OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

Polymeric Hard Gel Media-HPLC vs.Soft Gel-FPLC in the Separation of BioMolecules.

Increasing speed while maintaining resolution is critical for all who use chromatography to separate, analyze, concentrate, and/or purify biomolecules – whether in research, quality control, or process scale production.
For each, cost of operation varies closely with run time – just as it increases with the volume of waste generated.

In comparing the performance of hard gels in HPLC with that of soft gels in FPLC, flow rate, eluent volume, media stability, and media lifetime must all be included in calculating the cost of achieving required resolution.
Highly subject to collapse because of the low degree of cross-linking, soft gels require large volume columns and must run at low flow rates. Raising pressure causes loss of gel integrity, leaching of fines, and foreshortened life.
Properly designed and sythesized polymeric hard gels media, in contrast, retain integrity under high pressure, are void of fines, and enjoy prolonged lifetimes of use. What resolution can they achieve?

Chromatograms 1 & 2 compare separation of proteins in egg white by a 10 µm monodispersed soft gel bead column (5 mm ID), with that achieved using a narrow bore (2.1mm ID) column packed with a highly corss-linked, fully pervious hard gel media.The separation on the hard gel HPLC system is complete in the same time as in the soft gel, while using half the volume of mobile phase and sample.

Table 1. shows the operating parameters:

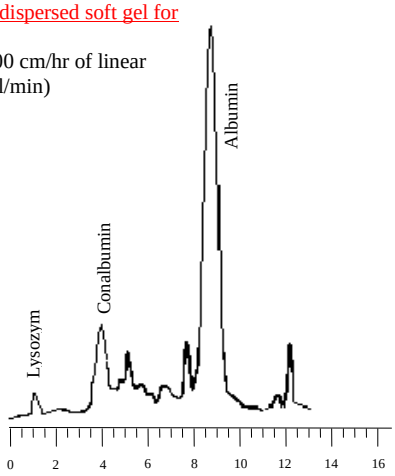
Columns (Strong Anion Exchangers)	**Monodispersed Soft Gel** 5x50 mm (chrom. 1) **STYROS™ HQ/NB** 2.1x150 mm (chrom. 2 and 3)
Mobile Phase	A: 20 mM Tris, pH = 8.2 B: A + 1 M NaCl
Gradient	0 to 10% B in 11 Column Volume (chrom. 1) 7 to 30% B in 18 Column Volume (chrom. 2 and 3)
Temperature	30°C
Detection	280 nm
Injection volume	4 µl (Chromatogram 1), 2 µl (Chromatogram 2 and 3)
Samples	Egg white (chicken) diluted 1 part to 9 in buffer A.

Separations can be optimized by increasing the rate of flow so long as the desired resolution is retained.

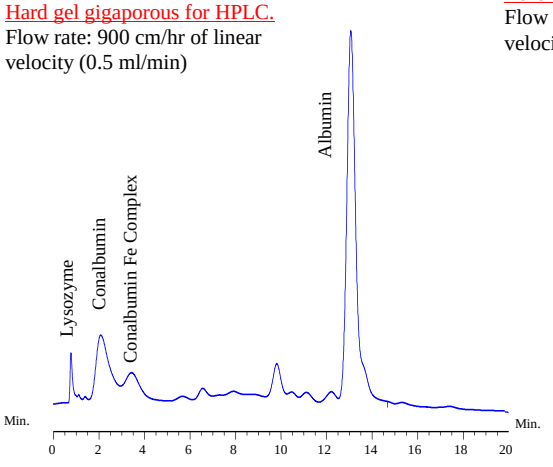
Chromatogram 3 illustrates separation by hard gel HPLC at a flow rate more than 10-fold greater than that tolerated by the soft gel in Chromatogram 1.

NB: The resolution remains essentially identical, while the total volume of eluent consumed is about two-thirds that required in the soft gel system. Run time is reduced from 12 minutes to under 4 minutes.

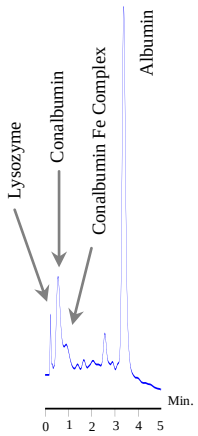
Chromatogram 1
10 µm monodispersed soft gel for FPLC.
Flow rate: 300 cm/hr of linear velocity (1ml/min)



Chromatogram 2
Hard gel gigaporous for HPLC.
Flow rate: 900 cm/hr of linear velocity (0.5 ml/min)



Chromatogram 3
Hard gel gigaporous for HPLC.
Flow rate: 3,500 cm/hr of linear velocity (2 ml/min)



Note well the linear flow rates, the times required for separation, and the total volumes of eluent.

STYROS ™ HQ/NB, the hard gel illustrated, tolerates pressures over 5,000 psi with no loss of integrity.
STYROS ™ hard gel media provide extraordinary life-times at highly competitive operating costs.