Trace Corrosion of Metallic Components from Common Mobile Phase Additives and the Deleterious Impact on Separations

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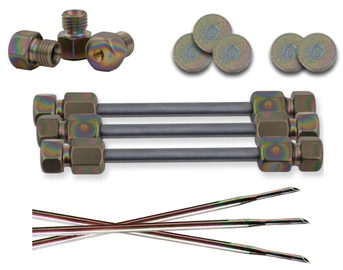
Introduction

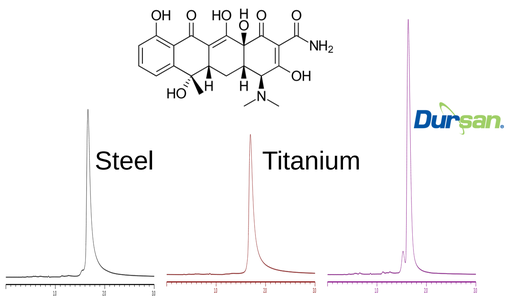
As separation and purification techniques move toward higher sensitivity and higher throughput, the need for a robust yet inert fluid pathway has become more critical. A typical HPLC instrument is made from stainless steel, PEEK, and/or ceramic components. The steel in these systems will have active sites on the surface that require routine passivation or priming to be effective when analyzing reactive compounds, especially at low detection limits. Additionally, stainless steel is not favorable for biological analysis as the material is not considered “bio-inert”. Metal ions can also leach into the flow path causing issues. For instance, oligonucleotides can suffer oxidation or degradation, proteins can experience irreversible aggregation, fermentation processes result in lower yields all due to elevated concentrations of various metal ions. Titanium typically replaces the stainless steel, but the metal surface can still be a source of difficulties when analyzing metal active compounds. PEEK is often used as a bio-inert, metal free surface; however, there are pressure and machinability issues involved with this material.

Here we investigate a different approach that would allow the utilization of metal alloys while reducing the amount of metal that can interact with solution: chemical vapor deposition (CVD) coated metal hardware. We investigate the amount of metal that leaches into water, methanol, and acetonitrile when in direct contact with stainless steel, titanium, MP35N and Hastelloy via ICP-MS. We also show how CVD coatings can bring the concentration of metals leached down to zero. Samples separations will be shown comparing the coated hardware to steel hardware for metal active compounds.

CVD coated hardware

Dursan® is an amorphous silica-like coating that can be applied to any non-plastic part in the flow path of an instrument (needles, tubing, columns, pumps, frits, etc). This coating is inert to most chemicals and has twice the wear resistance of stainless steel. It can handle both acidic and basic conditions.

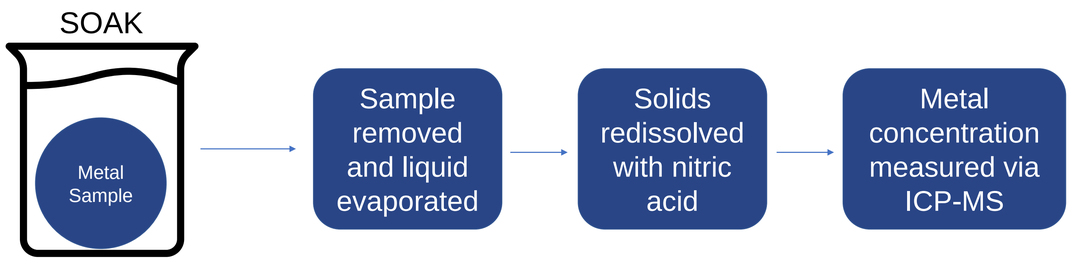




*Data gathered in cooperation with William Campbell – Phase Analytical Technology, LLC

Separation of tetracycline shows the coating can provide an inert surface for better peak height, less tailing, and separate out small impurities that may otherwise be missed.

Experimental



- Samples were 10 µm frits with the exception of MP35N samples (cylinders).
  - Surface area of the frits were measured via porosimeter and MP35N sample surface area was calculated via geometric measurements.
- Pure solvents were allowed to soak for one month at room temperature.
- TEAA, TEA+HFIP, TFA, and MSA samples were soaked for 1 week at 60°C at various ratios of aqueous and organic solvent as described in the data:
  - Triethylammonium acetate: Mobile phase A – 100 mM TEAA in water, Mobile phase B – 100 mM TEAA in acetonitrile (UV compatible oligonucleotide solvent)
  - Triethylamine and HFIP: Mobile phase A – 15 mM TEA + 400 mM HFIP in water, Mobile phase B – 50:50 v/v Mobile phase A + Methanol (Mass spec compatible oligonucleotide solvent
  - Trifluoroacetic acid and Methanesulfonic acid: 0.1% v/v in water or acetonitrile

Pure solvent results

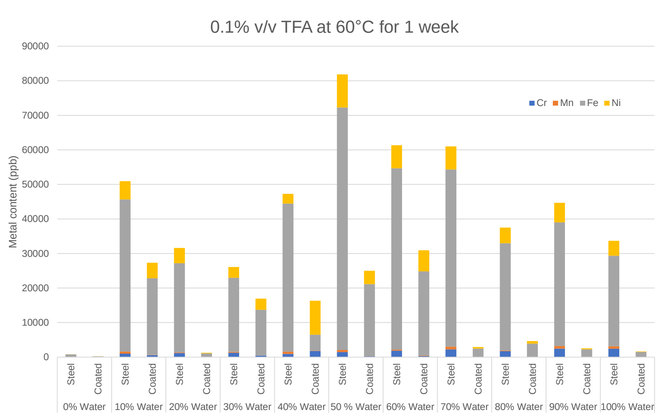
Metal Ions leached into solution after a 1-month soak

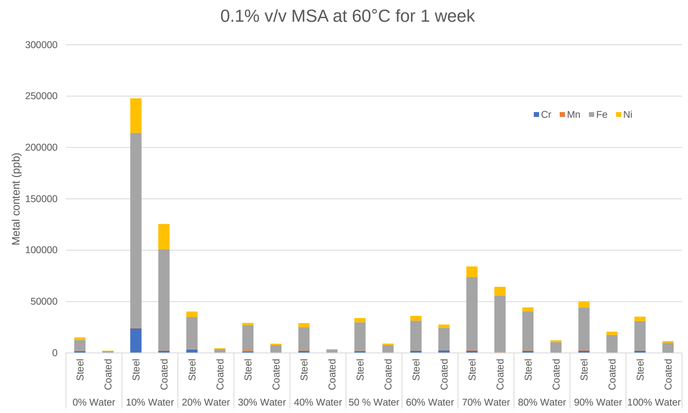
	316 Stainless steel	Titanium	MP35N	C-22 Hastelloy	Our coated coupons
UHPLC Grade DI water	Fe Cr Ni Mo	Ti	Ni Cr Mo Co	Fe Cr Ni Mo	All Metals
UHPLC Grade methanol	Fe Cr Ni Mo	Ti	Ni Cr Mo Co	Fe Cr Ni Mo	All Metals
UHPLC Grade acetonitrile	Fe Cr Ni Mo	Ti	Ni Cr Mo Co	Fe Cr Ni Mo	All Metals

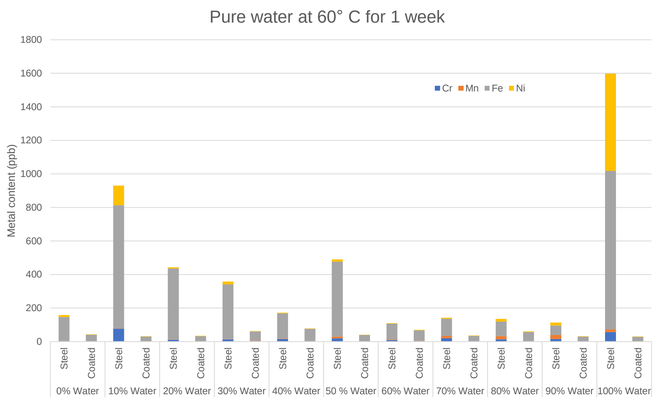
<1 ppb/m² <100 ppb/m² <1000 ppb/m² >1000 ppb/m²

For full details of this study, please use the QR code shown here:

Aggressive buffer solutions





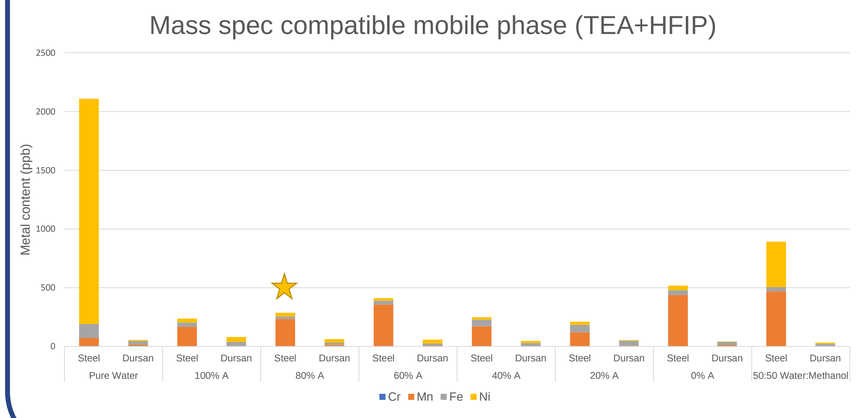


Key Takeaways:

- MSA is very aggressive compared to TFA (note scale bars)
  - This is not surprising as MSA is often used as a surface etchant for stainless steel aqueous cleaning agents
- Coatings do provide some protection in these conditions; however, the amount of metal leached is still significant
  - Work is being performed now on alternative coatings to decrease the amount of metal leached
- 10% Water acid solutions are significantly more corrosive than pure water or pure acetonitrile – reason unknown
- No obvious pattern among the mixtures, but results match limited literature publications[1]
- The elevated temperatures on pure water show larger amounts of metal leaching than the room temperature experiments that had a 4x longer soak time

1. M.Y. Cheung, J. Bruce, M.R. Euerby, J.K. Field, P. Petersson., *Journal of Chromatography A*. **2022**, 1668, 462888,.

Oligonucleotide solvent systems



General observations:

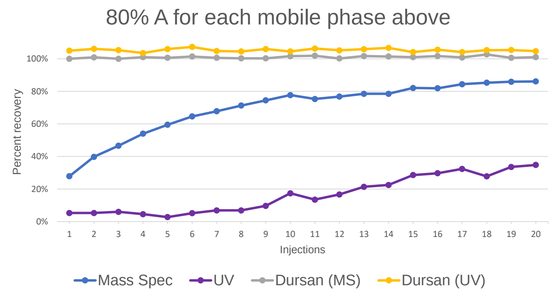
- Addition of TEAA causes an increase in metal ion leaching compared to pure water (compare to 100% A) or pure acetonitrile (compare to 0% A)
- Mn contributions are surprising as 316 steel has only 2% Mn in the bulk
- Aqueous solution attacks the nickel while organic solution attacks the chromium and iron
- Dursan significantly decreases the amount of metals that are leached

General observations:

- Addition of TEA + HFIP causes a decrease in the amount of metal ions leached compared to pure water and the 50:50 water and methanol mixture
- Manganese once again shows significant contributions compared to the bulk percentage of manganese in steel
- Dursan significantly decreases the amount of metals that are leached

★ Refer to “Oligonucleotide Recovery” section for impact of the metal ion leaching to non-specific binding

Oligonucleotide Recovery



Experimental details
- 20mer Oligonucleotide all phophorothioated
- 50 x 2.1 mm ID empty column (no separation intended, only looking for recovery)
- Peak area without a column is used as the 100% reference value

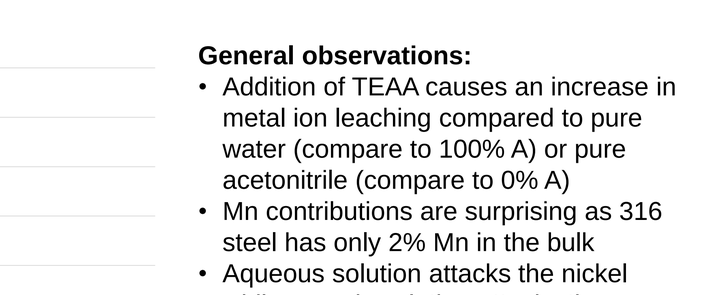
*Data courtesy of YMC America

Preventing column bleed

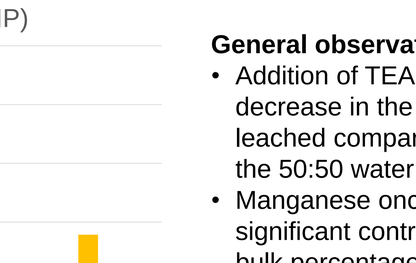
Separation of 6 mushroom alkaloids on a Restek Force Biphenyl column (UV detection):
- Bare Stainless Steel
- Medronic acid treated steel
  - Common treatment to eliminate metal contamination
- Dursan coated

- Chromatograms above are the first injection for each column
- Metal induced column bleed arises after 25 injections on the Dursan column due to metal sources upstream (Tubing, pumps, valves, etc in the HPLC instrument)
- The biphenyl end group is UV active and causes baseline drift

Phosphate separations



*Data courtesy of anonymous HPLC equipment manufacturer- run conditions and packing material identical in each injection



- Dursan shows the same metal free performance as PEEK
- Note that even after 10 injections, the stainless steel does not prime to the same recovery level as PEEK and Dursan.

*Data courtesy of Diego Lopez- Restek Corporation

Conclusion

Both metallic surfaces as well as metal ions leaching into the flow path are of concern for many industries that utilize LC separation techniques for both analytical work as well as purifications. No metal alloy here is immune to leaching oxidizing metallic ions; however, titanium seems to be the most promising material without a coating. Coating the metal surfaces with a silica-like CVD coating provides sufficient protection for column and equipment lifetime extensions. Dursan is an ideal coating for these conditions as it has a very wide operational pH range (0-14), is inert to most materials, and will not allow metal ions into the flow path to impact separations. All metal components (frits, tubing, columns) can be coated.

Frequently asked questions

How is the coating applied?

Can I get a coated sample?

Where can I get already coated product?