SP/XP 100x4.6mm
Mobile Phase	A: 20 mM Phosphate, pH = 7 B: A + 0.7 M NaCl
Flow rate	5 ml/min (1,800 cm/hr)
Temperature	30°C
Detection	280 nm
Samples	Egg white + IgG



The potential for a stationary phase to have industrial relevance depends on a number of characteristics it offers. These clearly include the media's dynamic capacity, which relates directly to the binding strength. The fast separation of large amounts of biomolecules at elevated flow rates reduces the process time and therefore the cost. Column lifetime, stability to a wide range of pH and chemical cleaning agents provide the end user the "Clean In Place" option that gives the stationary phase high values.