Metal Interactions in Chromatography

Abstract
While metal-analyte interactions in IMAC (immobilized metal affinity chromatography) are widely used to analyze and purify certain compounds, the same interactions can have a detrimental effect on peak shape in other modes of chromatography. This effect has been known for a number of decades and led to the development of high purity silica in the 1980s. However, there are other sources of metal within the chromatographic system, which can cause deterioration of peak shape and sensitivity.

Sources of Metal in Chromatography
Any surface that comes in contact with the sample has the potential to interact with the sample components. Since the surface areas involved are rather small (compared to the surface area of the stationary phase) these interactions manifest themselves in the form of asymmetries of the elution peak.

Metal interaction can occur on the stainless steel of the transfer tubing, the frit and the column body. Stainless steel contains ~60 % Fe, 20 % Ni and 20 % Cr, making it predominantly hard Lewis acids.

Another source of metal can be trace impurities in the silica (Fe, Ca, Al, Mg and Ti). With modern silicas the purity of the matrix improved dramatically but the surface area is several orders of magnitude larger than the other system components the effect of impurities can still be significant.

ProteCol™ Column Hardware
In order to minimize non-specific interactions the inner walls of the column tubing is coated by either glass or PEEK™ and a porous PEEK™ frit is employed allowing the estimation of the hydrophobicity of the column while the relative LC system.

Application: Tetracycline Antibiotics
Tetracycline is a member of a group of antibiotic drugs commonly used in human and veterinary medicine. The molecule has a metal chelating part of the cyclopirox molecule, an anti-fungal drug. In this experiment, a stainless steel column with metal frit and a PEEK™-lined column with porous PEEK™ frits were packed with the same packing material. The columns were attached to the HPLC system with stainless steel capillaries or with PEEK™-lined fused silica.

In the test mix uracil is a non-retained compound and marks t a value needed for further calculations. The capacity factors (k') for tetracycline and most of the other compounds in the mixture are large enough to allow the estimation of the hydrophobicity of the column while the relative retention between tetracycline and tetracycline gives an estimate of the selectivity of the column.

The chromatogram on the ProteCol™-lined column with porous PEEK™ frits was packed with the same packing material. The column was tested with the following conditions:

Table 1: Pearson's Hard/Soft Classification of Lewis Acid and Bases

Metal Chelates
Chelates are formed when the organic partner contains two or more Lewis bases in a favorable position. The resulting 5- or 6-ring structures (Fig. 1) are entropically favored to comparable monodentate complexes. Classical examples of chelates are EDTA (Fig. 2) and imino diacetic acid.

Metal-Free Chelates
Metal-free chelates are formed when the organic partner contains two or more Lewis bases in a favorable position. The resulting 5- or 6-ring structures (Fig. 1) are entropically favored to comparable monodentate complexes. Classical examples of chelates are EDTA (Fig. 2) and imino diacetic acid.

Results displayed as Figure 6 highlight the importance of excluding any exposed metal of getting in contact with the sample - connection capillaries, frits, column body and low activity stationary phase.

Application: Tetracycline Antibiotics
Tetracycline is a member of a group of antibiotic drugs commonly used in human and veterinary medicine. The molecule has three potential chelating sites for iron aligned at one site. The drugs are known to bind metal ions as dietary calcium and iron can render them ineffective.

Chromatographic conditions:
Sample: 1 mg/mL tetracycline
Flow-rate: 1.0 mL/min
Temperature: 23 °C
Injection volume: 1 μL
Mobile-phase: A: 80 % acetonitrile/H2O
Detection: 254 nm

Figure 7 demonstrates that changing from stainless steel column to a PEK™ coated column increase the sensitivity (peak area) of the tetracycline peak by 35 %. There is also noticeably less peak broadening on the base of the peak. Inset: the tetracycline molecule depicting the three potential chelating groups.

Conclusions
Non-specific metal interactions anywhere in the system can have a negative effect on chromatographic performance. The extent of adverse effects are related to the amount of metal surfaces present and the strength of the interaction between the analyte and the metal surfaces. By using a combination of high purity silica, non-metallic connection capillaries and a metal-free column design it is possible to suppress non-specific interactions and significantly gain in sensitivity in the analysis of chelating samples.

Chromatographic conditions:
Sample: 0.5 mg/mL N-hydroxypyridine-2-on
Flow-rate: 1.0 mL/min
Temperature: 25 °C
Injection volume: 1 μL
Mobile-phase: A: 50 % Acetonitrile/water
Detection: 254 nm

The column was tested with the following conditions:

Figure 4: Test conditions for the NIST SRM870 test mix

Figure 3: Inert Column Designs for ProteCol™ Columns

Figure 2: EDTA-H complex

Figure 1: Stable chelate conformations

Figure 5: Chromatograms of the NIST SRM870 test mix on a wide range of columns.

Figure 6: Effect of exposed metal in the flow path on the Chromatography of Chelating compounds.

Figure 7: Chromatogram of tetracycline and its major degradation product

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