Achieving Metal Free HPLC Analysis with a Bio-inert Chemical Vapor Deposited Coating

Jesse Bischof, Ph.D.
SilcoTek Corporation
2/25/2020

Overview

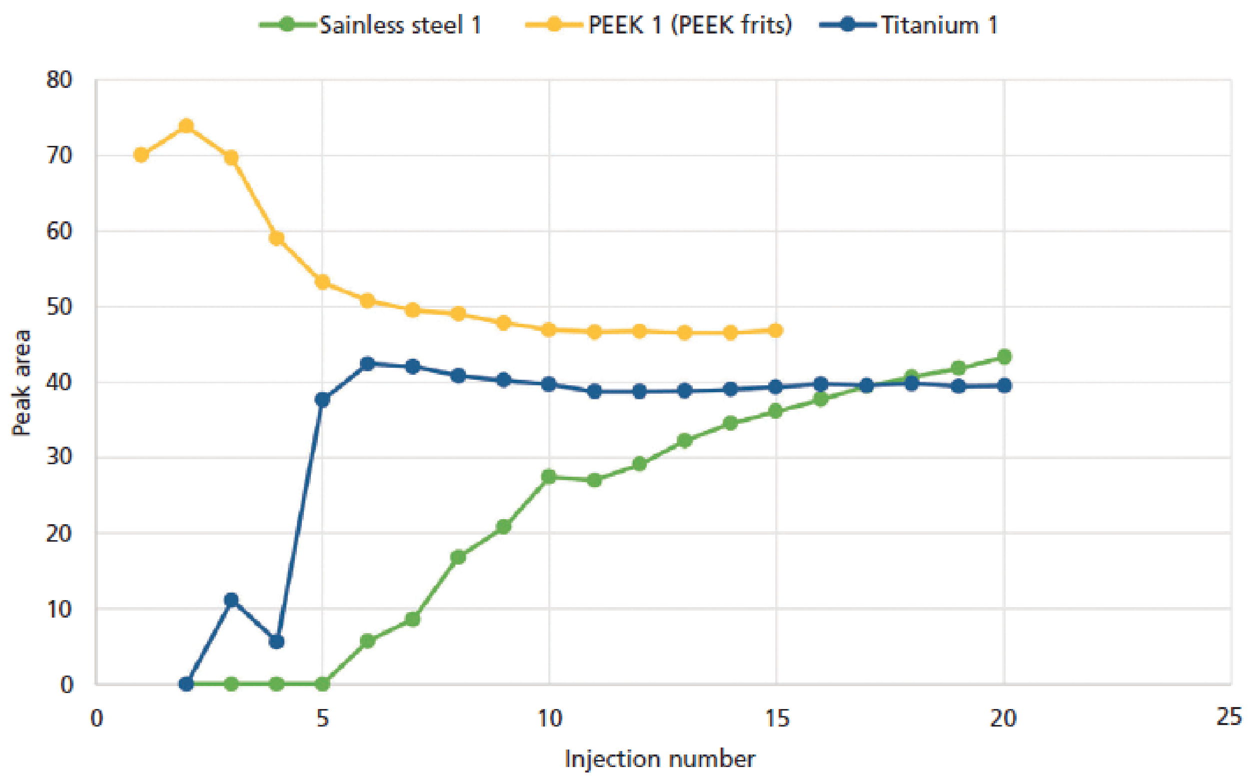
- Current hardware materials and potential issues
- Overview of SilcoTek Technology
- Materials properties of Dursan®
  - Inertness
  - Corrosion resistance
  - Anti-protein fouling properties
- Dursan® used in HPLC columns

Current HPLC materials

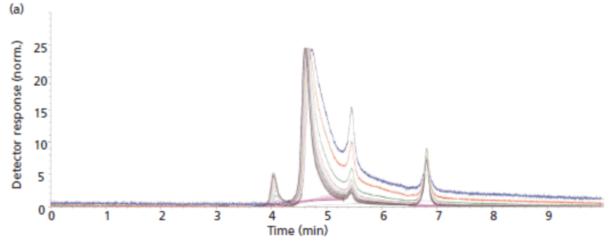
Material	Mechanically strong	Bio-inert	Metal free	Chemical incompatibility
Stainless Steel	✓	✗	✗	Halogens, some acids, pure organic solvents
Titanium	✓	✓	✗	Some acids, Methanol and other organic solvents
PEEK	✗	✓	✓	THF, some acids slowly attack

Stainless steel: Inertness issues

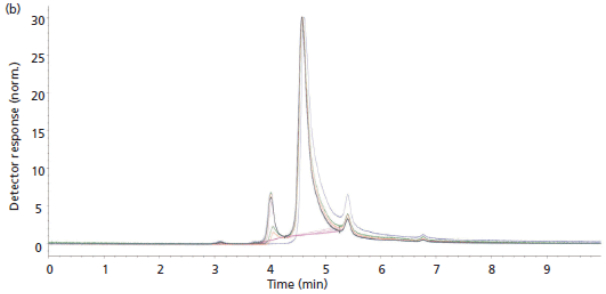
SEC analysis of γ-globulin and ovalbumin by Phenomenex:



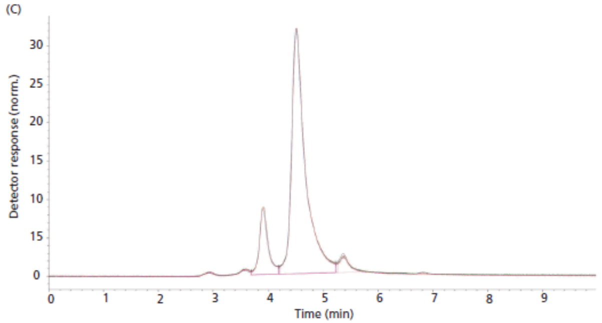
Steel:



Titanium:



PEEK:



Anspach, J.A., Rao, S., Rivera, B., "Bioinert Versus Biocompatible" LCGC Magazine, Volume 36, Issue 6, p. 24-29.

Titanium's Compatibility with Methanol

SilcoTek

NASA TECHNICAL NOTE

NASA TN D-3868

c. 1

NASA TN D-3868

STRESS-CORROSION CRACKING OF Ti-6A1-4V ALLOY IN METHANOL

by Robert L. Johnston, Robert E. Johnson, Glenn M. Ecord, and Willard L. Castner

Manned Spacecraft Center
Houston, Texas

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • FEBRUARY 1967

Technical Note

Effect of "Pure" Methanol on the Cracking of Titanium*

*E. G. HANEY and W. R. WEARMOUTH**

The failure of titanium under stress in methanol-water-chloride solutions is characterized by a dependence upon the water content. As water in the methanol is increased, cracking of titanium is increasingly detrimental until water has been added in substantial amount to inhibit the cracking process.[1,2]

The resulting minimum in the curve of time-to-failure plotted against the water content of the methanol has been shown to intensify and its position to shift toward higher water contents when the chloride content was increased from 10^{-4}N NaCl to 10^{-2}N NaCl. Whether the chloride was added as NaCl or HCl was not significant at the 10^{-2}N level.[1]

However, in current literature, there is some contention as to the necessity for the presence of anything other than liquid methanol to promote cracking in titanium and its alloys. Some investigators were not able to obtain failures within the duration of the exposure unless some activating agent was also present such as certain halide ions.[2-4] Mori[4] *et al* was able to crack titanium with about 10^{-3}N HCl in methanol but not with methanol alone. Others have reported failures with methanol,[5-8] but there is no indication that the solutions were checked for possible traces of chloride contamination.

The purpose of the present work was to define the effect of chloride contents between 10^{-2}N HCl and "pure" methanol on the cracking of two titanium alloys in methanol-water solutions.

Experimental

Two commercial alloys, one an alpha alloy and the other a beta alloy, namely, 35A grade of pure titanium and Ti-13V-11Cr-3Al, were investigated. The alloys were tested in foil form, approximately 0.0035 inch thick, having been rolled on a Sendzimer mill and sheared to quarter-inch wide strips and cut parallel with the rolling direction by automatic slitting equipment at the mill. The Ti-13V-11Cr-3Al foil was received in the annealed condition. The Ti-35A foil was cold rolled as-received and was annealed in the laboratory at 1300 F (705 C) in sealed vycor tubes under a reduced pressure of helium. The mill compositions were reported in weight percent as shown in Table 1.

Tensile tests were performed on an Instron[(1)] testing machine. The results, in Table 2, show the ultimate tensile strength and 0.2% offset yield strength in pounds per square inch and the elongation as percent in two inches.

The apparatus for loading and exposing the foil specimens was designed for constant stress conditions and total immersion of specimens during exposure. After degreasing with methyl-ethyl-ketone, the 4-inch long foil specimens were threaded through 60 ml polyethylene bottles, used to contain the methanol and sealed with

(1) Instron Engineering Corp., Quincy, Mass.

TABLE 2—Tensile Properties

	Y.S., psi	T.S., psi	Elongation
Ti-35A	34,900	47,700	28.8
Ti-13V-11Cr-3Al	139,600	140,400	30.7

TABLE 1 – Mill Compositions in Weight %

	Al	V	Cr	Fe	O	C	N	H
Ti-35A	–	–	–	.07	.07	.023	.010	.004
Ti-13V-11Cr-3Al	3.2	13.8	10.6	.21	.12	.04	.03	.013

*Submitted for publication November, 1968.
*Mellon Institute of Carnegie-Mellon University, Pittsburgh, Pa.

Vol. 25, No. 2, February, 1969 87

ELSEVIER

Corrosion Science
Volume 78, January 2014, Pages 287-292

Passive film-induced stress and mechanical properties of α-Ti in methanol solution

Zhi Qin [a], Xiaolu Pang [a], Yu Yan [a], Lijie Qiao [a], Hai T. Tran [b], Alex A. Volinsky [b, a]

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https://doi.org/10.1016/j.corsci.2013.10.011 Get rights and content

ELSEVIER

Applied Surface Science
Volume 303, 1 June 2014, Pages 282-289

Water molecules effect on pure Ti passive film structure in methanol solution

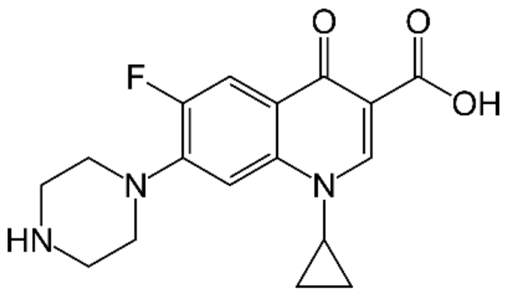
Zhi Qin [a], Xiaolu Pang [a], Lijie Qiao [a], Mehdi Khodayari [b], Alex A. Volinsky [b]

⊞ Show more

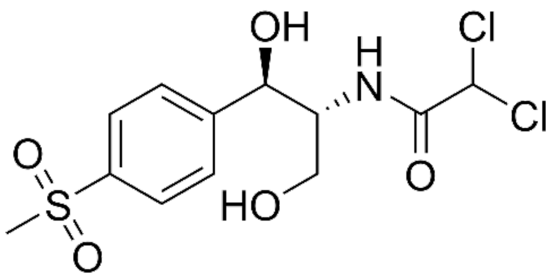
https://doi.org/10.1016/j.apsusc.2014.02.167 Get rights and content

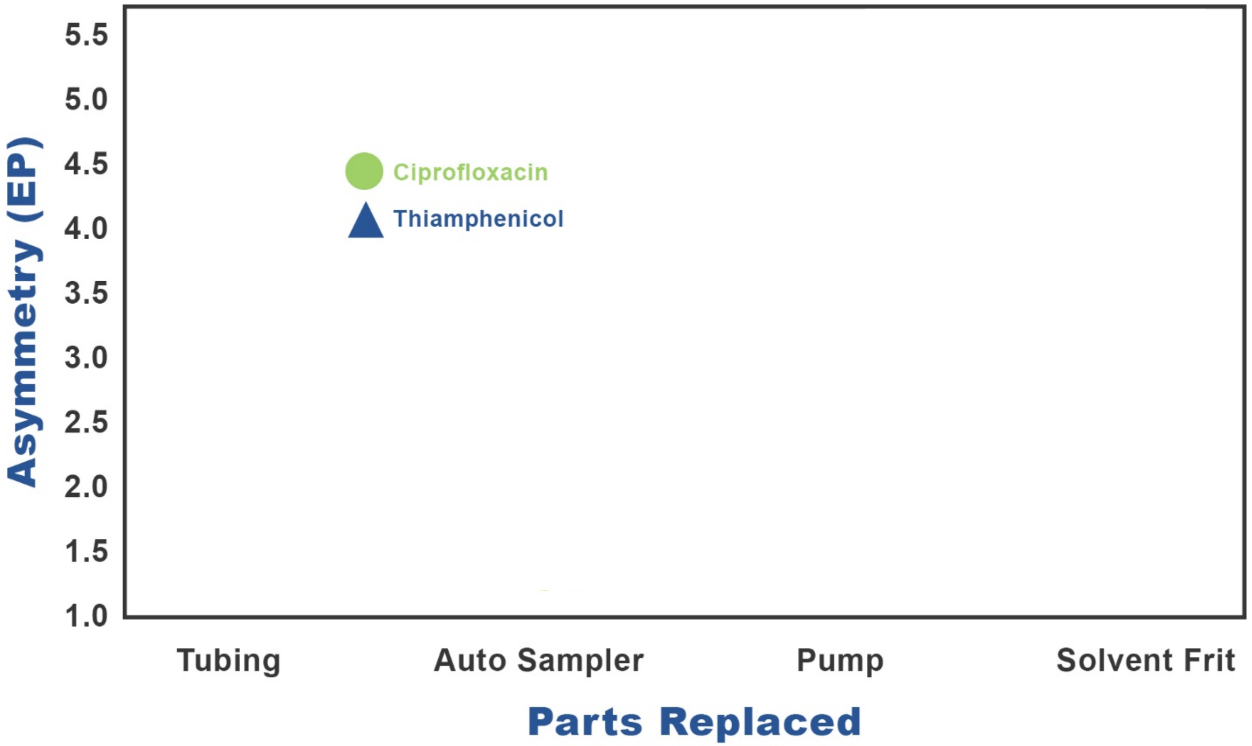
Biocompatible ≠ Metal free

Ciprofloxacin:



Thiamphenicol:





M. De Pra, G. Greco and M.P. Krajewski et al., *Journal of Chromatography A*. **2020**, 1611, 460619.

Silica bed is poisoned by Ti ions

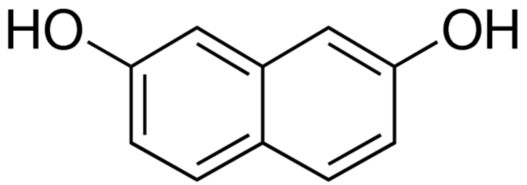
- Once the titanium ions reach the silica bed, they cannot fully be removed
- Less of an issue on well bonded C18 columns, but could be an issue at low level detection
- But this is just a titanium issue?
  - Nope…

M. De Pra, G. Greco and M.P. Krajewski et al., *Journal of Chromatography A*. **2020**, 1611, 460619.

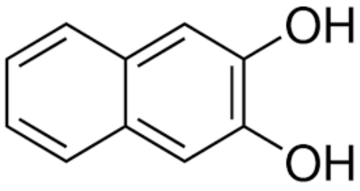
Stainless steel does it too…

- Kromasil C18 column stored under:
  - A: Methanol for 6 days
  - B: Methanol for 1 day
  - C: ACN:Water (60:40) for 7 days

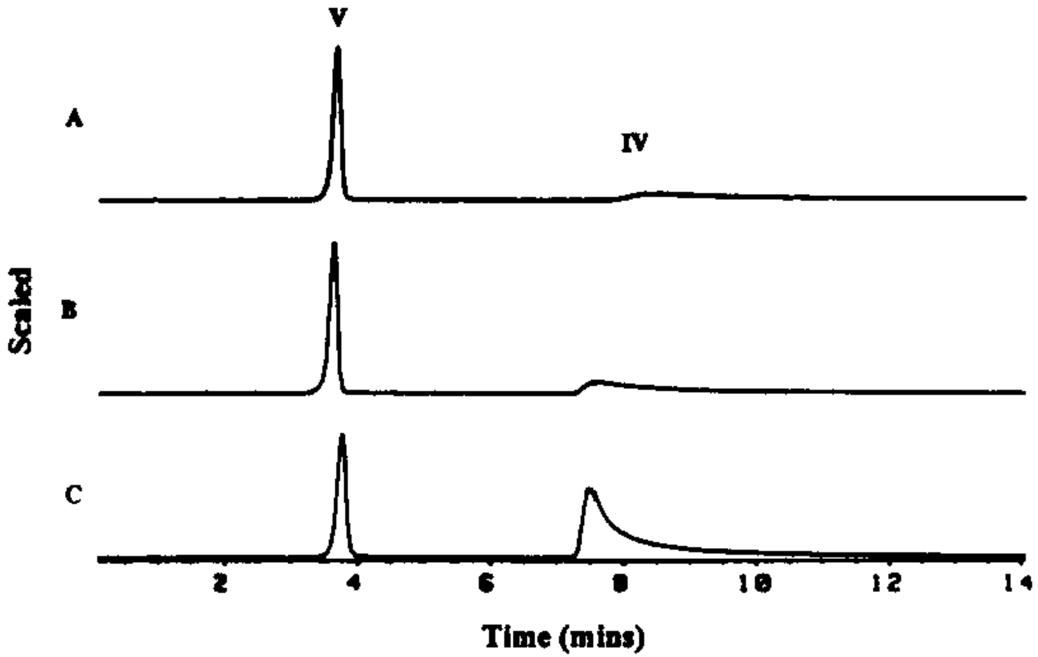
Peak labeled "V"



Peak labeled "IV"



Euerby, M.R., Johnson, C.M., Rushin, I.D., Tennekoon, D.A.S., *J. Chromatogr. A.* **1995**, *705*, 229-245.

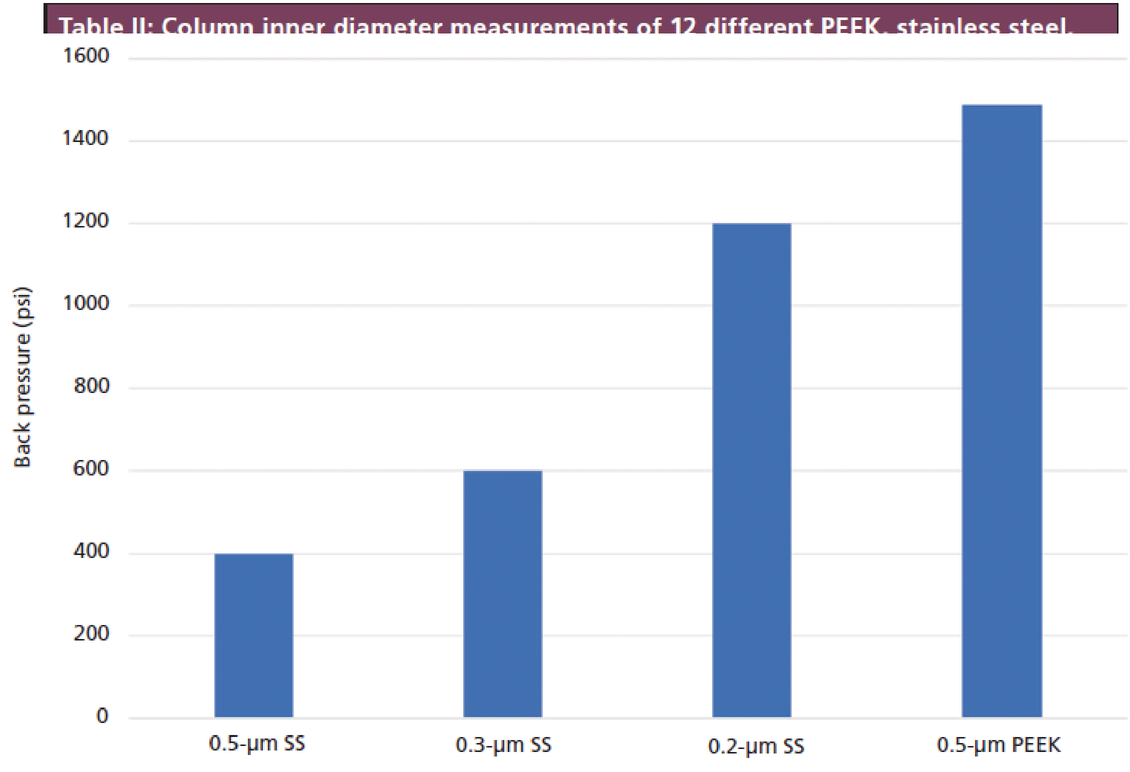


"Examination of the stationary phase material showed wide variation in colour ranging from white to dark orange."

"Atomic absorption spectroscopy of silica revealed levels of 250 ppm total iron."

PEEK issues:

- Mechanically not as robust as metal
- Machining inconsistencies
- Frit inconsistencies
- Potential swelling and incompatibility with organic solvents
  - Especially noticeable with THF



Back pressure when flowing 100% IPA at a rate of 40 mL/min

Anspach, J.A., Rao, S., Rivera, B., “Bioinert Versus Biocompatible” LCGC Magazine, Volume 36, Issue 6, p. 24-29.

Our solution: Dursan® coating on stainless steel:

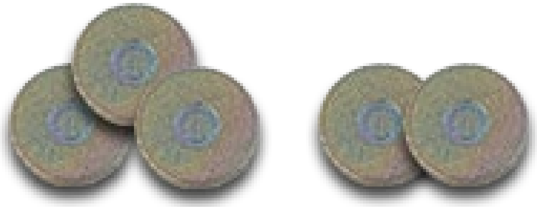
- Non-line of sight coating (can coat frits)
- Usable in wide pH range: 0-14
- Molecularly bound to the substrate: Good adhesion
- Wear: 2x more resistant than 316 Stainless steel
- Inert to most chemicals

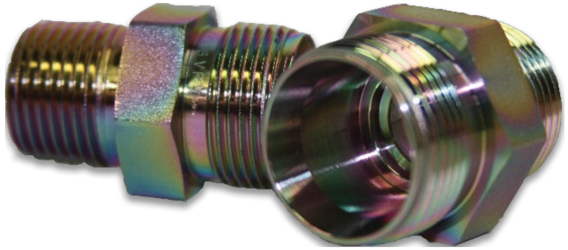
Current HPLC materials

Material	Mechanically strong	Bio-inert	Metal free	Chemical incompatibility
Stainless Steel	✓	✗	✗	Halogens, some acids, pure organic solvents
Titanium	✓	✓	✗	Some acids, Methanol and other organic solvents
PEEK	✗	✓	✓	THF, some acids slowly attack
Dursan®	✓	✓	✓	Similar to silica incompatibilities (HF)

The CVD process

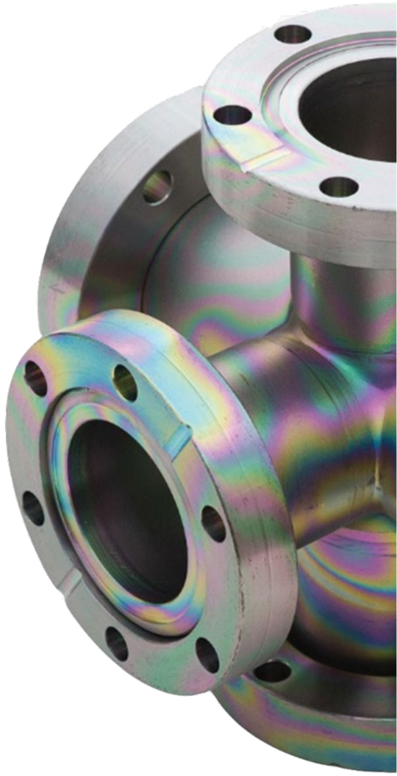
CVD Coated parts:





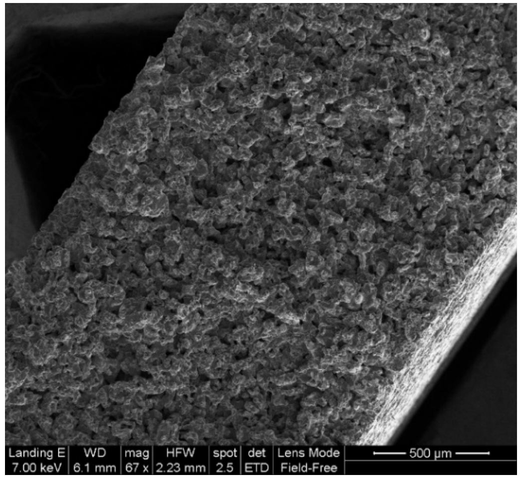
Advantages of CVD Coatings

- Non-line-of-sight deposition; uniformly treats 3D, high aspect ratio part geometries
- Molecular adhesion to base substrate. Won't flake nor delaminate.
- Scalable, versatile, and highly reproducible

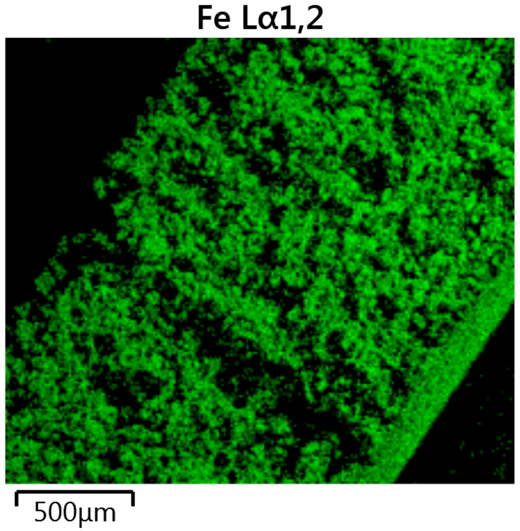


Example of non line of sight coating:

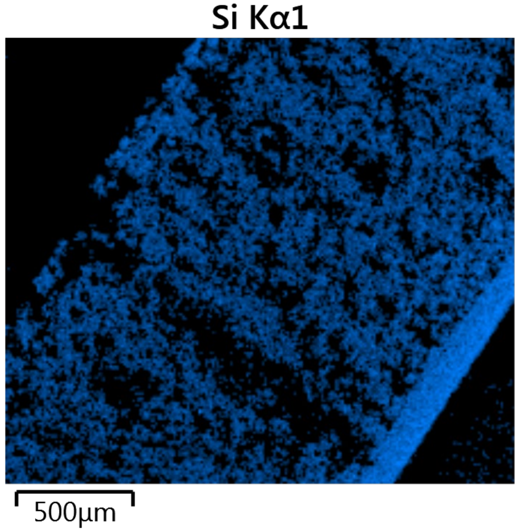
- Cross section of a 2µm nominal pore size frit after Dursan coating:



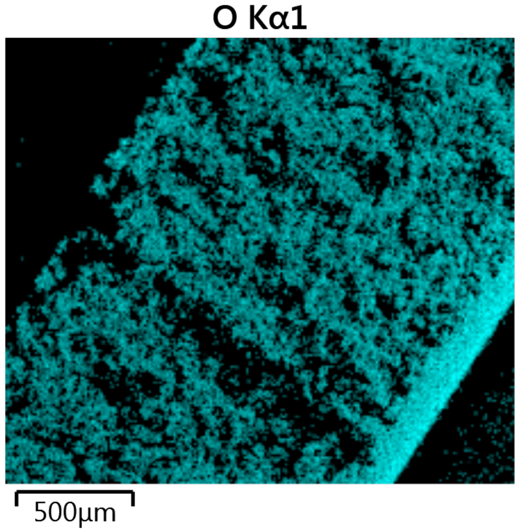
SEM micrograph



Iron EDS map



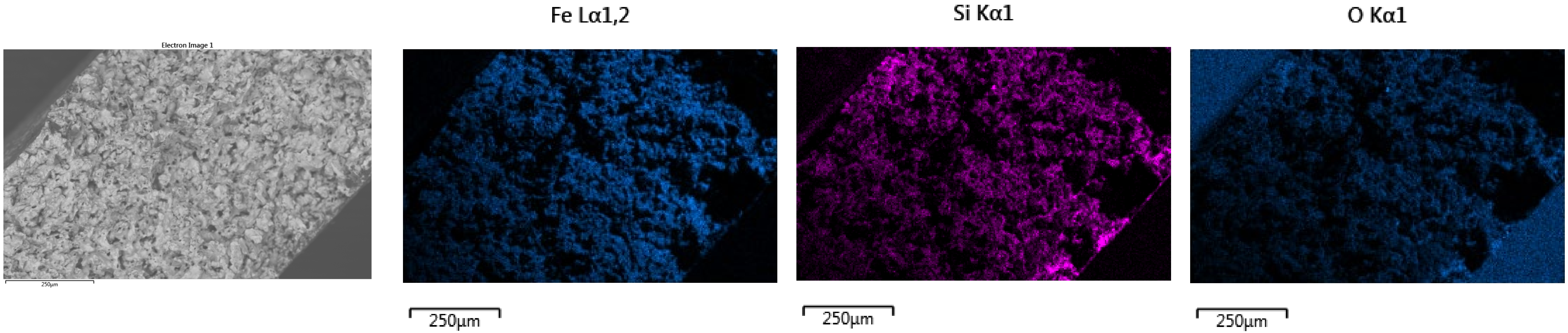
Silicon EDS map



Oxygen EDS map

Example of non line of sight coating:

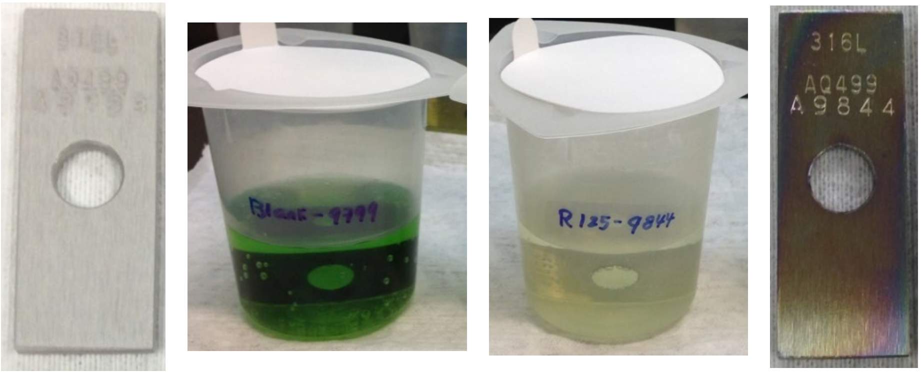
- Cross section of a 0.5 μm nominal pore size frit after Dursan coating:



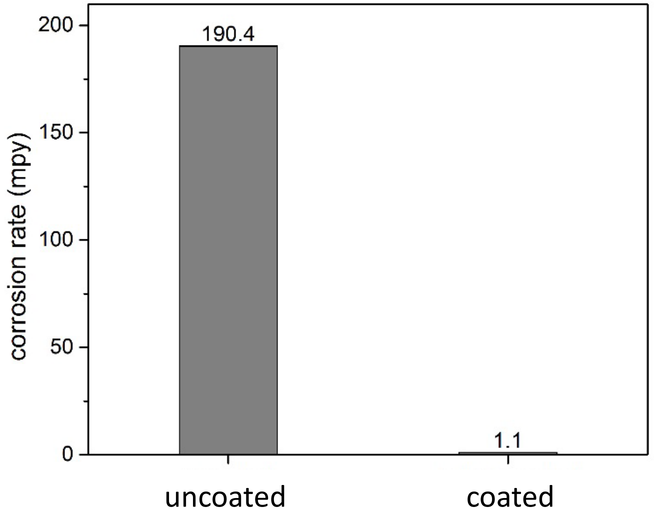
SEM micrograph | Iron EDS map | Silicon EDS map | Oxygen EDS map

Corrosion resistance in hydrochloric acid

- ASTM G31 guidelines
- 20% (6M) HCl room temperature immersion 24 hours
- Over 170x improvement with coating

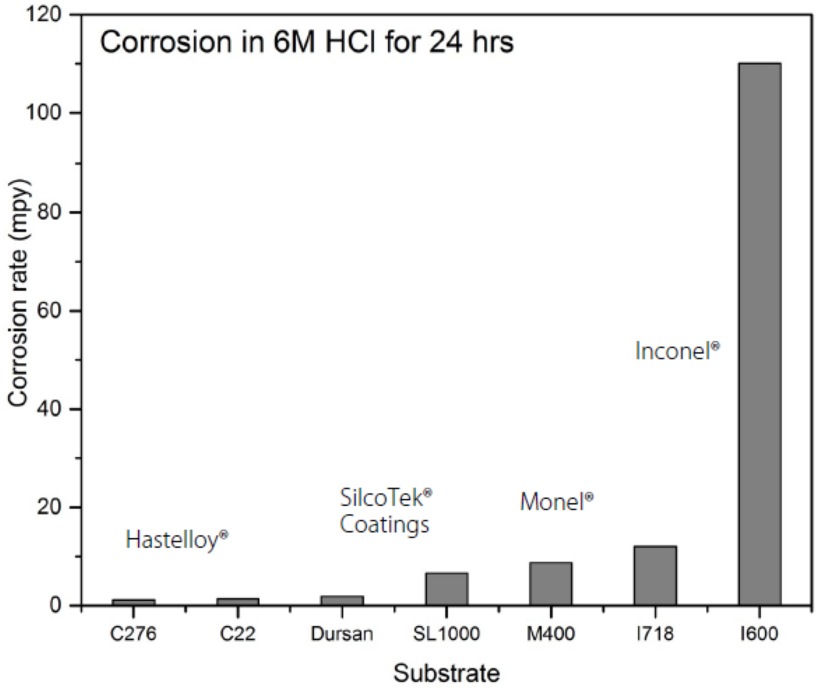


uncoated coated



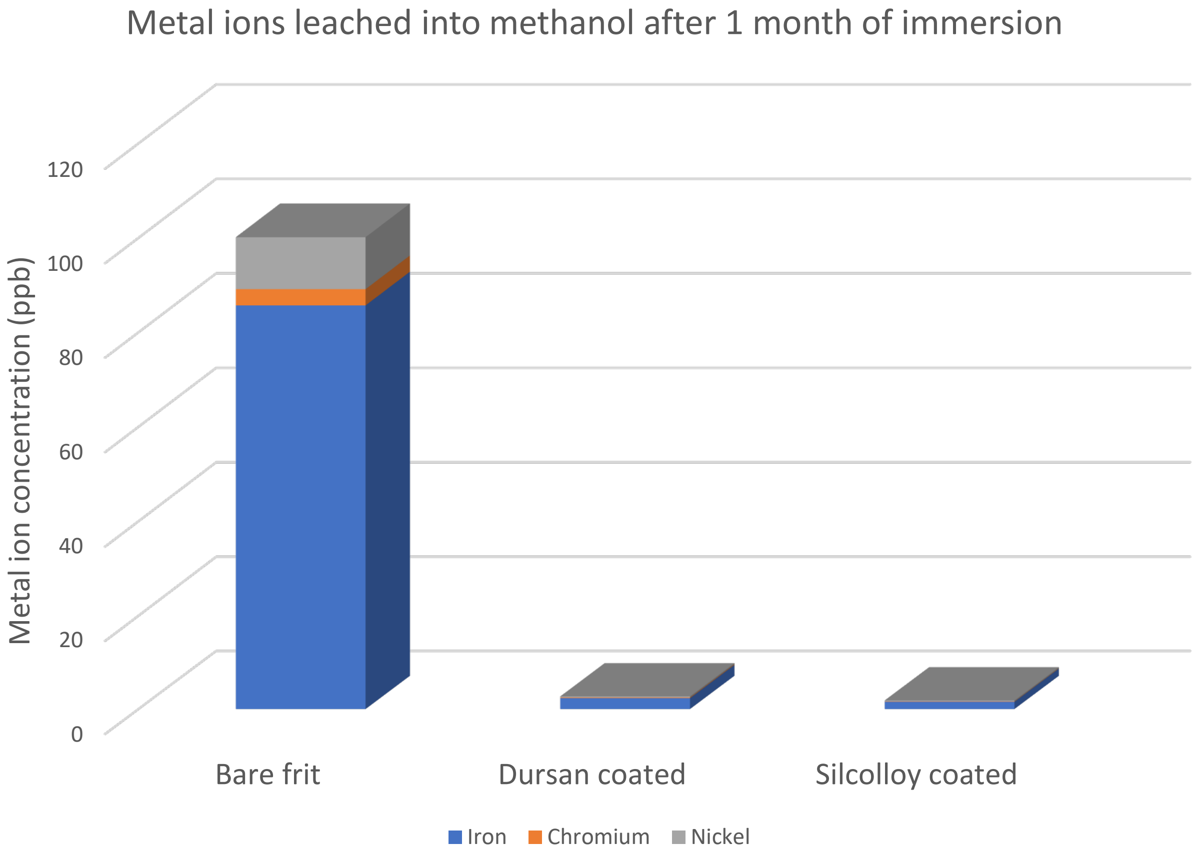
Additional corrosion resistance

Corrosive media	Bare Stainless Steel (MPY)	Dursan coated steel (MPY)	Improvement multiplier
6M HCl @ 50°C	3116.1	23.5	133x
Concentrated H_2SO_4	78.45	0.15	523x
48% HBr	2.05	0.29	7x
Bleach	1.70	0.10	17x
Concentrated H_3PO_4 @ 80°C	2.14	0.53	4x
2% TFA	No corrosion, change in CA	Unaffected	-



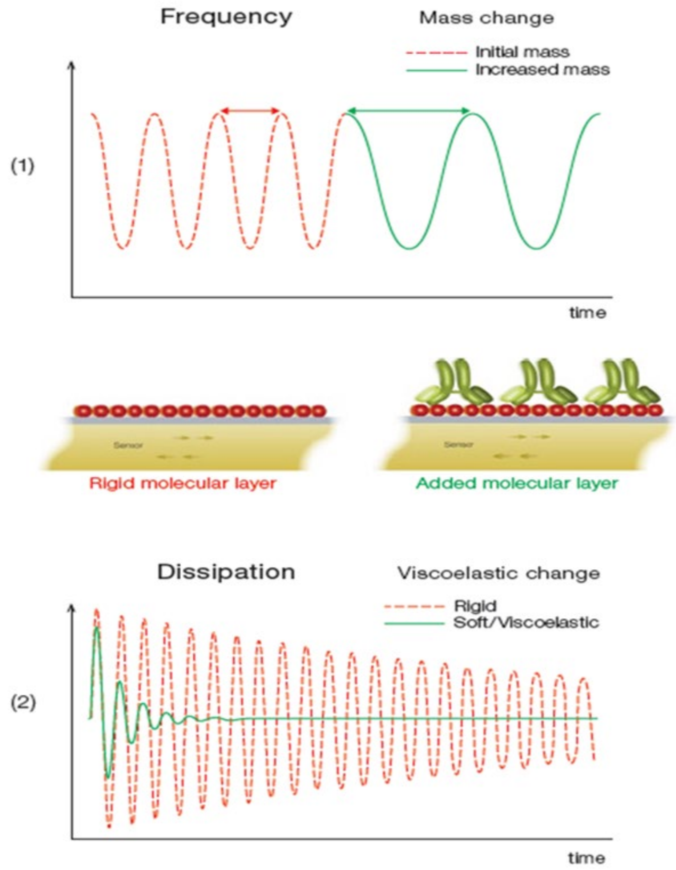
Protection against organic solvents

- Frits were left to soak in methanol for 1 month
- Results showed significantly higher levels of iron, chromium, and nickel in the uncoated frit container
- Future studies will replicate this with titanium substrates



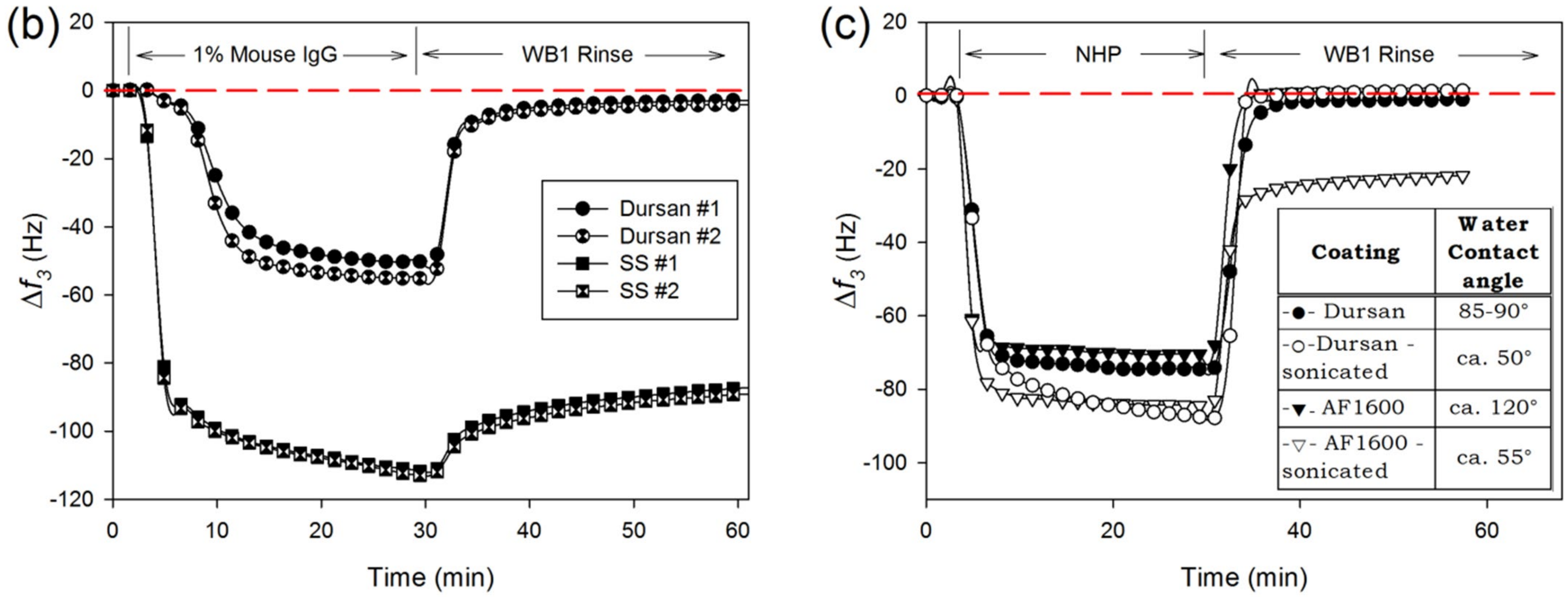
Bio-Inertness: Non-specific protein adsorption studies

- Collaborative study between Abbott Laboratories and SilcoTek on protein adsorption
- QCM-D with a thin layer of 316L SS was coated with Dursan
- Protein solutions were flowed over the sensor and the frequency was monitored over time



Vaidya, S.V.; Yuan, M.; Narvaez, A.R.; Daghfal, D.; Mattzela, J.; Smith, D. *Appl. Surf. Sci.* **2016**, *364*, 896-908.

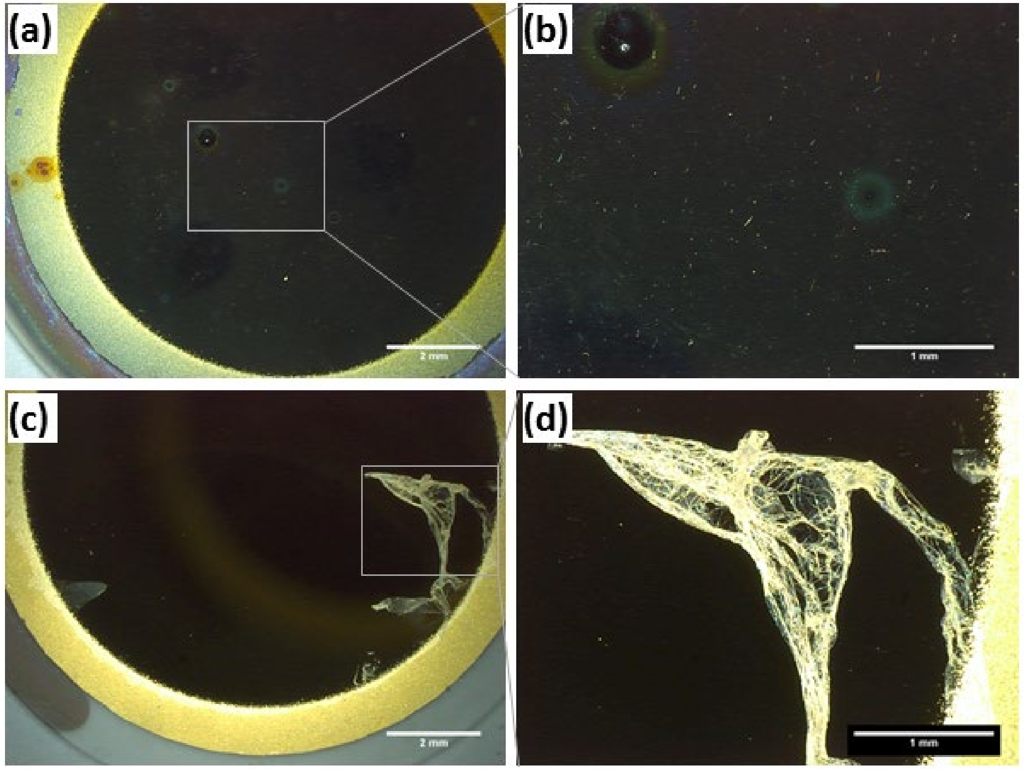
Mouse immunoglobulins and normal human plasma adsorption



Vaidya, S.V.; Yuan, M.; Narvaez, A.R.; Daghfal, D.; Mattzela, J.; Smith, D. *Appl. Surf. Sci.* **2016**, *364*, 896-908.

Dursan® remains attached to the steel surface after sonication.

30x 100x



Dursan

AF1600

Delamination of the polymer coating leads to active metal sites being exposed.

Vaidya, S.V.; Yuan, M.; Narvaez, A.R.; Daghfal, D.; Mattzela, J.; Smith, D. *Appl. Surf. Sci.* **2016**, *364*, 896-908.

Summary of Dursan® benefits

- Conformal coating that can be applied to as built columns, frits, tubing, pumps, sampling needles, etc.
- Molecularly bound to the surface and will not delaminate
- More wear resistant than stainless steel and PEEK
- Protects the substrate from corrosive attack
- Provides an inert surface to decrease the amount of molecules and/or proteins that adsorb to the surface

Dursan® coated HPLC columns: Customer testimonials

“The coating has opened up a whole new window of opportunities; providing a high quality problem-solving solution for the industry and our company.”

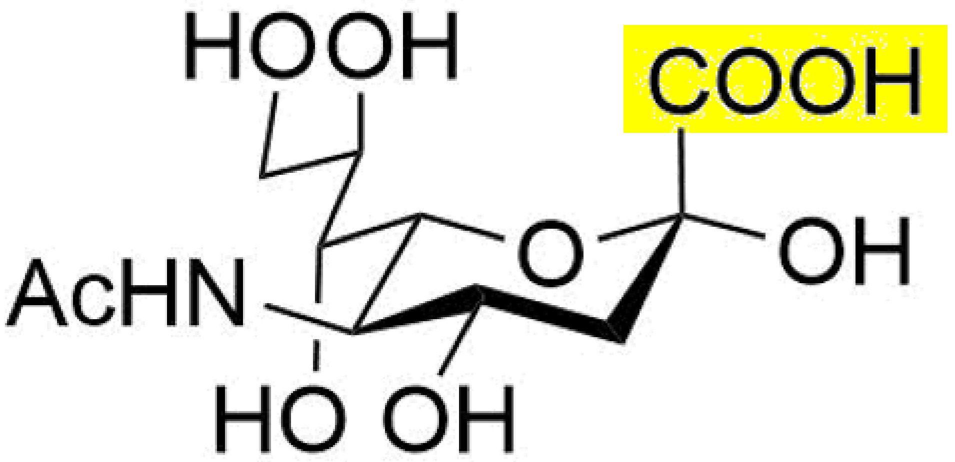
“We have tried the treated tubes on two different column phases and we are seeing an increase in efficiency and improved peak shape.”

“The coated columns have so far passed all tests bravely. The chromatographic separation in standard samples remains unchanged; in the case of biomolecules the results were, as expected, much better than in normal steel columns, but also better than in the case of pure PEEK columns.”

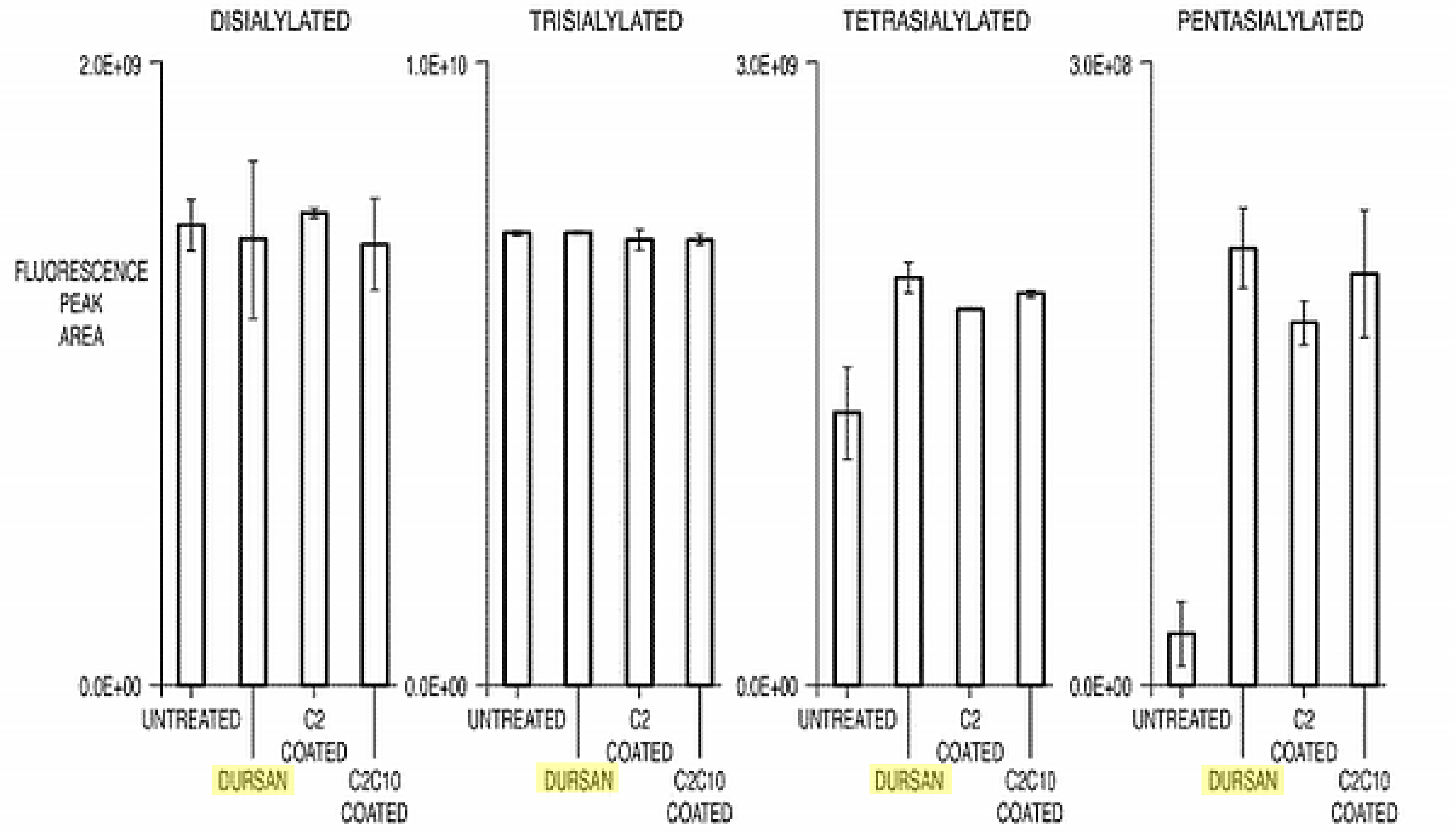
Analysis of N-Glycans

- Deglycosylation of a protein is standard in protein and biologic analysis
- Sialylated glycans contain carboxylic acids that have weak affinity toward metals

N-Acetylneuraminic acid:



Analysis of multiple sialic acid residues:



Lauber, Matthew A., et al. "Use of vapor deposition coated flow paths for improved chromatography of metal interacting analytes." U.S. Patent Application No. 16/133,089.

Our studies on HPLC columns:

SHEPARD INDUSTRIES

Phase
Analytical Technology LLC

phaseanalytical.net

acme

Our studies on HPLC columns

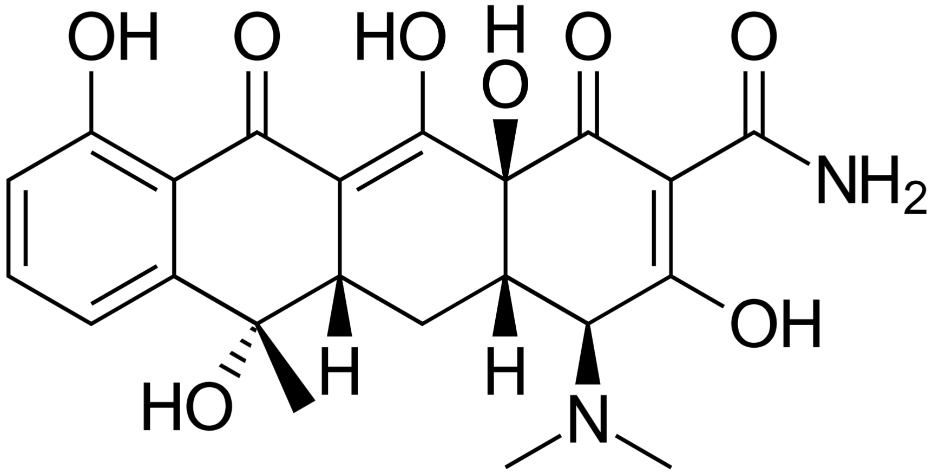
- Three 2.1 mm ID x 100 mm columns were packed of each of the following:
  - All Stainless Steel (A)
  - All Dursan coated (B)
  - Dursan coated frit and stainless steel column (C)
  - Titanium frit and stainless steel column (D)
- Biphenyl peak in standard HPLC mix was used to measure efficiency of column
  - No change in efficiency, asymmetry, or capacity factor after 800 injections

Column type	Efficiency (avg of 3)	Asym (avg) of 3
A	15195 plates	1.028
B	15233 plates	1.026
C	15069 plates	1.020
D	14263 plates	1.044

- Columns made by Shepard's Machine Shop
- Frits purchased from Mott Corporation
- Packed with ACME PLUS C18 and analysis done by Phase Analytical Technology, LLC
- Shows no detrimental effects in packing efficiency
  - Since the coating is quite thin, this was expected.

Tetracycline

- Tetracycline is an antibiotic, commonly used for acne and skin infections
- The molecule has numerous chelating groups that bind readily to metal sites
- Dursan can make the steel column more inert toward metal loving molecules like tetracycline

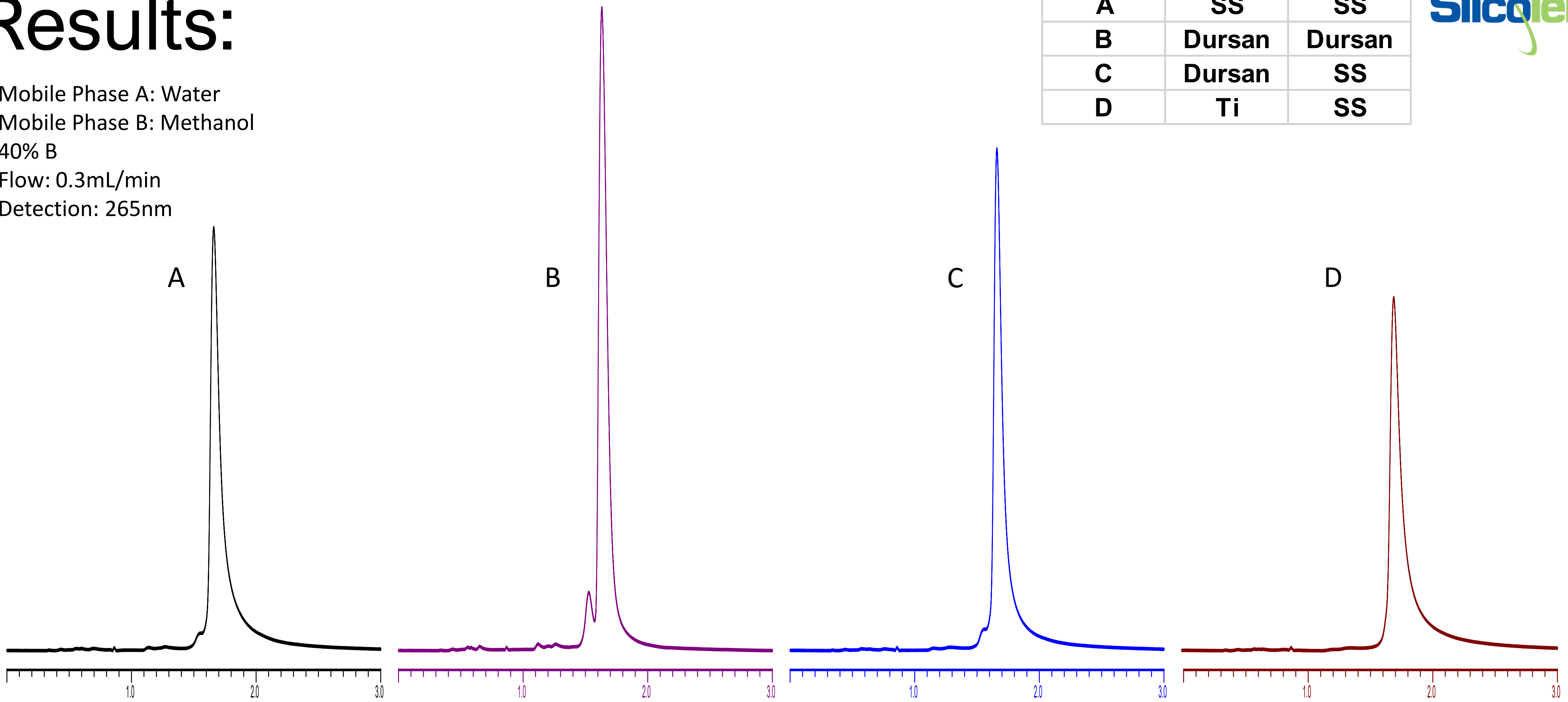


Molecular structure of tetracycline

Results:

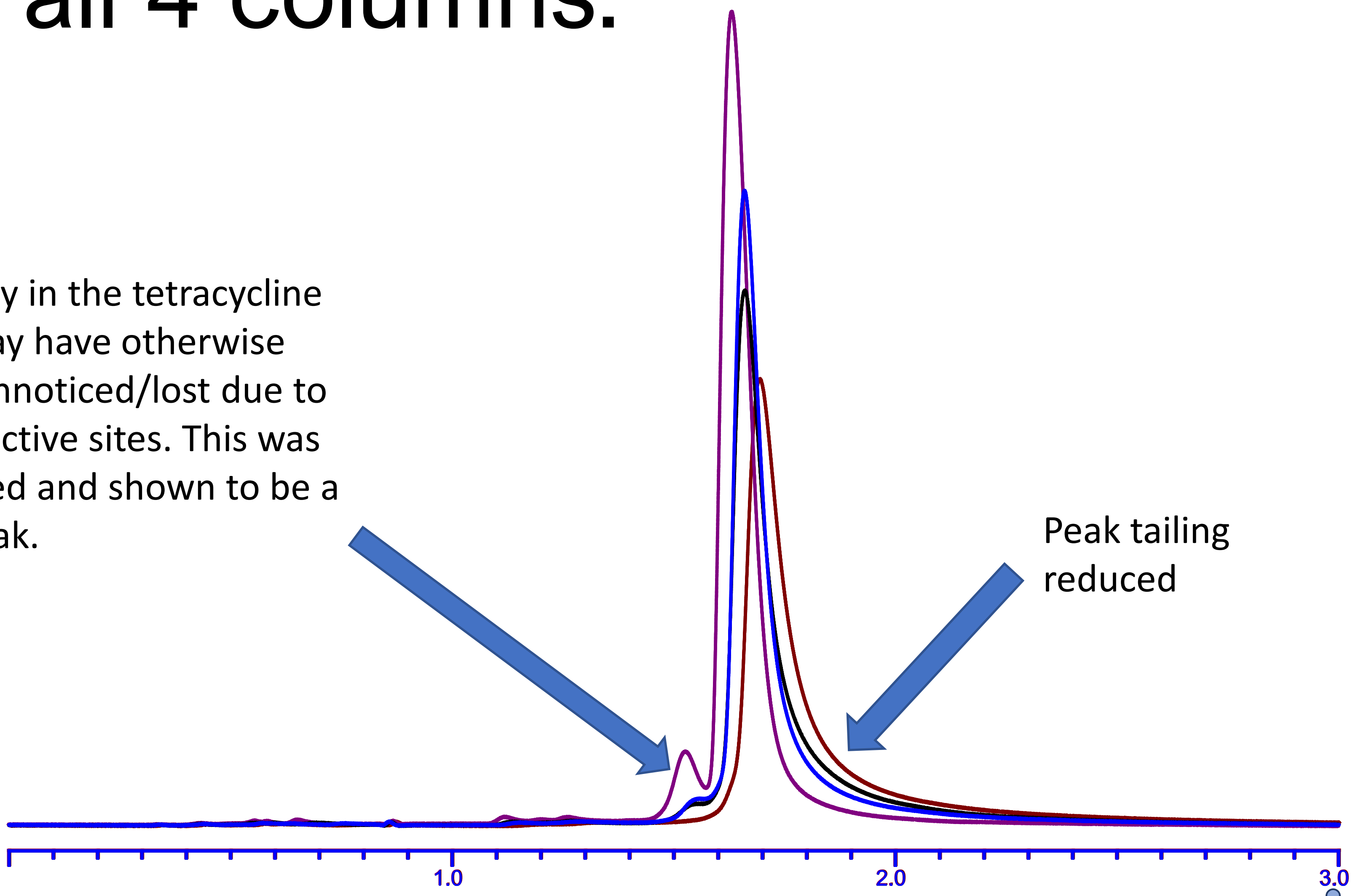
Mobile Phase A: Water
Mobile Phase B: Methanol
40% B
Flow: 0.3mL/min
Detection: 265nm

Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS



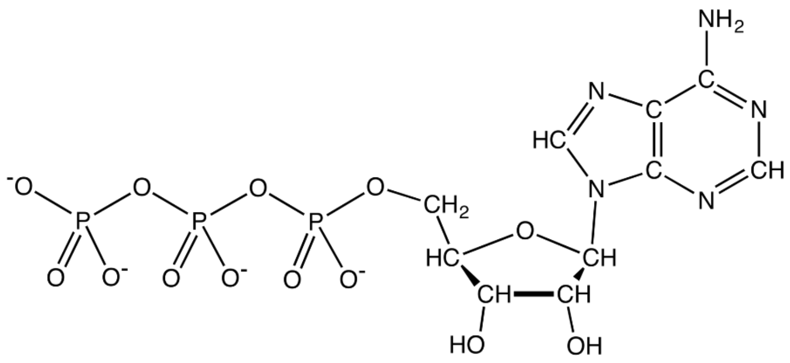
Overlay of all 4 columns:

Impurity in the tetracycline that may have otherwise gone unnoticed/lost due to metal active sites. This was repeated and shown to be a real peak.

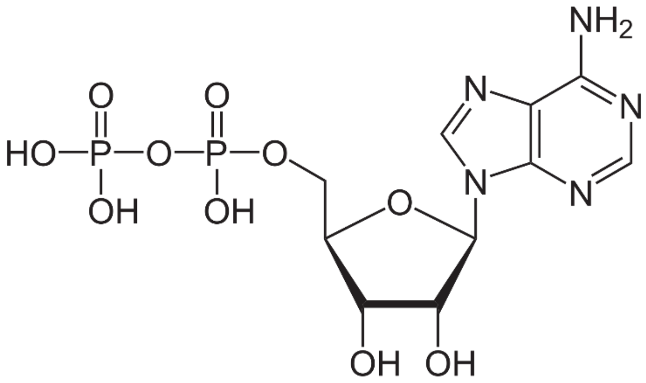


Adenosine triphosphate and diphosphate (ATP and ADP)

- ATP provides energy to drive numerous processes in living cells
- It is typically converted to ADP or AMP
- Phosphates are well known to have severe peak tailing during HPLC analysis due to the phosphate-iron interaction



ATP

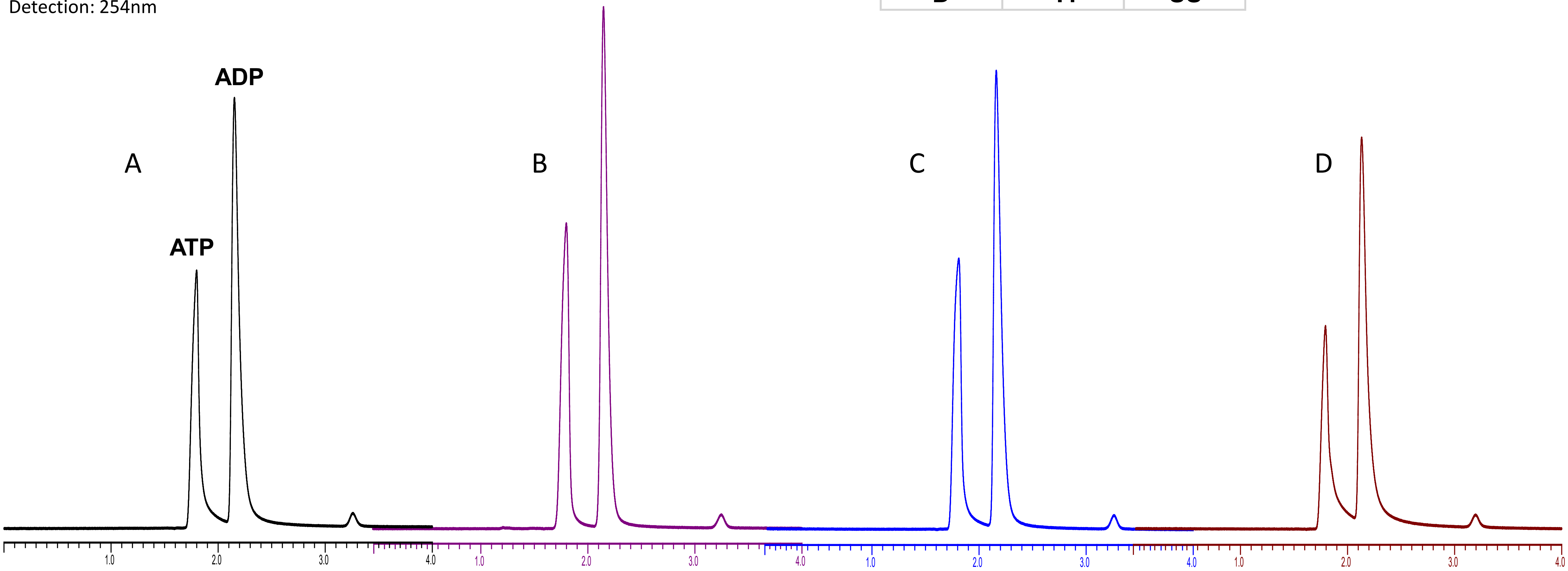


ADP

Results:

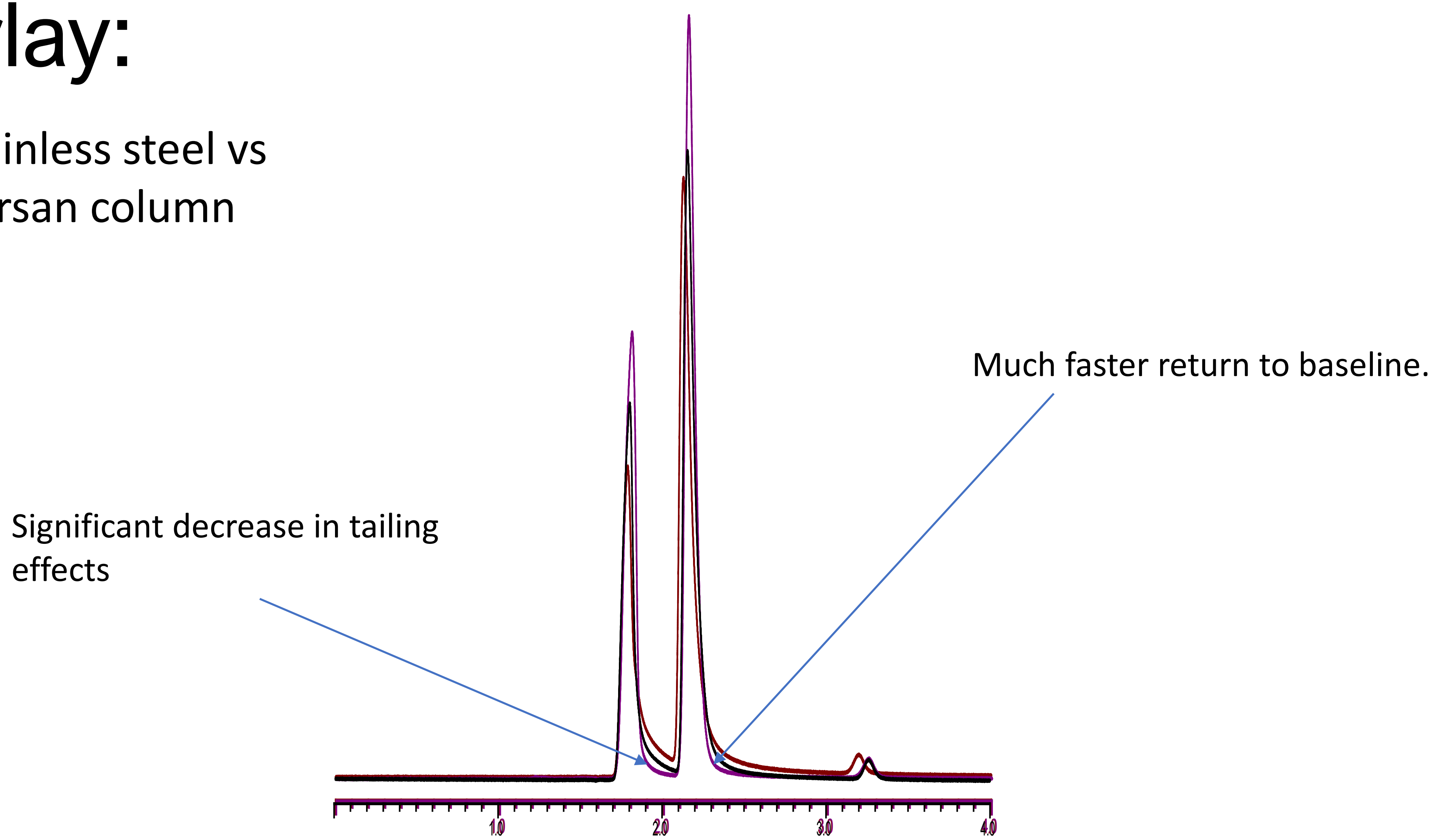
Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS

Mobile phase: Water / 10mM Ammonium Acetate
Flow: 0.2mL/min
Detection: 254nm



Overlay:

All stainless steel vs
all Dursan column



2-pyridinol-1-oxide

- Ciclopirox is an antifungal agent typically used in topical fingernail and toenail infections
- 2-pyridinol-1-oxide is the chelating part of this antifungal agent
  - It is a very powerful metal chelating agent
- The chromatograms show significant loss of signal due to metal interactions in the separation
  - This highlights the interaction that the column wall has with the analyte and there is a need for column coating as this interaction is not negligible.

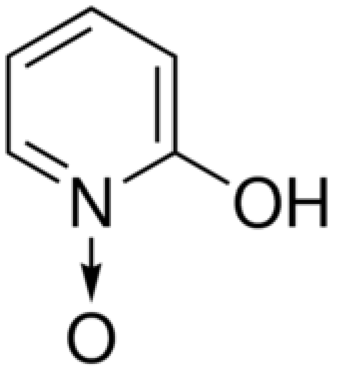
Results:

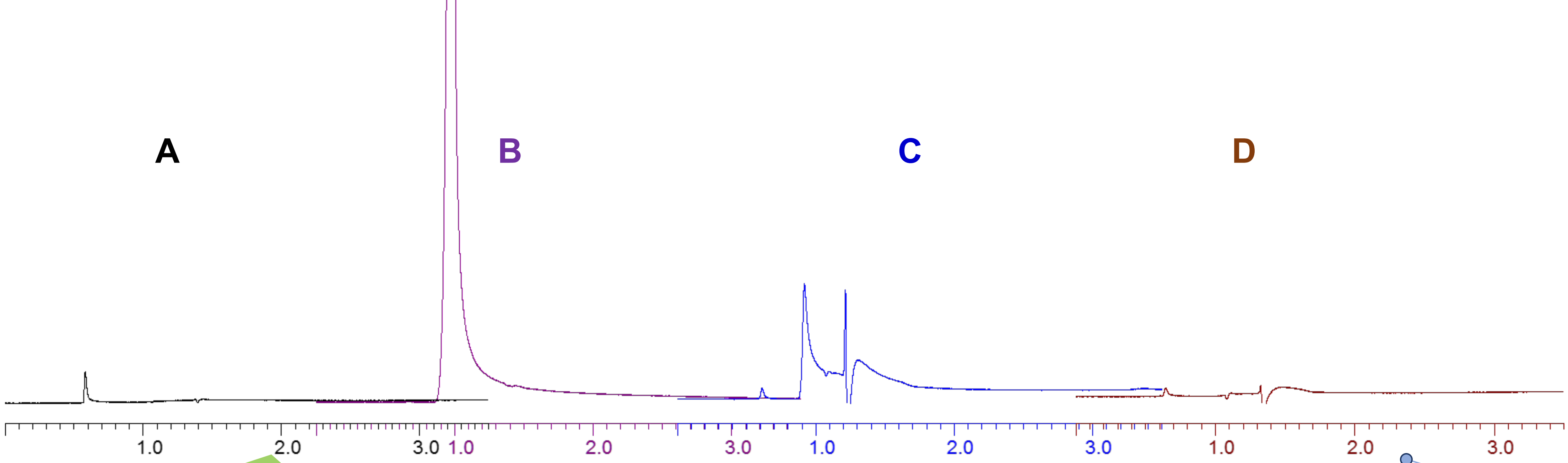
Mobile phase: Water no buffer
Flow: 0.3mL/min
Detection: 230nm

100 X 2.1

Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS

2-Pyridinol-1-Oxide

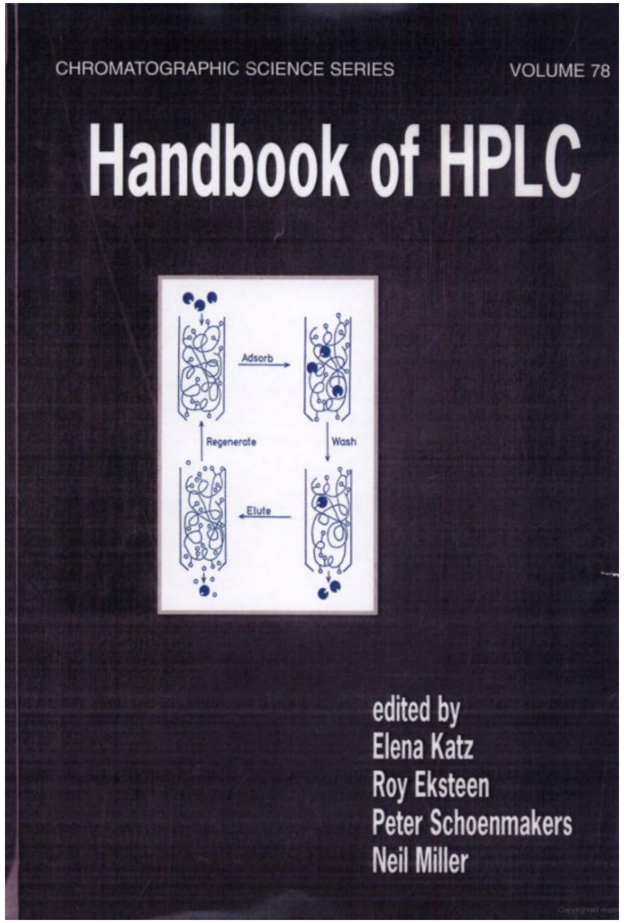




Things that I’ve been told at trade shows:

- “We can just passivate our system; it’ll do the same thing…”
- “Bio-inert is just a marketing ploy. If you use high quality materials, there’s no sticking problems…”
- “I can understand coating the frit. It has a huge surface area, but the coating on the column is unnecessary…”

Their opinions aren't unfounded…

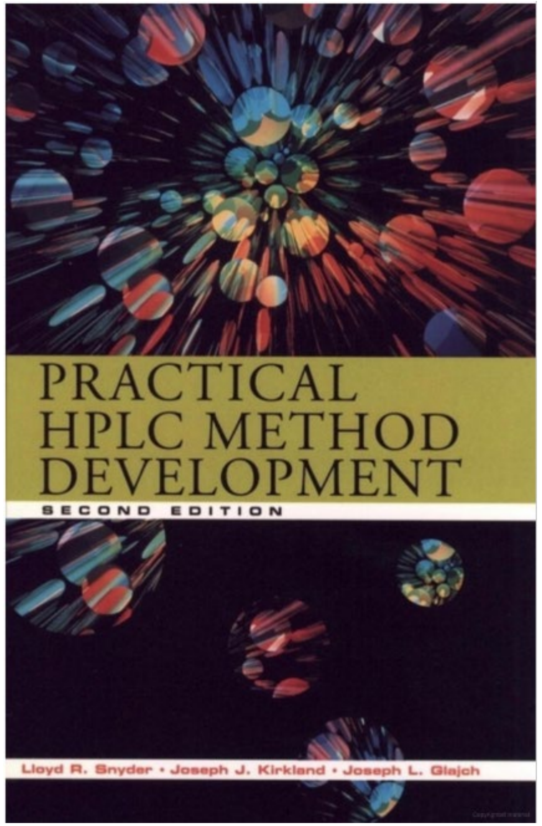


IV. BIOCOMPATIBILITY AND MATERIALS OF CONSTRUCTION

Generally, pumps and the high-pressure flow path are made from stainless steel, usually 316 grade. Stainless steel resists corrosion by forming a dense oxide surface over the metal, which renders the metal inert for most applications. The slight risk of corrosion with halides can be managed by occasional passivation by flushing the system with nitric acid, which can be a practical option for the routine user of HPLC. [Consult your owner's manual for details on the specific protocols for passivation.] The other elements in the flow path are listed in Table 6.

It is hard to separate the need for pumps made from other materials from the marketing hype that some firms used in promoting their pumps. In the early days of HPLC, Nester-Faust marketed syringe pumps with glass walls and Teflon pistons. Few found them to be required, and the option was dropped after the firm was purchased by Perkin-Elmer. About a decade later, Pharmacia introduced an integrated glass and plastic system specifically designed and marketed for protein chromatography. Pharmacia was very successful in communicating to prospects the potential problems of metal contamination from stainless steel systems or adsorption of proteins on metal oxides. The claim was that only glass and plastic were "biocompatible." Other firms continued using stainless steel or offered other materials, such as sapphire, titanium, polyetheretherketone (PEEK), and others.

Their opinions aren't unfounded…



Commercial columns made from glass, glass-lined stainless steel, and plastic also are available for special applications where samples might interact deleteriously with stainless steel. However, few problems of this type with stainless-steel columns are documented. The surface area of the column internal wall is quite small, so the opportunity for unwanted interaction is low. Samples that strongly complex with the components of stainless steel (iron, chromium, and nickel) are most likely to cause problems.

Porous frits close the ends of columns and retain the packing particles. Typically, 2- and 0.5 µm-porosity stainless-steel frits are used for 5- and 3-µm particles, respectively. Any problems arising from stainless-steel columns usually can be traced to the inlet stainless-steel frit, which has a much higher surface area than column walls for possible deleterious sample interaction. Poor peak shapes and low sample yields are indications of possible frit problems. Less-interactive porous titanium and polymer frits are available for this infrequent problem.

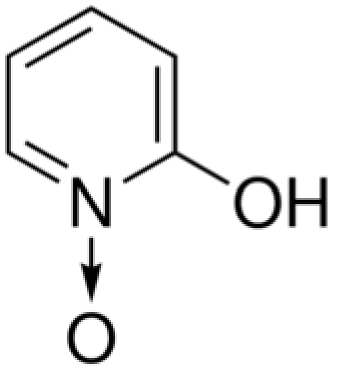
Results:

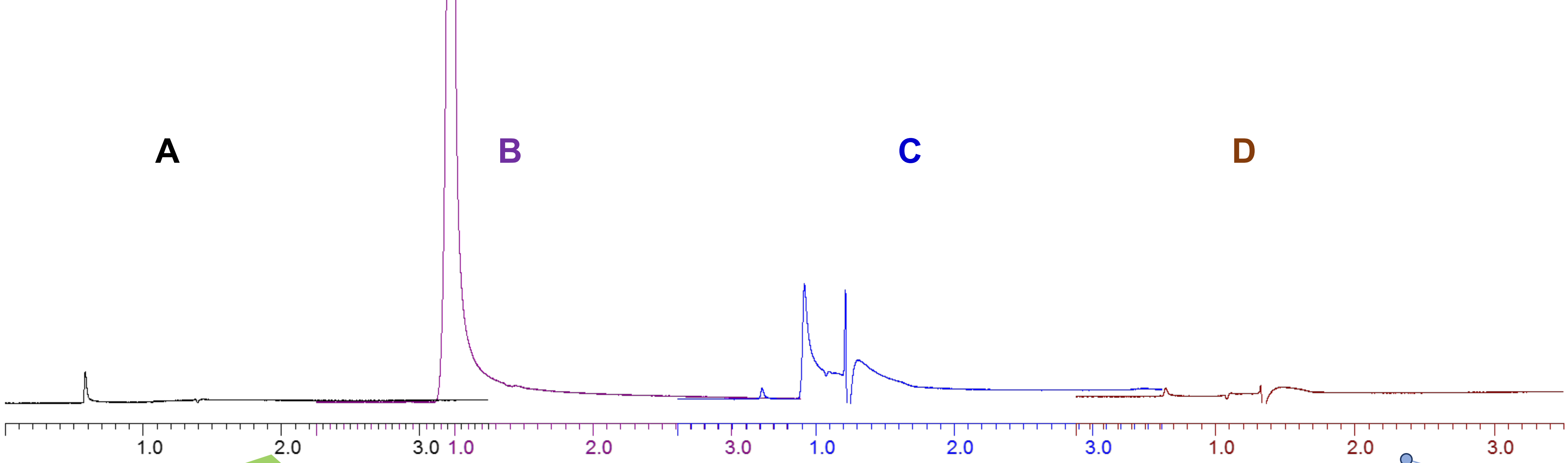
Mobile phase: Water no buffer
Flow: 0.3mL/min
Detection: 230nm

100 X 2.1

Group	Frit	Column
A	SS	SS
B	Dursan	Dursan
C	Dursan	SS
D	Ti	SS

2-Pyridinol-1-Oxide





Planned future studies

- Analysis of pesticides such as glyphosate and its metabolites
  - Phosphinate and carboxylic acid groups can make them difficult to analyze with stainless steel components
- Chemically passivated steel vs Dursan® coated steel
- Coating the entire flow path while using polar embedded stationary phases which can retain metal ions

Conclusion

- Dursan® can provide a bio-inert coating to all as-built stainless steel components
- Increased corrosion resistance without the possibility of swelling and delamination due to various solvents
- Decreased non-specific protein and chelating agent adsorption while still having the robustness of stainless steel
- No need for priming or passivation. A protective, barrier layer allows for reliable, reproducible data.