

QCM-D IN DRUG FORMULATION AND STORAGE

Protein drug aggregation can occur during manufacturing or formulation stages. Examples of surfaces where proteins aggregate include glass, oils, plastic polymers and metals in concentrators, tubings, containers or bags. Understanding proteins' aggregation propensity is crucial to minimize loss of often expensive therapeutic proteins during manufacturing and to ensure the development of a fully functional drug component. Quartz Crystal Microbalance with Dissipation monitoring, QCM-D, has been successfully used to study such aggregation processes.

INTRODUCTION

Protein aggregation is an important factor in drug development and manufacturing. Better knowledge about these processes can also help increase shelf-life of the end-product through additives such as stabilizers and surfactants. Although there is a great need for understanding protein aggregation in drug discovery and development, there is a current lack of mechanistic understanding of these events.

In the two studies reported here QCM-D was used as an efficient way of monitoring the adsorption and aggregation of therapeutic monoclonal antibodies and proteins. By the use of QCM-D both mass changes and viscoelastic properties of the proteins were analyzed in real-time. In addition to protein-surface interactions, the effect of surfactants and concentration was also investigated.

EXPERIMENTAL

In the first study¹ two monoclonal antibodies (mAb1 and mAb2) with significant differences in hydrophobicity and self-oligomerization behavior in solution were studied. The adsorption behavior of the antibodies onto four dif-

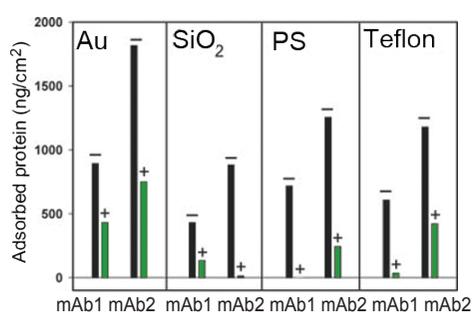


FIGURE 1 A. Mass of irreversibly bound mAb1 and mAb2 (1 mg/ml) determined by viscoelastic modeling of QCM-D data. + and – represent the presence or absence of the surfactant PS-80. Control measurements provided an average value for adsorbed surfactant. Protein mass has been adjusted accordingly.

ferent surfaces (silicone dioxide (SiO₂), polystyrene (PS), Teflon AF1600 (Teflon) and gold (Au)) was investigated in the presence and absence of the surfactant polysorbate 80 (PS-80). The concentration dependence of the adsorption behavior was also examined.

In the second study² the protein drug Abatacept and its interaction with the silicone oil/water interface was analyzed. The drug is administrated through a silicone oil lubricated syringe which is known to cause severe aggregation. SiO₂ sensors were spin-coated with silicone oil to form a homogeneous film. The adsorption of Abatacept was monitored in the presence

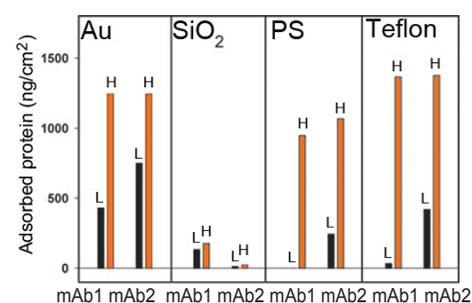


FIGURE 1 B. Mass of irreversibly bound mAb1 and mAb2 as a function of concentration. L refers to 1 mg/ml and H refers to 50 mg/ml. All data was generated in the presence of PS-80. Control measurements provided an average value for adsorbed surfactant. Protein mass has been adjusted accordingly.

and absence of two surfactants (PS-80 and Poloxamer 188).

The mass changes were extracted in QTools from the change in frequency (Δf) and Dissipation (ΔD) respectively. Both studies were conducted using Q-Sense equipment at 25 °C.

RESULTS AND DISCUSSION

In the antibody study¹ it was concluded that mAb2 adsorbed stronger than mAb1 on all types of surfaces used (Fig. 1A). Addition of the surfactant PS-80 did significantly reduce the adsorption for both proteins. On the Au surface both mAb1 and mAb2 remained

adsorbed to a large extent even in the presence of the surfactant (Fig. 1A). This is probably due to the strong electrostatic interaction between the positively charged protein surface and the negatively charged metal, which the non-ionic surfactant does not easily disrupt.

The concentration dependence of the adsorption was also evaluated and showed differences in adsorption level for the PS, Teflon and Au surfaces but had less impact on the SiO₂ surface (Fig. 1B). The surfaces which displayed concentration-dependent adsorption also gave rise to higher level of softness in the protein film, indicating increased hydration (data not shown).

In the protein drug study² the adsorption of Abatacept to the silicone oil/water interface was investigated. Silicone oil spin-coated QCM-D sensors provided an excellent platform to address these questions. It was concluded that the surfactant PS-80 reduced the amount of adsorbed Abatacept whereas Poloxamer 188 did not alter the binding significantly (Fig. 2). Injection of PS-80 also reduced the overall softness of the Abatacept film (lower $\Delta D/\Delta f$), whereas Poloxamer 188 did not (data not shown).

One possible explanation for the observed trend that PS-80 inhibited aggregation could be attributed to the fact that PS-80 displays significantly faster adsorption kinetics - about 2 minutes - onto the interface than the Abatacept molecule on its own. Abatacept alone reached equilibrium after 12 minutes

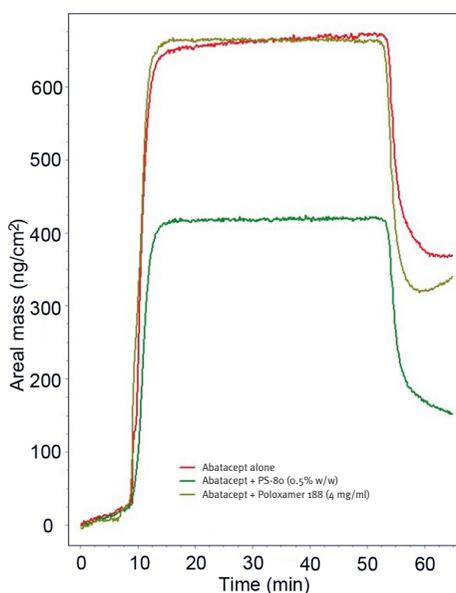


FIGURE 2. Changes in estimated mass adsorbed at the oil/water interface as a function of time upon injection of 10 mg/ml Abatacept without PS-80 (red), with PS-80 (dark green) and Poloxamer 188 (light green). The mass changes were calculated based on the assumption that the underlying silicon oil film does not affect changes in f and D upon protein adsorption. This data was analyzed with permission from the authors².

(Fig. 2). It is possible that PS-80 then forms a protective shield, whereas Poloxamer 188 binds more slowly - about 10 minutes - to the interface and hence fails to prevent aggregation (Fig. 3).

CONCLUSIONS

QCM-D is shown to be a valuable tool in monitoring aggregation of proteins and antibodies onto different surfaces used in production or manufacturing in the pharmaceutical industry. By gaining better understanding of the aggregation processes of therapeutic protein drugs, both minimization of product loss and a more accurate end-product can be attained.

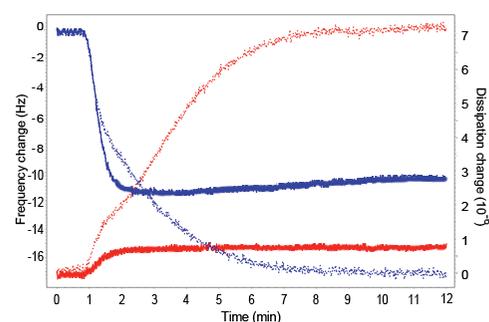


FIGURE 3. Changes in f (blue) and D (red) upon injection of PS-80 and Poloxamer 188 alone onto the oil/water interface. Thick curves show PS-80, dotted curves show Poloxamer 188.

ACKNOWLEDGEMENTS

We thank Eli Lilly and Bristol-Myers Squibb for their collaboration to prepare this note.

REFERENCES

[1] Anna Oom, Mark Poggi, Jennie Wikström and Mupalla Sukumar. *Surface interactions of monoclonal antibodies characterized by Quartz Crystal Microbalance with Dissipation; Impact of hydrophobicity and protein self-interactions*. Journal of Pharmaceutical Sciences, 2011, 101, 519-529.

Joint publication between Eli Lilly and Biolin Scientific.

[2] Jinjiang Li, Swathi Pinnamaneni, Yong Quan, Archana Jaiswal, Fredrik I Andersson, Xiaochun Zhang. *Mechanistic understanding of protein-silicone oil interactions*. Pharm Res. 2012 [Epub ahead of print].

Joint publication between Bristol-Myers Squibb and Biolin Scientific.