Reducing Adhesion of Proteins on Stainless Steel Components by the Application of a Carboxysilane Coating

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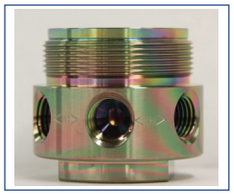
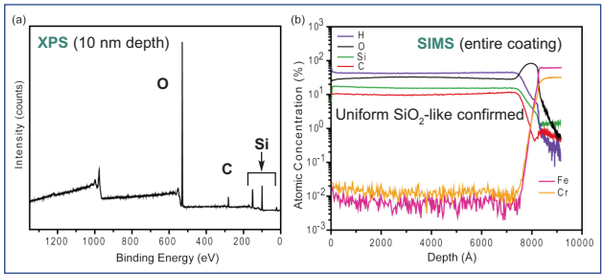
Background

- Prevention of non-specific protein adsorption to surfaces is highly desirable in many fields including the food, marine and medical industries.
- Collaborative work by Abbott and SilcoTek has demonstrated prevention of residual adsorption of protein fouling to the surface of stainless steel by Dursan® – a hydrophobic carboxysilane CVD coating developed by SilcoTek.
- Chemical Vapor Deposition
  - 3-dimensional coating that can coat complex geometries and small holes.
  - Ideal substrates include stainless steel, glass, ceramics and high performance alloys.
  - Coating thickness is in the range of 0.4 – 1.6 μm, and color varies with thickness.

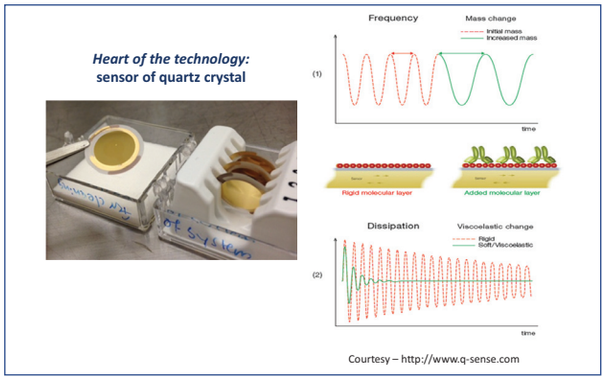


- About Dursan®
  - Dursan is comprised of a SiO_2-like bulk framework, with the surface functionalized by alkyl groups to render the coating chemically inert and hydrophobic.
  - The composition and purity of the Dursan coating were confirmed with SIMS and XPS.



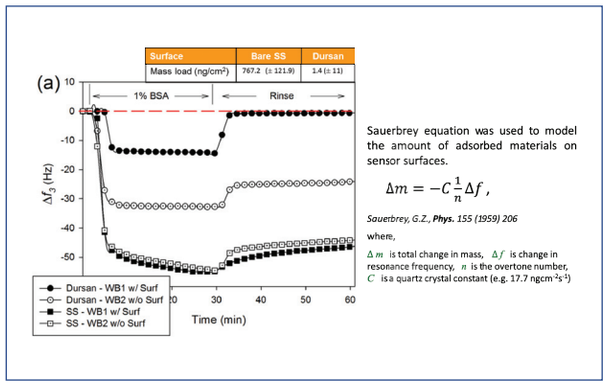
Methods

- QCM-D
  - A highly sensitive mass sensor that can detect mass uptake or release on the ng/cm² scale. It works by interpreting changes in the quartz resonance frequency.
  - **Coatings** AT-cut quartz crystal sensors coated with a uniform thin layer of 316L grade Stainless Steel (QSX 304), and AF1600 (QSX 331) – an amorphous fluoropolymer – were purchased from Biolin Scientific, Inc.
  - The Dursan coating was applied on stainless steel sensors via a 3-dimensional chemical vapor deposition process. The coating thickness is about 200~300 nm on the QCMD sensors.
  - Protein solutions were flowed (0.150 mL/min) over sensors maintained at 25±0.1°C using a peristaltic pump (Ismatec IPC-N4) attached to the Q-Sense E4 unit. Changes in **frequency (f) and dissipation (D)** at the 3rd, 5th, 7th and 9th overtones of the base resonance of Quartz crystal (5 MHz) were monitored over time.
  - Bare stainless steel sensors were cleaned after each experiment by storing in a 1% solution of Hellmanex™ in HPLC water for a minimum of 30 minutes followed by thorough rinse with HPLC water, sonication for 10 minutes in 190 proof ethyl alcohol, drying by nitrogen gas, and exposure to UV/Ozone treatment (BioForce Nanosciences Procleaner™ 110) for 10 minutes. AF1600 and Dursan coated sensors were cleaned similarly with exception of not performing sonication and UV/Ozone treatment as both methods were found to considerably lower the static contact angle of water on these surfaces transitioning them from hydrophobic to hydrophilic.

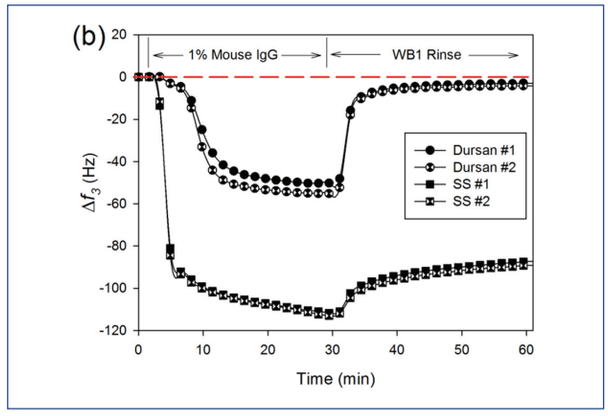


Results

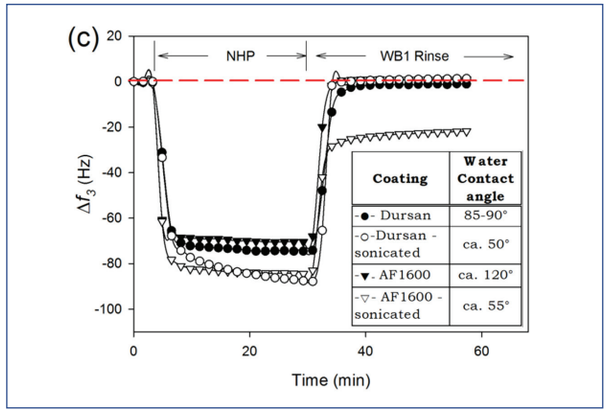
- Effect of coating and surfactant
  - A wash solution with non-ionic surfactant (Brij 35) removed all residual BSA proteins from the Dursan-coated sensor surface. The same wash solution had no effect on protein loading on bare stainless steel.

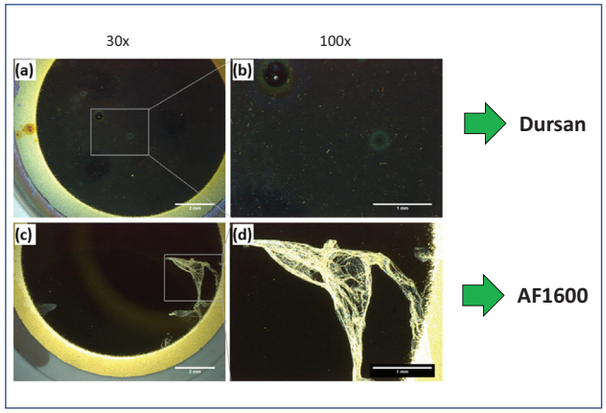


- Performance with immunoglobulins
  - The combination of Dursan coating and non-ionic surfactant in the wash solution led to effective reduction of protein loss to the sensor surface.



- Effect of sonication on coating performance
  - Wear resistance test using sonication showed robustness of the CVD Dursan coating. In contrast AF1600 coating lost efficiency due to coating delamination.





- Optical micrograph of the post-sonication QCM-D sensors showed intact Dursan coating (a and b), compared to delaminated AF1600 coating (c and d).

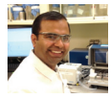
Discussion

- Biofouling in biotechnological applications can be prevented by following these 3 precautionary measures:
  - *pre-exposure*, i.e. by modifying the surface chemistry (i.e. **coatings**) to prevent non-specific adsorption;
  - *during-exposure*, i.e. by changing the solution properties such as pH, ionic strength and by using excipients such as ionic or non-ionic **surfactants** to alter electrostatic as well as hydrophobic interactions;
  - *post-exposure*, i.e. by using **wash/rinse** solutions containing caustic chemicals.
- These measures on its own cannot provide an universal fix to the biofouling problem, but various combinations of these three can work. Combination of the robust Dursan® coating and a non-ionic surfactant in the wash buffer we reported here is an example of a step forward, specifically in the case of automated assay analyzers, reagent manufacturing, and filling setups.

Conclusions

- The protein resistance performance of Dursan was compared to those of bare stainless steel and an amorphous fluoropolymer coating (AF1600) using quartz crystal microbalance with dissipation monitoring (QCM-D).
- A combination of Dursan coating and a wash step containing a non-ionic surfactant (Brij 35) facilitated 100% removal of tested proteins including bovine serum albumin (BSA), mouse immunoglobulin G (IgG), and normal human plasma proteins. The same wash solution had no effect on protein loading on bare stainless steel.
- Comparison studies between Dursan and AF1600 showed that sonication degraded the protein resistance properties of AF1600 due to delamination of the polymer coating, but had no negative impact on the Dursan performance demonstrating the coating's durability toward mechanical wear.

The Authors



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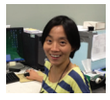

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