

APPLICATION NOTE

Separation of Cytochrome c Isoform on **STYROS™ SE**: Strong cation exchanger.

The characterization of closely related forms of proteins, known as “isoform”, is essential as they may indicate:

- a- Specific structural change during the purification process or storage.
- b- Post-translational changes such as glycosylation, phosphorylation or lipidation.
- c- Substitution of one or more amino acids as a result of gene mutation.

Since the isoelectric point (pI) of protein isoforms are very close, it is important to use very efficient HPLC columns to properly separate them.

Ion exchange chromatography with **STYROS™** columns has been very effective in achieving high level of separations.

The following examples show:

- 1- the separation of 2 close isoform of Cytochrome c from bovine and horse heart (figure 1)
- 2- the separation of normal hemoglobin A from the abnormal form S (figure 2).

The column is a strong cation exchanger **STYROS™** SE with medium capacity.

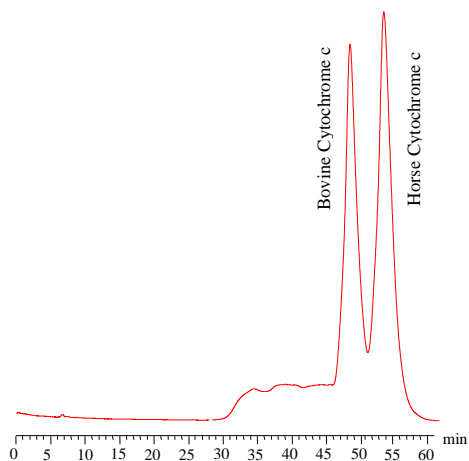


Figure 1

STYROS™SE/XP, Strong Cation Exchanger
4.6 x 250 mm. 0.5 ml/min (180 cm/hr), at pH= 7

Table 1. Operating Parameters.

HPLC System.	HP 1100
Columns	STYROS™ SE/XP 4.6 x 250 mm
Mobile Phase	A: 20 mM Bis-Tris-HCl, pH = 7 and 6 B: A + 1 M NaCl, Ph=7 and 6
Flow rates	As indicated
Gradient	8 to 17 % B in 9 cv for the separation of Cytochrome c, 4 to 30 % B in 11 cv for the separation of Hemoglobin.
Temperature	30°C
Detection	280 nm
Injection volume	25 µl
Samples	Cytochrome c from horse and bovine heart (5 mg/ml each). Hemoglobin A and S (5:1.5 mg/ml)

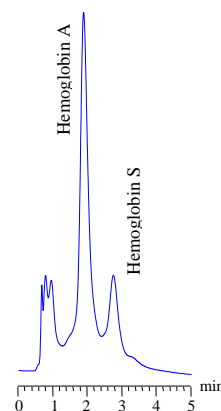


Figure 2

STYROS™SE/XP, Strong Cation Exchanger
4.6 x 250 mm. 5 ml/min (1,800 cm/hr), at pH=6

Hb S differs from Hb A by only one amino acid in position 6 of the β-chain: the substitution of Glutamic acid by Valine. The α-chain of the α₂ β₂ heterotetramere are similar.

The Cytochrome C variants chosen here differ only by 3 amino acids, yet their separation was performed at linear flow rates of 1,800 cm/hr.

Notice the linear velocity of 1,800 cm/hr (based on an empty column), far exceeds those used with either soft gel or non pervious media.