OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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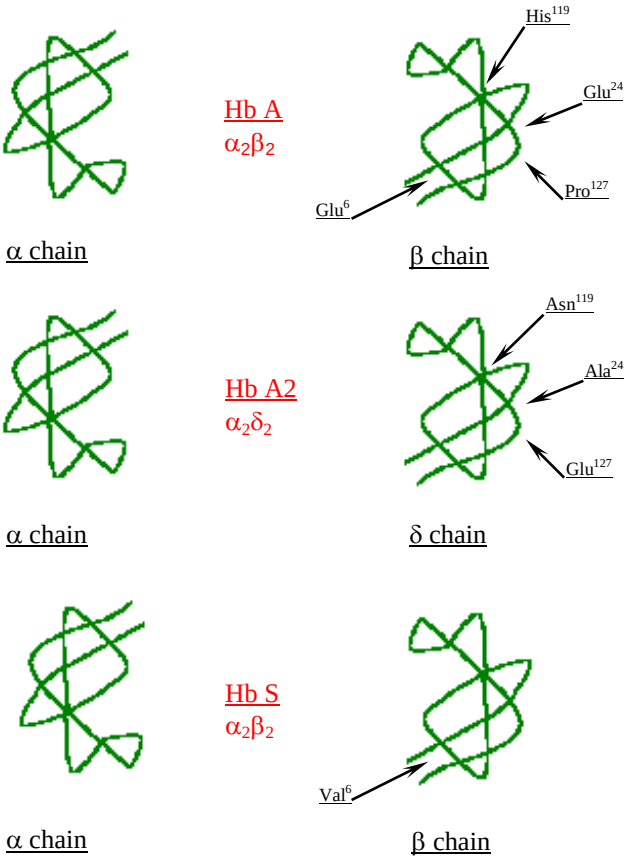
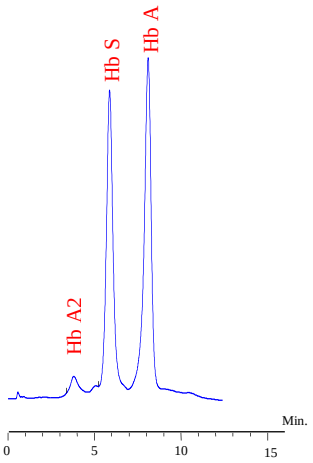
APPLICATION NOTE

Fast Separation of Protein Isoforms with Fully Porous Hard Gel Media: Hemoglobins.

Separation of nearly identical protein variants (isoforms) is a very difficult challenge, whether for characterization, quantitation, and/or purification.
Ion exchange chromatography with highly porous polymeric hard gels can provide a high degree of resolution far more efficiently than traditional soft gel or reversed phase chromatography.
The chromatogram at right shows hemoglobin (Hb) isoforms separated on a narrow bore DEAE **STYROS**™ column. The structure of Hb A (the principal isoform in the adult) is a heterotetramer : $\alpha_2\beta_2$.
Hb A2, a second Hb species in the adult, varies at the three δ-chain aminoacids (diagram).
Hb S, the pathologic variant in sickle cell anemia, differs only in the substitution of Glutamic acid by Valine in the β-chain. It is nevertheless sharply separated by **STYROS**™.





The chromatographic conditions are summarized in the following table.

HPLC System.	HP 1100
Column	**STYROS**™ DEAE/NB 250x2.1mm
Mobile Phase	A: 20 mM Tris, pH = 8.2 B: A + 1 M NaCl
Flow rate	1 ml/min (1,800 cm/hr)
Gradient	1 to 15 % B in 18 Column Volume
Temperature	30°C
Detection	280 nm
Injection volume	5 µl
Samples	Hb A, Hb S (2.5 mg/ml each) Hb A2 (as impurity)

Note: the linear flow rate is 1,800 cm/hr, to be compared with the usual rate of 180 cm/hr available with soft gel media, a 10 x advantage.
Separation is complete: there is essentially no loss of resolution compared with that at lower flow rates.
The high dynamic capacity (80 mg/ml) of **STYROS**™ media avoids the saturation artifacts commonly seen with lower capacity media.
Regeneration and re-equilibration of the **STYROS**™ column requires less than 5 minutes. The useful life of the column is 3-5 times longer than stationary phases based on silica or soft gels.
Revalidation requires proportionally less time.