OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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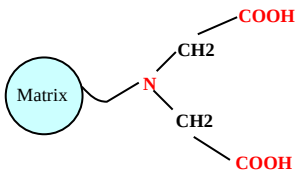
APPLICATION NOTE

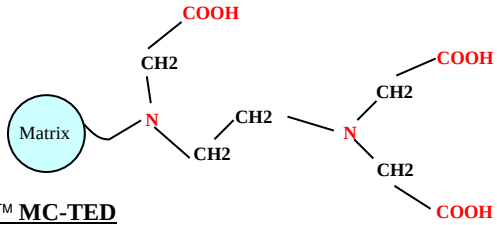
Metal Chelate Liquid Chromatography on Hard Gel Gigaporous Polymeric Media: Tri-dentate IDA versus Five-dentate TED:

The structure of the complex formed with a particular metal depends upon the metal used, the composition of the buffer and the number of chelates forming the functionalities of the stationary phase.

STYROS™ MC-IDA (Iminodiacetic acid) is a three-dentate metal chelate media whereas **STYROS™ MC-TED** [Tris (carboxymethyl) ethylenediamine] is a five-dentate metal chelate stationary phase.

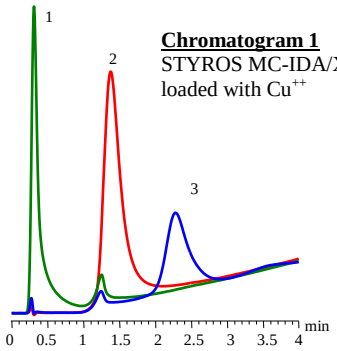


STYROS™ MC-IDA
(Iminodiacetic acid)

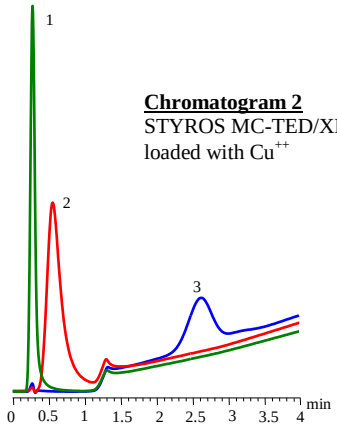


STYROS™ MC-TED
[Tris (carboxymethyl) ethylenediamine]

Each of these stationary phases offers a different alternative, **STYROS™ MC-TED** being a weaker absorbent than the corresponding **STYROS™ MC-IDA.**
Both metal chelates can be loaded with up to 50 µmole/ml of Cu^{++} providing a full range of retentivity.
There is no need to saturate the column at the metal loading stage. Indeed it is recommended that the column be loaded gradually until the optimum loading is reached.
In contrast to soft gel, the column can be used under high pressures (up to 4,000 psi) and high flow rates, therefore reducing the run time considerably.
The following chromatograms compare the two stationary phases under similar conditions.



Chromatogram 1
STYROS MC-IDA/XP 4.6 X 50 mm loaded with Cu^{++}



Chromatogram 2
STYROS MC-TED/XP 4.6 X 50 mm loaded with Cu^{++}

Table 1. Operating parameters

HPLC System.	HP 1100
Column	As indicated
Mobile Phase	A: 20 mM Sodium Phosphate, 1 M NaCl, pH = 7.5 B: 20 mM Sodium Phosphate, 1 M NH4Cl, pH = 7.5
Flow rate	2.5 ml/min (900 cm/hr)
Gradient	0 to 100% B in 12 Column Volume
Temperature	30°C
Detection	280 nm
Injection volume	5 µl
Sample: (5 mg/ml each respectively).	1: Cytochrome c, 2: Lysozyme, 3: Myoglobin (dissolved in 50 % buffer A) Proteins are assessed by the supplier to be 99% pure.

A typical **STYROS™ MC-IDA** column loaded with Cu^{++} can be used in as many as 50 separation cycles before it requires any regeneration.