Improved Biomolecular Analysis with Inert CVD Coating that Prevents Metal Interactions and Non-Specific Protein Binding

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Overview

- Metal ions and surfaces can have dramatic impacts on fermentation, purification, aggregation, analysis, etc.
- Most equipment is made of stainless steel – iron is a huge problem
- Rather than moving toward exotic alloys or plastic, a silica like coating could be used (Dursan is the name of our coating)

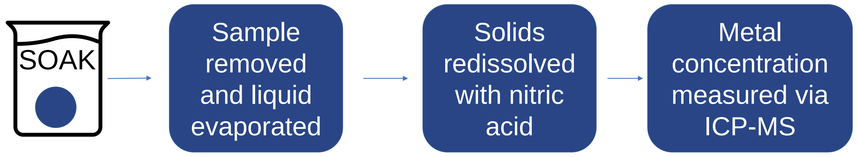
Introduction

Stainless steel contains iron which can cause issues such as oxidative stress, irreversible aggregation, and disrupt otherwise controlled fermentation processes. Options to avoid steel include use of alternative, iron free metals, or move toward plastic materials. These too have issues that may be difficult to overcome.

Here we investigate an approach that would allow the utilization of stainless steel 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. Sample separations will be shown comparing the coated hardware to steel hardware for metal active compounds. Finally, the CVD coating's resistance to non-specific protein binding from a clinical diagnostic application will be demonstrated.

Methods

- 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

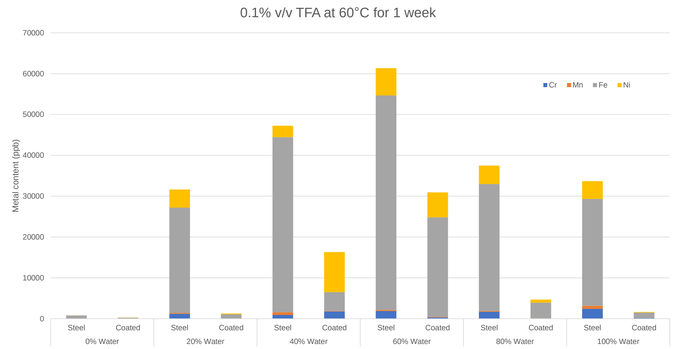
Metal Ions leached into solution after a 1-month soak

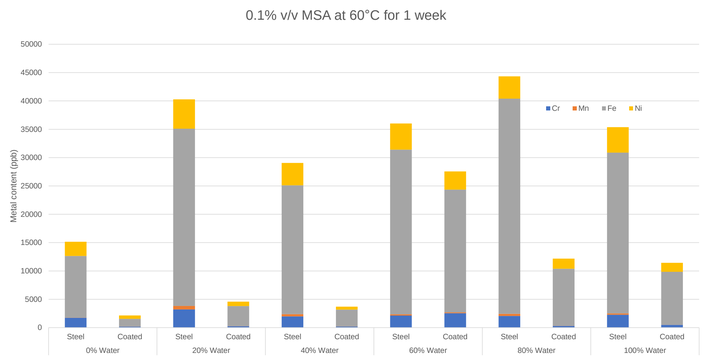
	316 Stainless steel	Titanium	MP35N	C-22 Hastelloy	Our coated coupons
UHPLC Grade DI water	Fe Cr Ni Mo	Ti	Ni Cr Mo Co	Fe Cr Ni Mo	All Metals
UHPLC Grade methanol	Fe Cr Ni Mo	Ti	Ni Cr Mo Co	Fe Cr Ni Mo	All Metals
UHPLC Grade acetonitrile	Fe Cr Ni Mo	Ti	Ni Cr Mo Co	Fe Cr Ni Mo	All Metals

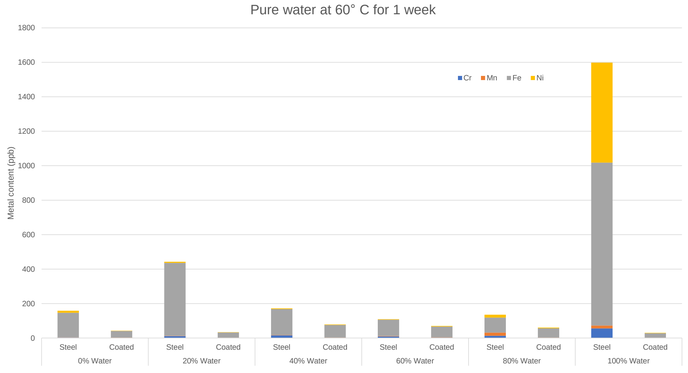
<1 ppb/m² <100 ppb/m² <1000 ppb/m² >1000 ppb/m²

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

Aggressive buffer solutions





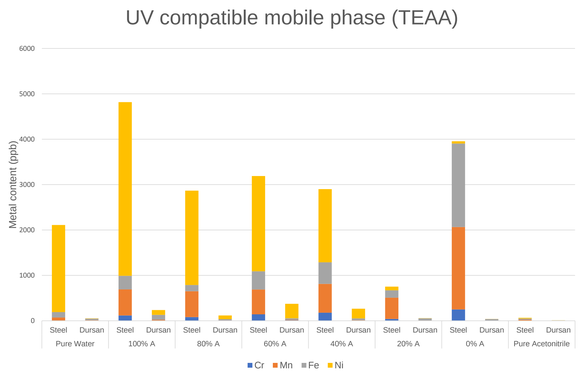


Key Takeaways:

- 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
- 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

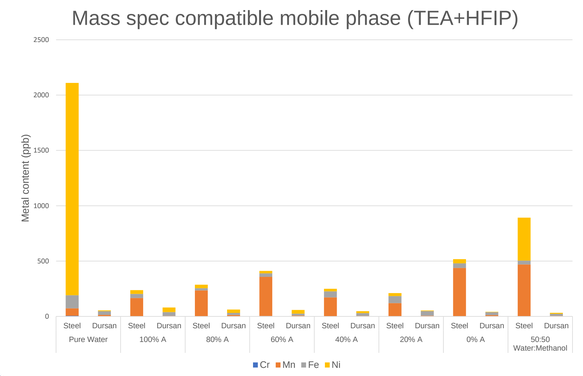
1. M.Y. Cheung, J. Bruce, M.R. Euerby, J.K. Field, P. Petersson., *Journal of Chromatography A*. **2022**, 1668, 462888,.

Oligonucleotide solvent systems



General observations:

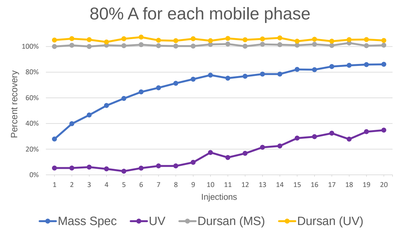
- Addition of TEAA causes an increase in metal ion leaching compared to pure water (compare to 100% A) or pure acetonitrile (compare to 0% A)
- Mn contributions are surprising as 316 steel has only 2% Mn in the bulk
- Aqueous solution attacks the nickel while organic solution attacks the chromium and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

Oligonucleotide Recovery



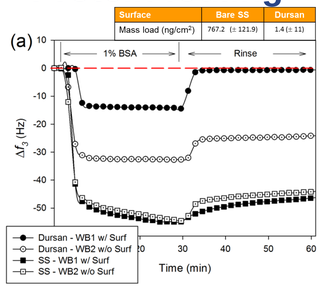
Experimental details

- 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

Protein fouling

Surface	Bare SS	Dursan
Mass load (ng/cm²)	767.2 (± 121.9)	1.4 (± 11)



The combination of Dursan and a surfactant allows for near-zero non-specific binding of BSA and many others. See paper for full details:

2. Vaidya, S.V.; Yuan, M.; Narvaez, A.R.; Daghfal, D.; Mattzela, J.; Smith, D. *Applied Surface Science* **2016**, *364*, 896-908.

Conclusions:

Metal ion leaching

- Dursan on stainless steel allows for minimal metal ion leaching
- Lower metal leaching from hardware provides the scientist with more control of fluidic environments

Non-specific binding

- Dursan allows for the highest recovery of oligonucleotide through HPLC hardware
- Dursan in combination with surfactants provide minimal non-specific binding of proteins like BSA