Technical White Paper

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Using QSense QCM-D to assess Stability and Material Compatibility of Biopharmaceuticals

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[Progress Together]

Analyze adsorption of biologics and excipients to materials used in manufacturing, storage and administration

Abstract

Thanks to the treatment efficiency for various diseases, biologics, such as therapeutic monoclonal antibodies, are on a steady rise and expected to continue to grow in popularity. Although holding great potential in terms of health gains, there are challenges associated with drug development using these relatively large and complex molecules. One challenge is that of surface interaction. Throughout their lifespan, from manufacturing to storage and administration, the therapeutic drug will interact with many different surfaces which may result in adsorption, loss of concentration and/or formation of proteinaceous particles. Late discovery of incompatibilities may pose a risk to product development timelines and pose large financial costs. Screening for surface – induced instabilities during development could help reduce the risk of incompatibilities and prevent challenges that may occur during manufacturing, storage, or administration. We propose QCM-D analysis to assess antibody adsorption to gain insight into when and why incompatibilities could occur and to identify ways to mitigate. As a proof of concept, we demonstrate the capabilities of the technology to measure antibody adsorption to different materials and the effect of added surfactant.

1. The lack of established methods to screen for surface-induced instabilities increases the risk of late discovery of incompatibilities that could jeopardize product development timelines and pose large financial costs

Over the past couple of decades, the pharmaceutical industry has increasingly turned their attention away from small molecules and moved towards biological macromolecules, such as monoclonal antibodies (mAbs). Small molecule drugs still dominate the pharmaceutical market, but due to the treatment efficiency for various diseases, biologics, which is today a billion-dollar industry, is on a steady rise and expected to continue to grow in popularity.[1,2] In spite the great promise of these molecules to be able to address disease where no treatment options currently are available, however, the road towards new revolutionizing drugs is not straight as there are several challenges associated with the development of biopharmaceuticals. Compared to the small molecule drugs, the biological macromolecules are large and much more complex. They are difficult to manufacture, sensitive to the ambient environment, and impossible to fully characterize. One aspect that is relevant in the context of characterization is that of surface interaction. Biological macromolecules are prone to interact with surfaces, and doing so, they may adsorb, which could result in loss of concentration and/or particle formation. Over their life span, from manufacturing to storage and administration, the therapeutic molecules will meet many different surfaces, and every interface that they meet after the terminal filtration step presents a risk of reducing the concentration or generating particles. Depending on drug and dose, the consequence may be an unacceptable decrease in concentration or increase of particles, posing a risk to product develop-

ment timelines and a risk of large financial costs such as failing product release specifications during manufacturing. Surface - interaction analysis during development and formulation could provide an indication of challenges that could occur in, or during, manufacturing, storage, and administration. However, methods to screen surface-induced instabilities have not yet been established, which increases the risk of late discovery of such unwanted scenarios.

2. Time-resolved analysis of molecule – surface interaction can decrease the risk of late discovery of potential incompatibilities

Quartz Crystal Microbalance with Dissipation monitoring, QCM-D, is a surface sensitive technology which has been used to analyze molecule - surface interaction in general, and biomolecule - surface interactions in particular, for more than two decades. The method has for example been used to study the interaction between proteins and material surfaces used in biopharmaceutical production and container closure systems.[3-8] The time-resolved information on mass, thickness and viscoelastic properties provided by the method enables insight into phenomena such as molecular adsorption, desorption, and structural rearrangement of molecular layers at the sensor surface.

Thanks to its high sensitivity and flexibility of substrate materials and solvent conditions, QCM-D technology offers a fast and low-sample consumption approach to study molecule-surface interactions at the nanoscale and get an early indication of potential incompatibilities as well as a mechanistic understanding of molecular processes and behavior at the solid-liquid interface. The detailed insight into the surface interaction phenomena can provide key information that helps predict challenges which may occur in, or during, manufacturing, storage, and administration, when drug product interfacing materials are introduced as well as support material development, guidance, and selection.

3. A fast and low sample consumption approach to get an early indication of potential incompatibilities

As discussed above, QSense QCM-D technology has offered insight into biomolecular interactions at surfaces and interfaces for more than two decades, including analysis in the context of biopharmaceutical production and container closure systems. To further demonstrate the capabilities of the technology, we here present two case examples that show how QSense QCM-D can be used in biopharmaceutical drug development to assess and reveal initial adsorption of biologics and excipients on different substrates and provide insight into to the mechanistic behavior of the system under study.

Selected materials and experimental approach

Pre-filled syringes are commonly used for storage and administration of biopharmaceuticals due to their ease of use and potential self-administration. The drug, such as a monoclonal antibody, will come in contact with several materials with different properties during storage and administration. These materials are typically a syringe barrel made of glass, a cap made of plastics, silicone oil used to lubricate the plunger and the barrel, and a needle made of metal. Thus, we here wanted to show two examples that demonstrate QSense QCM-D as a robust and reproducible method to detect the interaction between mAbs and materials relevant for biopharmaceutical storage, with emphasis on pre-filled syringes.

In the first example, QCM-D is used to study mAb adsorption to plastics, glass, metal, and silicone oil coated surfaces . In the second example, we show how the method can be used to study the effect on mAb adsorption to glass and silicone oil in the presence of a non-ionic surfactant. In both examples, we used the automated instruments QSense Pro and QSense Omni to run measurements in parallel as an efficient way to get triplicates to evaluate variation and get good statistics.

QSense QCM-D is a surface sensitive time-resolved technology for label free analysis of molecular interactons at surfaces and interfaces. Monitoring changes in resonance frequency, *f*, and dissipation, *D*, of a quartz crystal sensor, processes at the solid-liquid interface can be characterized and quantified.

What you can do:

Analyze molecule - surface interaction, molecule-molecule interaction, and conformational changes of surface - adhering layers, time-resolved and label-free.

Events that you can analyze:

- Adsorption/desorption
- Binding
- Enzymatic activity
- Structural changes of the molecular layer

How to interpret the data

- **Δ*f* provides** information about mass changes at the surface. A decrease indicates mass uptake and vice versa
- **Δ*D* provides** information about the layer softness. As a rule of thumb, the higher the *D*, the softer and/or thicker the layer

Case A) Analyzing the adsorption of mAbs to plastics, glass, metal, and silicone oil

In this example, we demonstrate how QCM-D can be used to study adsorption of mAb to plastics, glass, metal and silicone oil coated surfaces.

The experimental protocol was as follows

- baseline in buffer, 1h
- mAb adsorption, 2h
- rinse with buffer, 2h

Results

The time resolved QCM-D raw data, Fig. 1, shows the mAb-material interaction dynamics and allows for analysis and comparison between the different materials studied.

Comparing the maximum Δf and ΔD responses in the mAb adsorption step, Fig. 2, it is noted that

- the mAb binds most strongly to the metal surface
- similar amounts bind to the plastic and the silicone oil, but that the ΔD shift is higher on the plastic
- the data also reveals that the interaction with the glass surface is weak

Mechanistic insights

Plotting the acoustic ratio, $\Delta D/\Delta f$, Fig. 3, additional aspects and insights emerge

- the higher $\Delta D/\Delta f$ -ratio for mAb adsorption on glass indicates a softer layer and difference in adsorption characteristics compared to the other materials
- the low $\Delta D/\Delta f$-ratio on silicone oil indicates that the mAb unfolds on this very hydrophobic surface when hydrophobic pockets on the protein bind to the sensor. This has been suggested as the driving factor for particulate formation and aggregation in silicon oil coated pre-filled syringes.[9]

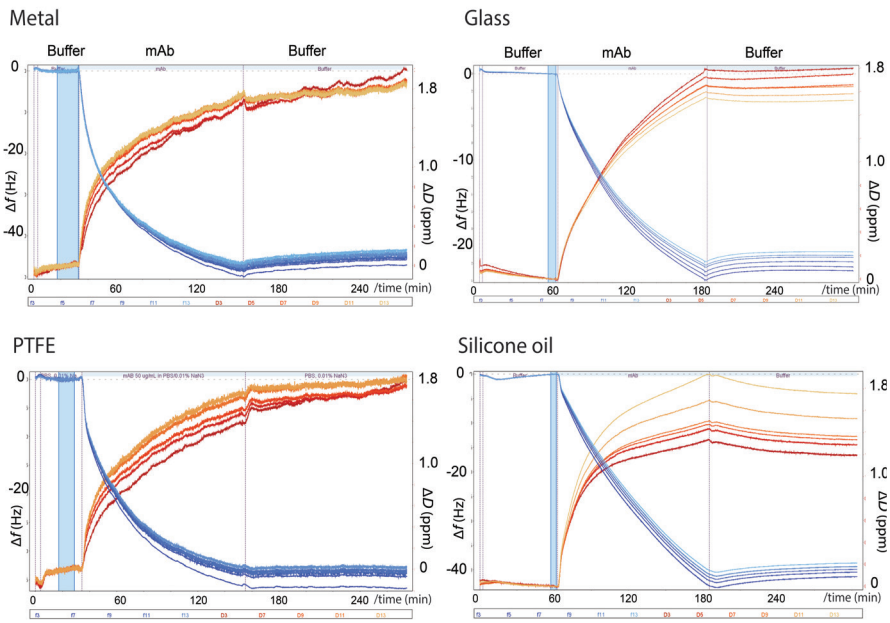

Figure 1. QCM-D raw data, Δf and ΔD, showing time resolved mAb-surface interaction with the pre-filled syringe mimicking materials steel, plastics, silicone oil and glass.

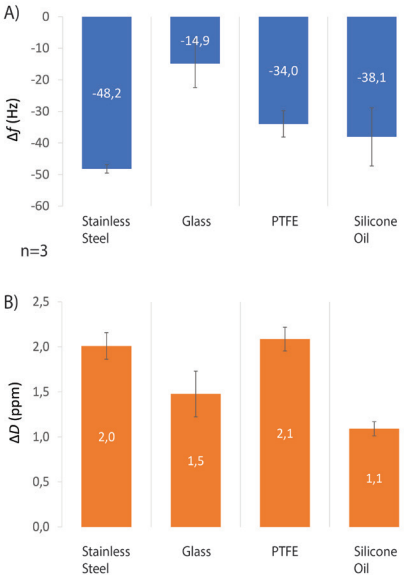

Figure 2. Comparison of the maximum QCM-D raw data A) Δf and B) ΔD, responses for mAb adsorption to the pre-filled syringe mimicking materials steel, glass plastics, and silicone oil revealing uptake and layer structure.

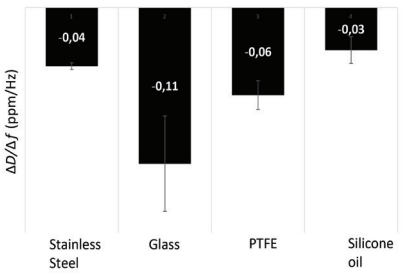

Figure 3. Comparison of the $\Delta D/\Delta f$ ratio for mAb adsorption to the pre-filled syringe mimicking materials steel, plastics, silicone oil and glass reveals differences in layer softness and adsorption characteristics on the different materials.

Use QCM-D to:

- *quickly assess the effect of actual manufacturing/storage/ administration materials on the full formulation*
- *quickly measure adsorbed amount of formulation candidate on relevant materials and identify ways to mitigate incompatibilities*
- *understand why and when there is likely to be incompatibilities*
- *understand surfactants action mechanism/potential as stabilizer in relevant context*

Case B) Analyzing the effect of surfactant on mAb adsorption to glass and silicone oil

Surfactants are often used in formulation to alter container-drug interactions.[10] In the second example, we therefore wanted to demonstrate how QSense QCM-D can be used to study the effect on mAb adsorption to silicone oil and glass when co-formulated with a non-ionic surfactant.

The experimental protocol for results shown in Figure 4A was as follows

- baseline in buffer, 1h
- non-ionic surfactant, 1h
- mAb adsorption together with surfactant, 2h
- rinse with buffer, 2h

Reference measurement of surfactant adsorption without mAb was also performed (Figure 4B) to better assess the surfactant and mAb adsorption characteristics.

Results

The time resolved QCM-D raw data, Figs. 4 and 5, reveal how the surfactant and the co-formulated surfactant-mAb interact with silicone oil, Fig. 4, and glass, Fig. 5.

Surfactant and surfactant – mAb interaction with silicone oil

Taking a closer look at the adsorption dynamics onto silicone oil, Fig. 4, we see that

- The surfactant binds with very fast kinetics, most likely saturating the surface in a monolayer (Fig 4, step I).
- The surfactant lowers the amount of bound mAb by 95% (comparison of data after rinse in Fig 1 -38,1 Hz, and Fig 4 -1,7Hz)
- The surfactant binds strongly to silicone oil and protects the sensor from mAb binding, Table 1.

Silicone oil	I, Δf at surfactant interaction	II, Δf after 30 min rinsing
Fig 4A	-13,8 Hz (n=3)	-8.8 Hz (n=3)
Fig 4B, ref	-11.6 Hz (n=2)	-9,5 Hz (n=2)

Table 1. Comparison of frequency shifts, Δf, at surfactant interaction with silicone oil before (I) and after (II) rinse and in presence of mAb (Fig 4A).

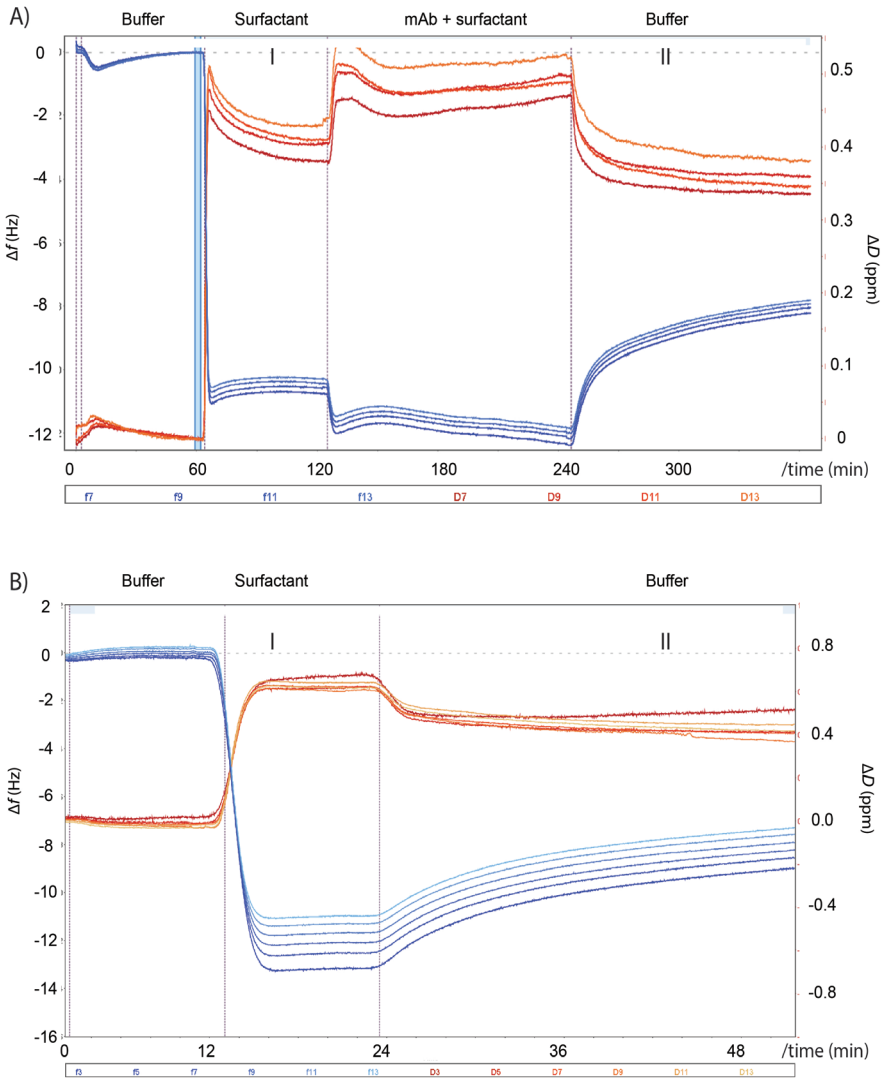

Figure 4. QCM-D raw data showing time resolved interaction of, A) surfactant (I) and thereafter mAb + surfactant, and B) surfactant interaction (I), with silicone oil. Both measurements end with a buffer rinse in (II).

Benefits QSense QCM-D

- Provides information on molecule-surface interaction and behavior at interfaces
- High mass sensitivity allows for measurements of initial adsorption
- Flexibility of substrate coatings enables analysis of real, relevant materials
- Small sample volume
- Fast
- Tailor-made for aqueous samples
- Automated sample handling

Surfactant and surfactant – mAb interaction with glass

The adsorption dynamics onto glass, Fig. 5, reveal that:

- The surfactant binds weakly to the glass and does not protect the sensor from mAb binding (see Table 2), most likely due to the hydrophilic properties of the glass surface (Step I).
- The presence of surfactant in the solution increases the amount of bound mAb by 67%. (-25,5 vs -14,9 Hz). As previously seen, the surfactant and the mAb most likely interacts strongly and binds as larger complex.
- The surfactant + mAb complex seems to bind less strongly to glass than without surfactant, which can be seen in the increased removal upon rising when comparing Figs. 1 and 5.

We could also see in the QCM-D data that the mAb adsorbed more, and probably unfolded, on the silicone oil (as revealed by a high Δ*f* shift and a low Δ*D* shift) compared to glass (low Δ*f* shift, high Δ*D* shift). One clear difference between these two materials is the surface energy where the silicone oil is highly hydrophobic and the glass is highly hydrophilic, as measured by static water contact angle using an Attension Theta Lite instrument.

Conclusions from the Case examples

The two case examples demonstrate how QSense QCM-D was used to successfully assess the adsorption behavior on relevant container closure materials for biologics production. The data revealed that the mAb adsorption was highest on metal and decreased on silicone oil, plastics, and glass respectively. Due to their central role for pre-filled syringe systems, silicone oil and glass were studied further. The strong mAb adsorption behavior on hydrophobic silicone oil sensors could be verified and the non-ionic surfactant did lower this unfavorable interaction by 95%. On hydrophilic glass sensors, the adsorption of mAb was significantly lower and the mAb did not collapse to the sensor surface to the same

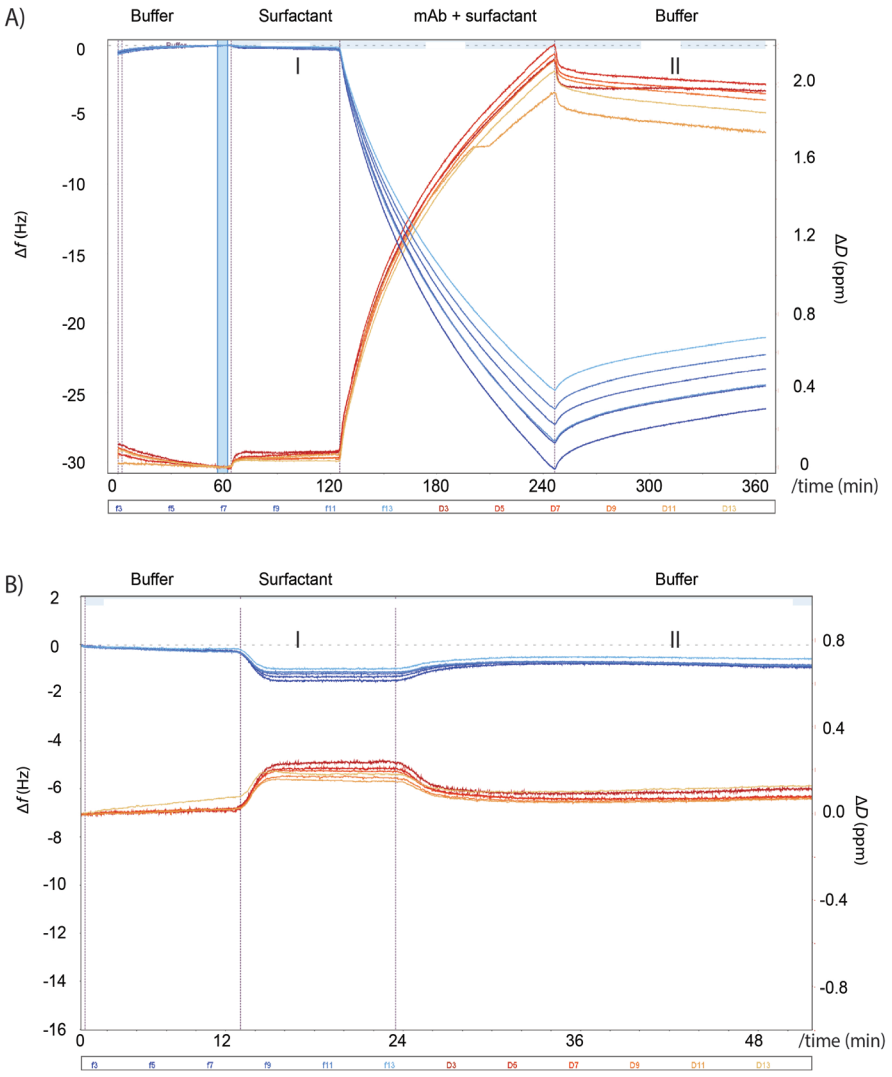

Figure 5. QCM-D raw data showing time resolved interaction of, A) surfactant (I) and thereafter mAb+surfactant, and bottom graph, surfactant interaction (I), with glass. Both measurements end with a buffer rinse in (II).

Glass	I, Δ*f* at surfactant interaction	II, Δ*f* after 30 min rinsing
Fig 5A	-0.1 Hz (n=3)	-25.5 Hz (n=3)
Fig 5B, ref	-2.1 Hz (n=2)	-0,1 Hz (n=2)

Table 2. Comparison of frequency shifts, Δ*f*, at surfactant interaction with glass before (I) and after (II) rinse and in presence of mAb (Fig 5A).

extent as for silicone oil. The surfactant did not lower the mAb adsorption, instead it likely created complexes with mAb in solution and bound at a higher rate on glass than the mAb alone.

4. Concluding remarks

As the pharmaceutical industry is shifting the attention towards biologics, there is an increasing need to test for protein adsorption to materials encountered during drug production, storage, and delivery to understand the risk of particle formation and loss of concentration and to mitigate potential challenges before they lead to program delays and unplanned costs. Surface - interaction analysis of antibodies and excipients during development and formulation could provide useful information and early on give an indication of challenges that could occur in later production or administration stages. However, there is lack of established methods to screen for surface-induced instabilities, which presents an unnecessary risk of late failure. QSense QCM-D is a robust and reproducible analysis method which can detect the interaction between biopharmaceuticals and materials relevant for manufacturing, storage, and administration. The case examples presented in this paper demonstrate how the adsorption behavior on relevant container closure materials for biologics production with emphasis on pre-filled syringes could be assessed.

Interested to learn more?

If you would like to learn more about QSense QCM-D and how it can help ypu in your work, please reach out and we will tell you more. We would love to hear from you.

References and further reading

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