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| **Dynamic Analysis of Lysozyme Adsorption Behaviour to a Borosilicate Substrate Measured by QCM-D** |
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| **Background:** There is an increased awareness of the effect of formulation on protein interfacial stability in primary packaging. However, a comprehensive understanding of the interplay between proteins, excipients e.g., buffering media and solid interfaces is required to help avoid the loss of therapeutic cargo during processing, formulation, storage, and delivery. Quartz crystal microbalance with dissipation (QCM-D) is a real-time, surface-sensitive technique for analyzing surface-interaction phenomena, layer formation and properties. It can also be used to screen for formulation effects and determine the effect of excipient choice on protein interfacial and conformational stability. The goal of this study was to investigate the effect of two non-amino acid buffers; sodium phosphate and sodium citrate, and two amino acid buffers; histidine and glycine at a fixed concentration of 10 mM using a pH range of 7.4 – 3.6 using QCM-D. With the revival of silicon-free, pre-filled delivery systems, evaluating the impact of fundamental protein formulation excipients like buffer type and pH on protein adsorption to naked borosilicate is crucial in ensuring judicious control over the critical quality attributes of protein drug products. |
| **Methods:** Lysozyme adsorption at the borosilicate surface was measured using a QSense® E4 QCM-D system. The sensors were equilibrated for 5 min to establish a stable baseline. Thereafter, lysozyme solutions (1 mg/mL) in the different buffers (sodium phosphate and histidine at pH 7.4, histidine and sodium citrate at pH 5.5 and sodium citrate and glycine at pH 3.6) were perfused for 15 min. During measurement, frequency decrease and dissipation increase for each sensor were recorded. Sensors were then flushed with the corresponding buffer for 15 min. All experiments were conducted at a flow rate of 100 μL/min. Average values of ΔF and ΔD (overtone #7-harmonic 7 above the fundamental note) were reported for analysis. As the changes in energy dissipation were low, the mass of lysozyme layers was calculated based on the Sauerbrey equation. |
| **Results:** QCD-M was performed to determine the impact of buffer type and pH on lysozyme adsorption behaviour to borosilicate. QCM-D frequency response is related to the quantity of adsorbed lysozyme, and the dissipation response, indicates the nature of layer formation on the sensor surface. The mass of adsorbed lysozyme displayed an adsorption behaviour that was primarily influenced by the pH of the solution. The effect of different buffers on lysozyme adsorption to borosilicate was quantified using the Sauerbrey Equation and the quantity of adsorbed lysozyme per surface area confirmed that lysozyme adsorption was primarily influenced by buffer pH, with buffer composition, at each pH, showing no effect. Lysozyme formed a thin, rigid, and non-viscoelastic layer on the substrate and demonstrated no changes in layer properties over time, indicating conformational stability on the surface of the borosilicate substrate. The kinetics of adsorption were also measured, and no considerable differences were observed except for the 10 mM glycine buffer which displayed slower and firmer interactions with the substrate over time.  |
| **Conclusions:** QCM-D analysis demonstrated lysozyme adsorption to the borosilicate substrate occurred was impacted by pH and buffer composition showed no significant effect. Layer formation was thin and rigid with no changes in conformation or adsorption kinetics.  |