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Q-Sense | [Application Note] 22

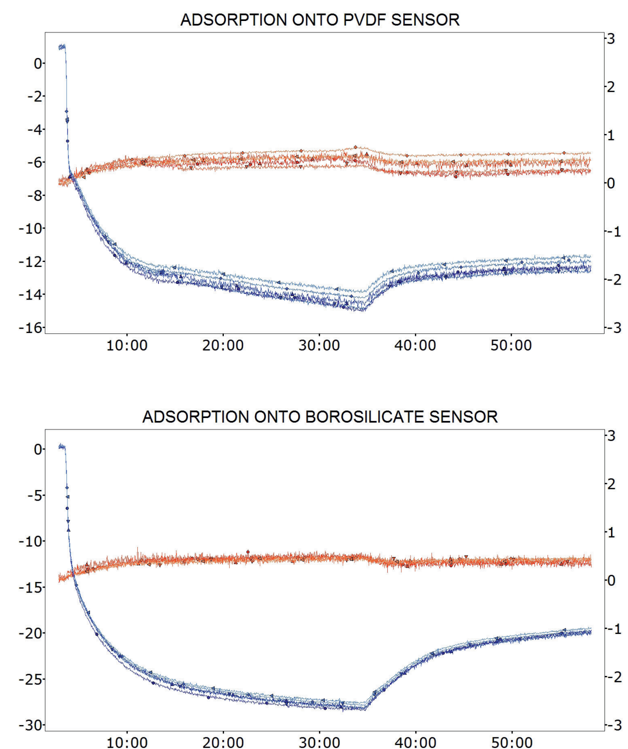
QCM-D as a powerful real-time screening tool for protein adsorption onto glass and plastic surfaces

Plastic and glass materials are continuously used in protein biotechnology applications. For example; syringes, concentrator filters and container systems are all composed of either glass or some plastic polymer (Fig. 1). Proteins have a tendency to passively adsorb to such surfaces via hydrophobic interactions, a phenomenon that has to be minimized or prevented in, for example, large-scale recombinant protein production. In such processes, extremely high protein concentrations, ranging from 40-100 mg/ml, are normally used. The proportion of any given protein that can adsorb onto surfaces ranges from 5-95%, so careful optimization of the used surfaces via special coatings is often required for any protein that is to be produced. In this application note, QCM-D is used as a real-time screening tool for protein adsorption onto PVDF and borosilicate.

Introduction

Quartz Crystal Microbalance with Dissipation (QCM-D) is a powerful acoustic technique where you, with nanogram sensitivity, can measure the mass and structural/viscoelastic changes that occur on the surface of a quartz sensor in real-time. An alternating voltage is applied to the quartz sensor which starts oscillating at its resonance frequency. The mass change at the sensor surface will be sensed as a change in frequency (Δf). In addition, the change in energy dissipation from the system (ΔD) when the power is shut off provides information of the viscoelastic properties of the film. In this study the quartz sensors used were coated with either PVDF or borosilicate glass to mimic surfaces used in protein production materials. Lysozyme was chosen as a model protein.

[Figure 1]: Illustration of equipment in which borosilicate and PVDF is used: a volumetric flask and a protein spin concentrator.



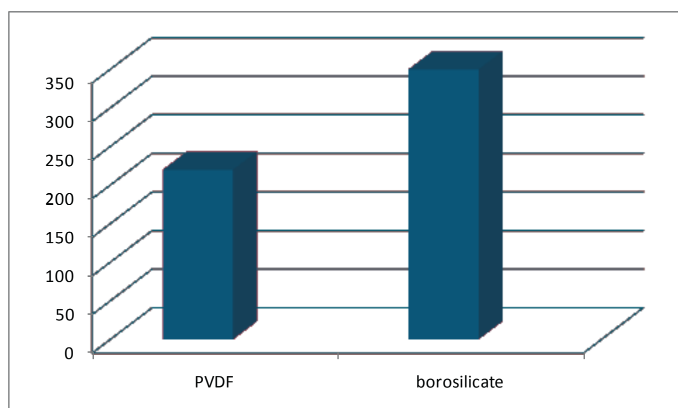
[Figure 2]: QCM-D response for A) Lysozyme (1 mg/ml) adsorption onto PVDF and B) Lysozyme (1 mg/ml) adsorption onto borosilicate.

Arrows indicate protein deposition and wash, respectively.

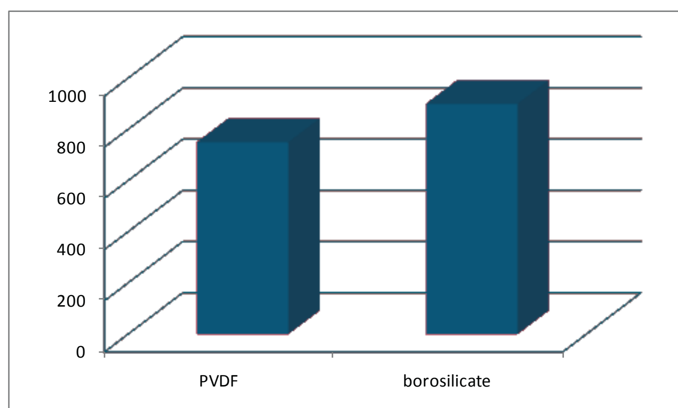
The change in frequency and dissipation is monitored in real-time. Here are shown overtones 3, 5, 7, 9 and 11.

Experimental

PVDF (QSX 999) and borosilicate (QSX 336) sensors, developed at Q-Sense, were equilibrated in buffer (PBS, pH 7.4) in the Q-sense E4 instrument until a stable baseline was observed. Lysozyme powder (Fluka chemicals) was dissolved in buffer to final concentrations of 1 mg/ml and 40 mg/ml. The two different lysozyme solutions were injected above the sensors at 50 µl/min at 25°C. The sample loading was done simultaneously with direct parallel comparison between the two different sensors. All measurements were repeated for accuracy. The mass adsorbed was calculated using QTools software*.



[Figure 3A]: Adsorbed mass onto PVDF and borosilicate sensors at the lower concentration of lysozyme (1 mg/ml).



[Figure 3B]: Adsorbed mass onto PVDF and borosilicate sensors at the higher concentration of lysozyme (40 mg/ml).

Results and discussion

At a concentration of 1 mg/ml, lysozyme was shown to irreversibly bind to both PVDF and borosilicate. The mass increase on the sensor surfaces is visualized by the decrease in frequency (blue lines) observed after washing with buffer (indicated with arrow) (Fig. 2A, B). The low dissipation values (red lines) measured indicates that the films are rigid, since a rigid film does not dampen the sensor oscillation very effectively. As the QCM-D analysis showed that the films were rigid, the Sauerbrey equation could be used to calculate the adsorbed mass. It was concluded that borosilicate adsorbed ca. 60% more of the model protein than PVDF in the case of the lower protein concentration (Fig. 3A).

As expected, the overall adsorbed mass for the more concentrated solution of lysozyme (40 mg/ml) was higher compared to that of the less concentrated sample (1 mg/ml). Similarly, lysozyme was again adsorbed more onto borosilicate than onto PVDF, ca. 20% in this case (Fig. 3B).

It should be noted that even though our model protein displayed no ob-vious structural re-arrangement after binding to the surfaces, many proteins that are routinely purified for pharmaceutical use (i.e. antibodies and human hormones) are known to display such behavior. By monitoring both the change in frequency and dissipation with QCM-D it is possible to probe both the adsorption kinetics and the structural changes simultaneously in real-time, which can give additional information about the adsorption process.

Conclusions

This study presents data that shows that QCM-D successfully can be used for real-time screening of protein adsorption onto various surfaces used in biochemical work, such as for example glass (borosilicate) and plastic (PVDF). By understanding these adsorption processes, thanks to the real-time analysis of both mass and possible structural re-arrangements, minimization of protein loss in protein production can be achieved.

References:

This study was performed by Q-Sense, BiolinScientific AB. For additional information contact info@q-sense.com.

*) QTools is an analysis software included in your Q-Sense QCM-D system.

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