OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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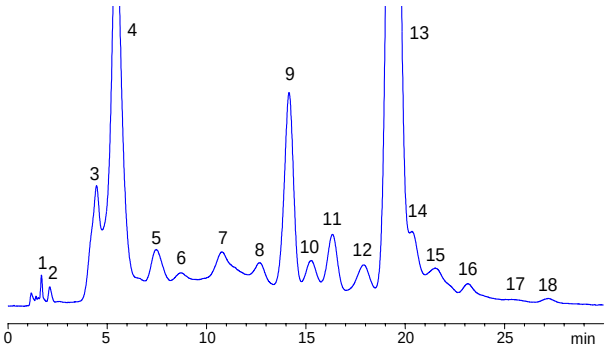
APPLICATION NOTE

Separation of Hen Egg White Proteins: STYROS™ HQ Compared with Mono Q.

The studies of hen egg white proteins aiming to elucidate the full range of their biological properties is an ongoing endeavor.
The challenge is to isolate the components without altering their biological structures.
HPLC and more specifically liquid chromatography on anion exchangers has been an efficient tool in this quest.
The limiting factor however, has been the stationary phase's performance.
The following chromatogram shows the separation of a sample of mucin free egg white on STYROS™ HQ column.

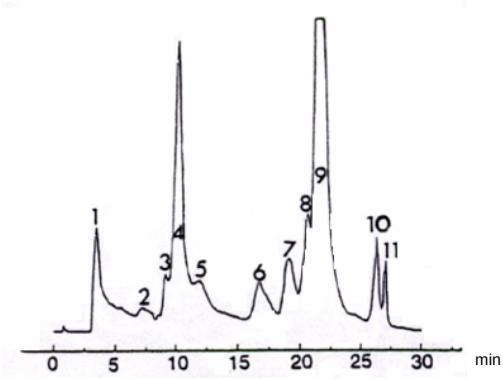


Chromatogram 1
Separation of mucin free egg white proteins on STYROS™ HQ/XH strong anion exchanger.
(Linear Flow Rate: 360 cm/hr)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ HQ/XH 4.6 X 100 mm
Mobile phase.	A: 20 mM TRIS, pH=8.2 B: A + 300 mM NaCl, pH=8.2
Flow rate	1 ml/min (360 cm/hr)
Gradient	2 to 100 % B in 30 min. (6 cv)
Temperature	30°C
Detection	280 nm
Injection volume	10 µl
Sample:	Mucin free egg white prepared in TRIS buffer (1:9 ratios).

At the operating pH of 8.2 only Lysozyme and Avidin are positively charged (with respective pI of 10.7 and 10). They will therefore not be retained on the anion exchanger column as free proteins.
The sample has been minimally handled and therefore all the proteins are in their native structures.
A total of 18 peaks are detected in the present chromatogram.
The most effective single run separation found in the literature so far belongs to A.C. Awadé and T. Efstathiou run on a Mono Q HR 5/5 (5cm L x 5 mm I.D.) column resulting in the separation of 11 peaks.



Chromatogram 2
Separation of mucin free egg white proteins on Mono Q HR
(Linear Flow Rate: 300 cm/hr)

Table 2. Operating parameters.

Columns	Mono Q, (5 cm L X 5 mm ID)
Mobile phase.	A: 20 mM TRIS, pH= 9 B: A + 500 mM NaCl, pH= 9
Flow rate	1 ml/min (300 cm/hr)
Gradient	Multistep gradient from 0 to 100% B in 30 min.
Temperature	25°C
Detection	280 nm
Injection volume	100 µl
Sample	Mucin free egg white prepared in 20 mM TRIS buffer with 10 fold dilution and 10 mM β-mercaptoethanol.

The use of STYROS™ polymeric columns allows a better separation under conditions that are close to the biological pH of egg white (pH=9) and there is no need for β-mercaptoethanol as reducing agent.
The increased number of peaks indicates the higher resolving capabilities of the media and therefore provides the researcher the added advantage of one step isolation of the proteins that make up the mixture.
The gradient used in the present case with the STYROS™ column is simple; further adjustments can be made to refine peaks of interest.
Since the media can tolerate high back pressure it is possible to run fast equilibrations in short periods.
Unlike Mono Q media, STYROS™ columns are available in many sizes for additional resolving capabilities.

Inc.