Application Note

Effect of pH on Protein Adsorption to Silicone Surface

Driven by both electrostatic and hydrophobic interactions, protein adsorption to solid surface is of major interest in pharmaceutical science. In this application, QCM instrument qCell T is used to study the extent and driving forces of the pH-dependent protein adsorption to heat-treated silicone surfaces.

Summary

Protein adsorption to solid surfaces is of major interest in pharmaceutical sciences. In this study we demonstrate the application of the qCell T instrument based on QCM technology to study pH-dependent adsorption of a model protein to heat-treated silicone surfaces. We further discuss potential driving forces that trigger protein adsorption.

Background

The resonant frequency of an oscillating quartz crystal in air or vacuum decreases linearly with the deposited rigid mass as first established by Sauerbrey [1]. The high mass sensitivity of the QCM derives from a high stability of the oscillator and a high resolution that in turn facilitate the detection of small frequency changes and mass depositions down to a few nanograms.

Driven by both electrostatic and hydrophobic interactions, proteins tend to adsorb to solid surfaces (Figure 1). In parenteral product packaging systems therapeutic proteins are exposed to silicone surfaces as present in cartridges, syringes and on rubber stoppers. The purpose of this study is to better understand the extent and driving forces of the pH-dependent protein adsorption to baked-on silicone surfaces.

Method

DC 365 35% silicone oil emulsion was diluted to 1.75% (w/w) using highly purified water. A thin film of the diluted silicone emulsion was heat-treated at approx. 300 °C for 12 min in a borosilicate glass bowl and subsequently redissolved in heptane. The silicone solution was used for spincoating gold-coated quartz crystals. Coating uniformity was analyzed using digital microscope with a 30x objective. Testing ink was employed to visualize the silicone coating. A monoclonal antibody was formulated in phosphate buffer at pH 4.0, 8.6 and 11.9 with constant ionic strength of 50 mM.

The quartz crystal microbalance was equilibrated in flow-mode at a rate of 60 µl/min with the particular placebo buffer at 25 °C until no further changes in the frequency (f) and damping (Γ) values were observed. 244 µl of the protein sample of the corresponding formulation were

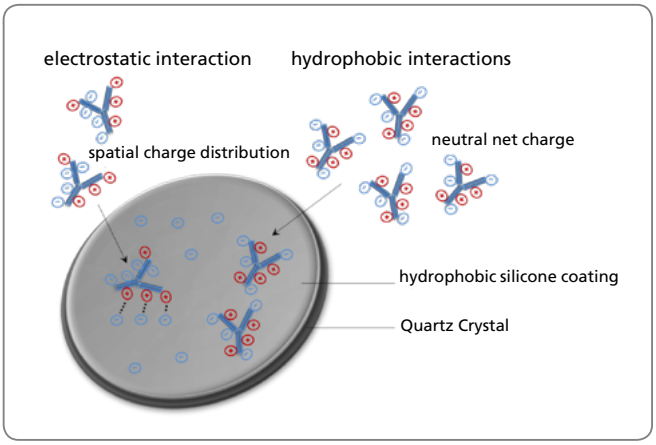

Figure 1. Electrostatic and hydrophobic interactions governing protein adsorption to silicone surfaces, exemplarily shown at pH 8.6.

introduced and allowed to remain in contact with the heat-treated silicone layer until equilibrium. The system was further rinsed with the placebo as running buffer until constant frequency and damping values were achieved. The frequency and damping shifts between the running buffer before and after protein injection were used to calculate the amount of protein that irreversibly adsorbed to the heat-treated silicone surface (Figure 2).

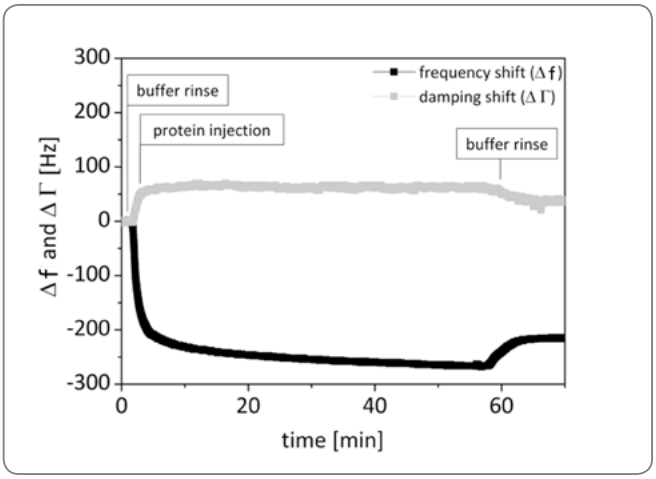

Figure 2. Typical QCM sequence of frequency decrease and damping increase upon adsorption of a model protein of 2 mg/mL at pH 4 to heat-treated silicone surfaces.

Results

The uniformity of the baked-on silicone surface was characterized by testing ink.The testing ink was evenly spreads over the gold-coated quartz crystal. Silicone coated areas lead to the retraction of testing ink (Figure 3). Spincoating was determined to be crucial to create a homogeneous silicone layer.

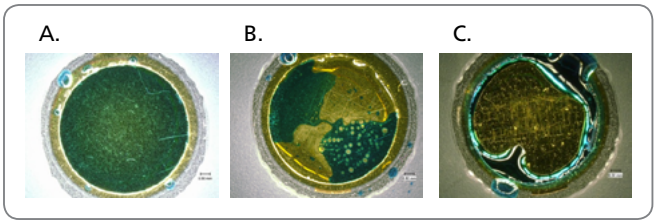

Figure 3. Development of a homogeneous heat-treated silicone coating on gold quartz crystals. The surfaces are covered with testing ink. A. gold-coated quartz crystal B. inhomogeneous silicone coating, manually coated. C. homogeneous silicone layer, spincoating.

The model protein markedly adsorbs at pH 4.0 and 8.6 with adsorbed masses of approx. 4.5 mg/m^2, whereas the adsorption is only 1 mg/m^2 at pH 11.9 (Figure 4).
At pH 4.0 the heat-treated silicone surface carries the least net charge (isoelectric point (pI) of silicone oil droplets in highly purified water was determined to be at pH 3.9). The adsorption of the positively charged model protein (pI 8.3) is suggested to be mainly driven by hydrophobic interactions and an increase in entropy due to the dehydration of the hydrophobic silicone surface [2].
Close to the protein pI the low net charge might trigger the initial adsorption to the hydrophobic silicone surface due to hydrophobic interactions. Furthermore the inter-

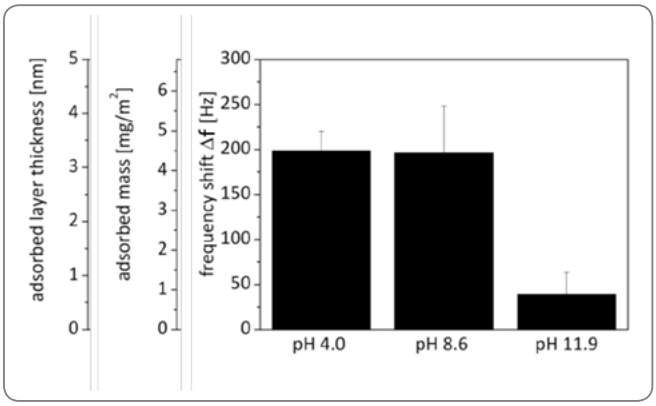

Figure 4. Protein irreversibly adsorbed to heat-treated silicone surfaces at equilibrium for pH 4.0, 8.6, 11.9 and 50 mM ionic strength.

protein repulsion is minimal around the pI which leads to a closer packaging of the protein molecules [3, 4]. Negatively charged anionic groups and positively charged cationic groups contribute to the overall neutral net charge. However, residual positive charge patches might induce protein adsorption to the negatively charged silicone surface.
At pH 11.9 where both the silicone oil surface and the protein molecules are negatively charged repulsion of the protein from the surface as well as amongst the protein molecules themselves impedes adsorption. But the hydrophobic interactions still provide a substantial driving force for the protein adsorption to heat-treated silicone surfaces [5]. Additionally, the repulsive overall charges might be shielded by the incorporation of ions into the adsorbed layer which facilitates protein adsorption even at high pH [2].

Conclusion

The pH dependency of protein adsorption to heated silicone surface was successfully monitored by qCell T in real time. The frequency and damping shift of qCell T suggested that the higher protein adsorption at pH 4.0 and pH 8.6 was driven by both hydrophobic interactions and charge interactions. At pH 11.9 protein adsorption was less due to strong repulsive electrostatic forces. But hydrophobic interactions still rendered substantial adsorption.

Reference

[1] G. Sauerbrey, Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung, Zeitschrift für Physik, 155 (1959) 206-222.
[2] W. Norde, My voyage of discovery to proteins in flatland … and beyond, Colloids and Surfaces B: Biointerfaces, 61 (2008) 1-9.
[3] J. Buijs, P.A. van den Berg, J.W.T. Lichtenbelt, W. Norde, J. Lyklema, Adsorption Dynamics of IgG and Its F (ab')2 and Fc Fragments Studied by Reflectometry, Journal of Colloid and Interface Science, 178 (1996) 594-605.
[4] J. Mathes, W. Friess, Influence of pH and ionic strength on IgG adsorption to vials, European Journal of Pharmaceutics and Biopharmaceutics, 78 (2011) 239-247.
[5] W. Norde, J. Lyklema, Thermodynamics of protein adsorption. Theory with special reference to the adsorption of human plasma albumin and bovine pancreas ribonuclease at polystyrene surfaces, Journal of Colloid and Interface Science, 71 (1979) 350-366.

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