Achieving PEEK-like Performance on Stainless Steel HPLC Components with Bio-Inert CVD Coatings

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Outline

• Why is an inert flow path critical?
• CVD basics and how a CVD coating differs from traditional coatings
• Chemical and physical properties of Dursan®: SilcoTek’s HPLC coating of choice
• HPLC data highlighting the use of Dursan® on HPLC columns
Why is an inert flow path critical?

- Allow analytes to get from injection to detector without the risk of adsorption
- Reduce costs and downtime
  - No need to re-test
  - Accurate profile of all components, both reactive and non-reactive
- Trust your results
  - Eliminates false negatives and ghosting
- No additional molecules/ions introduced
  - Stainless steel: chemical reactivity, corrosion, and abrasion can all lead to the introduction of molecules and ions that are not in your sample.
Inertness leads to faster response time

- 500 ppbv methyl mercaptan ran through 100 feet of 1/8 in diameter tubing
Inertness prevents loss of analytes

### H₂S Stability: Dursan vs. Stainless Steel

50ppmv, 300cc cylinder

<table>
<thead>
<tr>
<th>Time</th>
<th>Stainless Steel</th>
<th>Dursan Coated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:00</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>25 hours</td>
<td>19</td>
<td>.98</td>
</tr>
<tr>
<td>50 hours</td>
<td>10</td>
<td>96.6</td>
</tr>
<tr>
<td>75 hours</td>
<td>4</td>
<td>98.3</td>
</tr>
</tbody>
</table>
Inert flow path example for GC

SilcoTek’s Inert Coating

Competitor’s Inert Coating

High activity surface results in severe peak distortion (a) and missing peaks (b).
## Current inert flow paths for HPLC

<table>
<thead>
<tr>
<th>Material</th>
<th>Benefits</th>
<th>New issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titanium</td>
<td>Bio-inert</td>
<td>Metal surface can still have “hot spots” of reactivity</td>
</tr>
<tr>
<td>PEEK</td>
<td>General inertness</td>
<td>Solvent limitations</td>
</tr>
<tr>
<td></td>
<td>Flexible</td>
<td>Swelling causes back pressure</td>
</tr>
<tr>
<td></td>
<td>Lightweight and easily cut</td>
<td>Pressure limitations</td>
</tr>
<tr>
<td>Ceramics</td>
<td>Robust</td>
<td>Brittle</td>
</tr>
<tr>
<td></td>
<td>Chemically inert</td>
<td>Can be expensive</td>
</tr>
</tbody>
</table>
SilcoTek’s solution: Dursan®

- Functionalized silica-like coating
- Highly corrosion resistant
- Stable up 450°C
- Provides a barrier between your solvent/analyte, and the substrate
  - Can be applied directly onto existing parts
  - <1 µm thick so will not affect tolerances
The CVD Coating Process

- Silane based CVD coatings
- Most metal alloys and ceramics can be coated
- Provides an inert barrier between substrate and flow path
- Surface chemistry can be altered by varying R.

Gas Supply

Processing Chamber (vacuum)

~450°C

<1µm

Part to be coated

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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:
Coating Capabilities

• We apply CVD coatings to:
  • Tubing: as small as 0.10mm ID
    • Internal coating, 600m ++ lengths if coiled
  • Frits, valve components (wetted), manifolds, etc.
  • Entire analytical flow path
  • Parts can be bent or flexed without flaking
Corrosion resistance in hydrochloric acid

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

<table>
<thead>
<tr>
<th>Corrosive media</th>
<th>Bare Stainless Steel (MPY)</th>
<th>Dursan coated steel (MPY)</th>
<th>Improvement multiplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>6M HCl @ 50°C</td>
<td>3116.1</td>
<td>23.5</td>
<td>133x</td>
</tr>
<tr>
<td>Concentrated H₂SO₄</td>
<td>78.45</td>
<td>0.15</td>
<td>523x</td>
</tr>
<tr>
<td>48% HBr</td>
<td>2.05</td>
<td>0.29</td>
<td>7x</td>
</tr>
<tr>
<td>Bleach</td>
<td>1.70</td>
<td>0.10</td>
<td>17x</td>
</tr>
<tr>
<td>Concentrated H₃PO₄ @ 80°C</td>
<td>2.14</td>
<td>0.53</td>
<td>4x</td>
</tr>
<tr>
<td>2% TFA</td>
<td>No corrosion, change in CA</td>
<td>Unaffected</td>
<td>-</td>
</tr>
</tbody>
</table>

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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
Mouse immunoglobulins do not stick

Performs as well as fluoroplastic in non-sonicated environment
Dursan® coated HPLC columns: Customer testimonials

“The group that was using the coated column to isolate and purify their sensitive novel compounds have completed their study. The LC work was critical as it allowed further characterization of the compounds they synthesized.”

“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.”
Inertness to sulfur compounds

• In a recent paper\textsuperscript{1}, Dursan coated columns were used to purify and concentrate molecules that would readily react with stainless steel.

• The peak of interest is the furan molecule (A).
  • Note that the researchers were optimizing for clean separation and not necessarily peak efficiency.

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

<table>
<thead>
<tr>
<th>Column type</th>
<th>Efficiency (avg of 3)</th>
<th>Asym (avg) of 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15195 plates</td>
<td>1.028</td>
</tr>
<tr>
<td>B</td>
<td>15233 plates</td>
<td>1.026</td>
</tr>
<tr>
<td>C</td>
<td>15069 plates</td>
<td>1.020</td>
</tr>
<tr>
<td>D</td>
<td>14263 plates</td>
<td>1.044</td>
</tr>
</tbody>
</table>

• 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
Results:

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Frit</th>
<th>Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SS</td>
<td>SS</td>
</tr>
<tr>
<td>B</td>
<td>Dursan</td>
<td>Dursan</td>
</tr>
<tr>
<td>C</td>
<td>Dursan</td>
<td>SS</td>
</tr>
<tr>
<td>D</td>
<td>Ti</td>
<td>SS</td>
</tr>
</tbody>
</table>
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
Results:

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Frit</th>
<th>Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SS</td>
<td>SS</td>
</tr>
<tr>
<td>B</td>
<td>Dursan</td>
<td>Dursan</td>
</tr>
<tr>
<td>C</td>
<td>Dursan</td>
<td>SS</td>
</tr>
<tr>
<td>D</td>
<td>Ti</td>
<td>SS</td>
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</table>

ATP
ADP

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Overlay:

All stainless steel vs all Dursan column

Significant decrease in tailing effects

Much faster return to baseline.
# Peak analysis of ATP and ADP

- Note the increase in height and decrease in asymmetry values for the Dursan system.

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Ret.Time</th>
<th>Height</th>
<th>Width (50%)</th>
<th>Asym.</th>
<th>Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>ATP</td>
<td>1.799</td>
<td>50.71</td>
<td>0.072</td>
<td>1.45</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>ADP</td>
<td>2.152</td>
<td>84.45</td>
<td>0.072</td>
<td>2.03</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>ATP</td>
<td>1.804</td>
<td>60.09</td>
<td>0.072</td>
<td>0.95</td>
</tr>
<tr>
<td>Dursan Dursan</td>
<td>ADP</td>
<td>2.152</td>
<td>102.4</td>
<td>0.066</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>ATP</td>
<td>1.8</td>
<td>53.3</td>
<td>0.08</td>
<td>1.16</td>
</tr>
<tr>
<td>Dursan Frits only</td>
<td>ADP</td>
<td>2.151</td>
<td>90.13</td>
<td>0.073</td>
<td>1.93</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>ATP</td>
<td>1.783</td>
<td>39.96</td>
<td>0.068</td>
<td>2.28</td>
</tr>
<tr>
<td>Ti Frits</td>
<td>ADP</td>
<td>2.122</td>
<td>76.74</td>
<td>0.074</td>
<td>2.45</td>
</tr>
</tbody>
</table>
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

<table>
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<td>Dursan</td>
<td>SS</td>
</tr>
<tr>
<td>D</td>
<td>Ti</td>
<td>SS</td>
</tr>
</tbody>
</table>

2-Pyridinol-1-Oxide

![Chemical Structure of 2-Pyridinol-1-Oxide]
Planned future studies

• PEEK vs Dursan®
  • Customers have anecdotally told us results, but we want to test it for ourselves

• Durability studies in harsh environments
  • High and low pH solvents could degrade columns, does Dursan® provide sufficient protection?

• More case studies on difficult molecules and/or biologics
  • Antibodies like IgG1

• Moving past reverse phase HPLC
  • Does Dursan® help in HILIC, ion exchange, gel filtration, etc.
Acknowledgements
Conclusions

- Dursan® is a robust, inert, and reproducible coating technology that can be applied to as built stainless steel HPLC components.
- Corrosion resistance and non-fouling properties of Dursan® can be beneficial under harsh testing conditions.
- Coating columns and frits have shown benefits for troublesome molecules such as peak narrowing, and faster return to baseline, or in the case of powerful chelating agents, the ability to get a good peak at all.
- You can gain the inertness of PEEK while maintaining the robustness and reliability of stainless steel.
Next steps

• You can get parts Dursan-coated exclusively by SilcoTek, or request coated hardware from your preferred vendor

• [www.silcotek.com](http://www.silcotek.com) has an array of technical and educational resources for further learning

• To talk directly with a SilcoTek representative about your application, please e-mail [silcod@silcotek.com](mailto:silcod@silcotek.com)
Questions?