



Protein-Resistant Properties of SilcoTek's Dursan[®] Coating

Technical Insight

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Background

Prevention of non-specific protein adsorption to surfaces is highly important for many industries such as food, marine, and medical industries. For example, modern medical devices can suffer from the interference of unwanted molecules binding to the solid surfaces surrounding the antibodies, which leads to poorer detection limit because of a lower signal to noise ratio.

Existing coating solutions that impart protein adsorption resistance or facilitate fouling release properties experience either chemical instability issues in oxidative environments (e.g. air), or physical degradation (e.g. delamination) during use and wear. The authors of this paper examined Dursan[®] as an alternative coating solution that may provide better stability and durability in such applications.

Quartz crystal microbalance with dissipation monitoring (QCM-D) was used to characterize the anti-biofouling properties of Dursan[®] on a stainless steel surface. QCM-D monitors changes in oscillation frequency and dissipation of a planar crystal substrate upon adsorption of macromolecules. The authors compared 3 surfaces: uncoated 316L stainless steel (SS), Dursan-coated 316L SS, and AF1600-coated 316L SS (AF1600 is an amorphous fluoropolymer). Sonication was introduced in the test to induce rapid mechanical wear so durability of the coatings can be assessed.



Experimental

Experimental baseline for the QCM-D measurement was determined by flowing a wash buffer, referred to as WB1, over all sensors for ~ 4 minutes to clean the surfaces and priming them for protein exposure. Protein solution of interest was then flowed over the sensors for 20~25 minutes, followed by another rinse using WB1 for 25 minutes. A minimum of two distinct measurements were carried out for each protein-surface system.

A change in the oscillation frequency (Δf) of the QCM-D sensor indicates protein adsorption onto the sensor surface, and Sauerbrey equation ($\Delta m = -C/n \cdot \Delta f$) was used to correlate the adsorbed mass (Δm) to the changes in oscillation frequency (Δf). C is the mass sensitivity constant for the specific sensors used, and n is the overtone used for measurement. In addition, the ratio of change in dissipation to change in frequency can be used to determine the rigidity of the adsorbed protein layer.

Discussion and Data

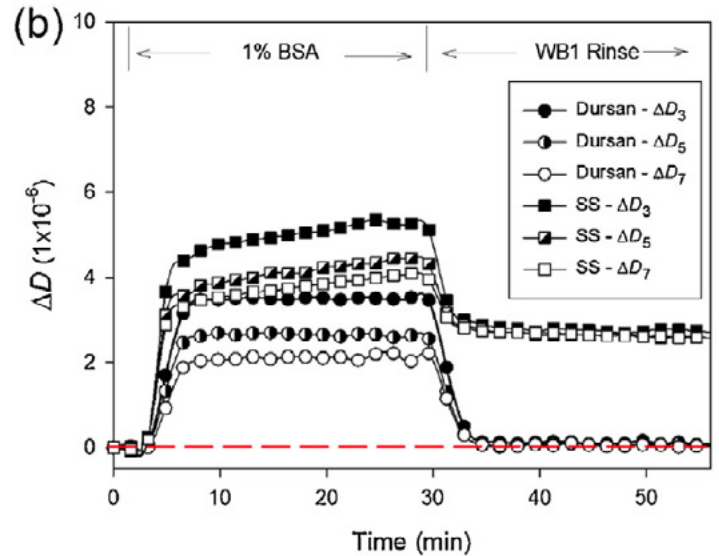
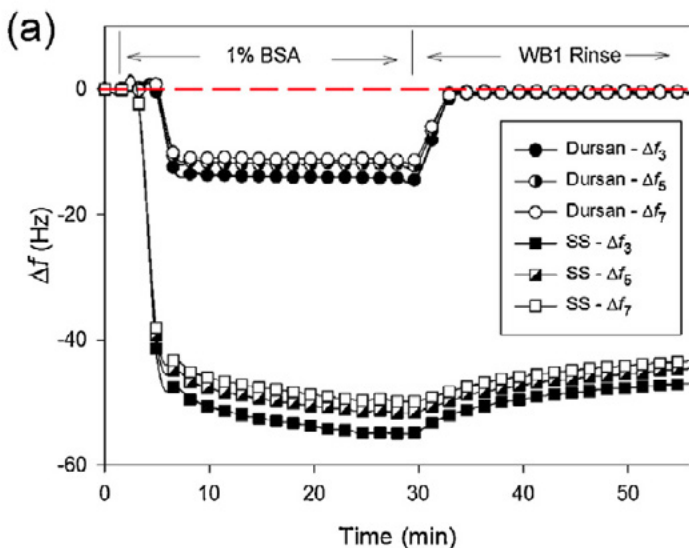


Figure 1) Comparison of BSA adsorption on Dursan-coated (circle) and bare SS (square) sensors. Panel (a) depicts sensor frequency change vs. time, and panel (b) depicts sensor dissipation change vs. time. The 3rd, 5th and 7th overtones were used for measurement.

Figure 1 shows the comparison between uncoated SS (square) vs. Dursan-coated SS sensors (circle) when exposed to bovine serum albumin (BSA). Both sensors experienced an immediate decrease in their resonance frequency upon initial exposure to the protein solution (panel a), indicating protein adsorption to the sensor surfaces. The frequency drop for the bare SS surface was 4 times higher than that of the Dursan[®] surface, due to more mass adsorption. When the sensors were rinsed with the wash buffer WB1, the bare SS surface saw a slight increase of the frequency, whereas the Dursan[®] surface reverted back to the baseline level, indicating near complete desorption of the BSA protein molecules, therefore greatly improved fouling release characteristics compared to the bare SS surface. The dissipation curves (b) indicate the same trend as discussed above. The calculated Sauerbrey mass (adsorbed BSA proteins) to the bare SS surface was 767 ng/cm², and negligible to the Dursan[®] surface.

The authors also compared the effectiveness of two wash buffer solutions used to rinse the sensors, and found that WB1, which contains a nonionic surfactant, was more effective in facilitating the removal of the proteins than a counterpart wash buffer solution without the nonionic surfactant. Their study highlighted the importance of combining the proper rinse solution and the right surface to optimize the protein-resistant properties.

In addition to BSA, the authors also investigated another protein system, mouse immunoglobulin G (IgG), and observed similar behaviors on bare SS and Dursan[®] surfaces. The calculated Sauerbrey mass (adsorbed mouse IgG proteins) to the bare SS surface was 1586 ng/cm², and 70 ng/cm² to the Dursan[®] surface.

In order to assess and compare the physical durability of different coatings during use, the authors exposed two types of coatings, Dursan[®] and AF1600, to 10 minutes of sonication in ethanol. Multiple sensors of each coating were sonicated, and all of the Dursan-coated sensors remained intact after the treatment, whereas a few of the sonicated AF1600-coated sensors exhibited coating delamination as shown in Figure 2.

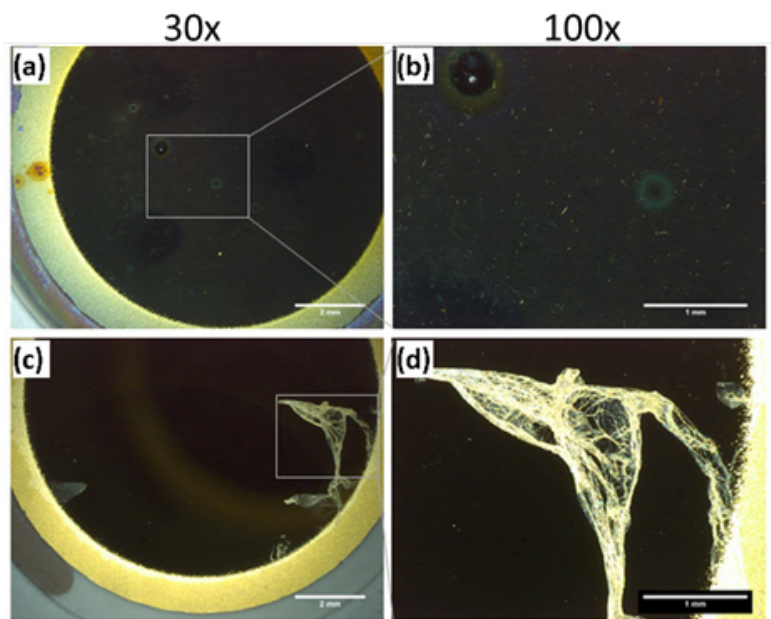


Figure 2) Optical micrograph comparison of Dursan[®] coating (a, b) and AF1600 coating (c, d) after cleaning and sonication treatment. Film delamination was observed in the AF1600 coating, while the Dursan[®] coating remained intact.

The protein adsorption properties of Dursan[®] and AF1600-coated sensors after cleaning and sonication (referred to as “treated”) were assessed by exposing them to normal human plasma (NHP), which is representative of patient samples utilized in diagnostics analyzers. The authors took care to only use AF1600 sensors that did not show any visible post-sonication delamination in this study. Even so, they observed loss of protein-resistant properties of the treated AF1600 surfaces, as shown in Figure 3, that the frequency and dissipation profiles of treated AF1600 (triangle) did not revert back to the baseline levels. In comparison, the Dursan[®] surface (circle) exhibited no performance change before and after treatment. The authors suspected that the AF1600 sensors are susceptible to microscopic structural change and deformity when exposed to sonication, so that even without visible delamination, they suffer the loss of the protein-resistance properties. The Dursan[®] coating was found to be more robust and reliable through the mechanical treatment.

Lastly, the authors investigated a synthetic amino-acid polymer poly-L-lysine (PLL), which exhibits a positive charge at neutral pH and is used extensively for patterning cell culture substrates. They found that PLL did not adsorb on the Dursan® surface at 0.25% or lower concentrations, but became challenging to remove at higher concentrations (1%). The authors believe the highly charged PLL macromolecules led to increased interfacial ordering of the polymer backbones, which made it more difficult to rinse and remove effectively. This system needs to be further studied and characterized.

Discussion and Data

The authors concluded that there is not a universal fix to all biofouling problems, and combinations of different measures should be used to provide more effective solutions. In their study, the combination of the durable and inert Dursan® surface, and an effective wash buffer that contains a non-ionic surfactant, proves to be a step in the right direction towards solving the complex problem of protein adsorption

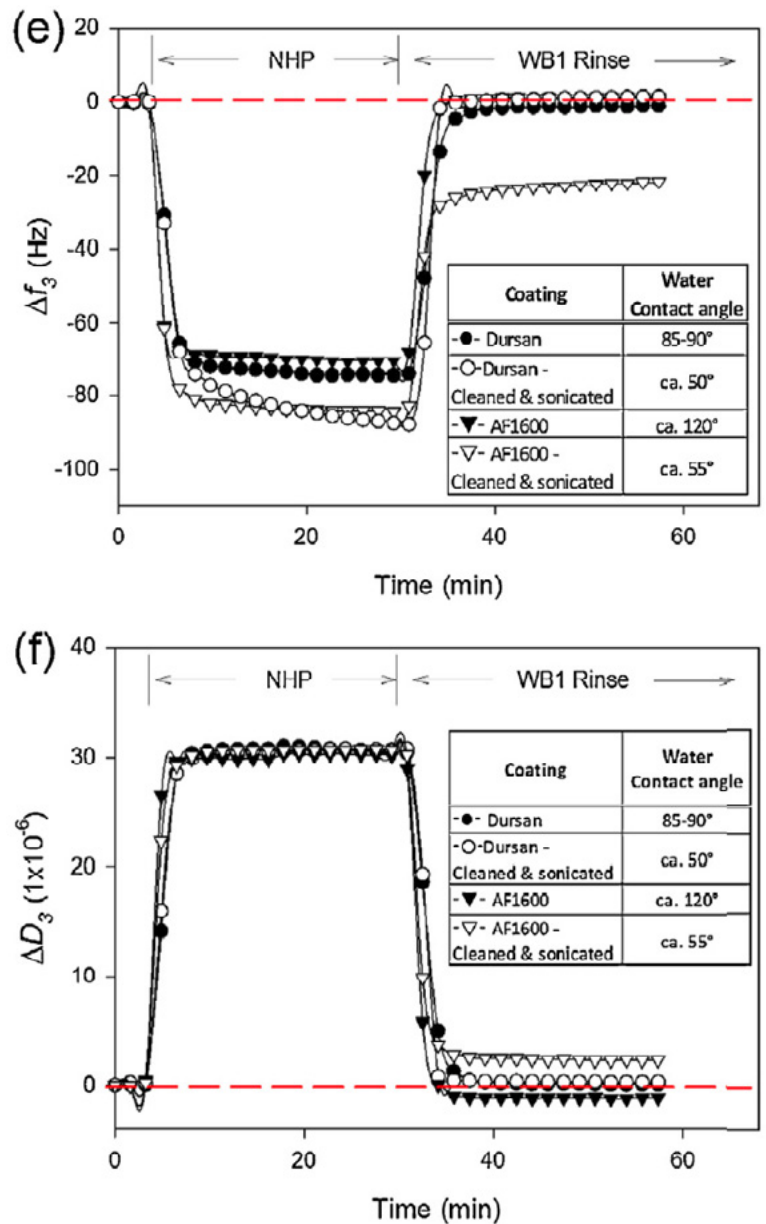


Figure 3) QCM-D frequency (e) and dissipation (f) profiles comparing the adsorption of NHP proteins on untreated and treated Dursan® (circle) and AF1600 (triangle) surfaces.

References:

Vaidya, S. V., Yuan, M., Narváez, A. R., Daghfal, D., Mattzela, J., and Smith, D. "Protein-resistant properties of a chemical vapor deposited alkyl-functional carboxysilane coating characterized using quartz crystal microbalance." Applied Surface Science 2016, 364, 896-908.



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