Experimental report

26/05/2020

Proposal: 9-13-844 **Council:** 4/2019

Title: Investigating how bioengineered monoclonal antibodies adsorb at the stainless steel water interface using neutron reflection

Research area: Physics

This proposal is a new proposal

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Samples: COE-3

Instrument	Requested days	Allocated days	From	To
D17	0	0		
FIGARO	3	3	29/07/2019	01/08/2019

Abstract:

Monoclonal antibodies are being used to develop cutting edge drugs to fight cancers and diseases. During fill-finish manufacturing of monoclonal antibody biopharmaceuticals interactions between the antibodies and stainless steel occur (in the form of storage containers and pipelines). These interactions can lead to negative phenomena including denaturing and aggregation which, in turn, lead to a reduction of bioactivity. MedImmune has provided a bioengineered monoclonal antibody (COE-3) and we aim to investigate its adsorption dynamics (using FIGAROs high flux). As well as this the adsorbed conformation and structure of COE-3 resulting from different solution conditions can be inferred. Our group have published papers investigating the adsorption dynamics of COE-3 at the SiO_2 interface. This study will extend on this revealing how different substrates impact the adsorption of COE-3. Understanding COE-3 adsorption at the stainless steel interface will increase the efficiency of biologicals production and potentially enable to production of some, otherwise unstable, biotherapeutics.

Experimental report on:

Investigating how bioengineered monoclonal antibodies adsorb at the stainless steel/water interface using neutron reflection

9-13-844 (29 July – 01 August 2019)

Background

Monoclonal antibodies (mAbs) are Y-shaped protein that make up a considerable portion of the therapeutic market. During fill-finish, transport, storage and administration therapeutic mAbs can spontaneously adsorb and desorb to interfaces present. Stainless steel is a key interface in therapeutic protein production as it is present in pumps, administration needles and storage containers. During the final fill/finish, product incompatibilities at the stainless steel/water can be costly resulting in failed batches and loss of product efficacy [1]. Understanding the adsorption of mAb can be used to develop mAbs that are more resistant to incompatibilities with common material.

Method

Specular neutron reflection (SNR) was used to investigate the adsorption of COE-3 (a mAb provided by AstaZeneca) in varying conditions. D_2O and H_2O based buffers were used to provide multiple solvent isotropic contrasts. SNR measurements were performed in time-of-flight mode on FIGARO providing a Q range from 0.012 – 0.30 A^{-1}. The silicon block was sputter coated with a stainless steel was measured in D_2O and H_2O as a baseline prior to adsorption. COE-3, its crystallisable fragment and its antigen-binding fragment were adsorbed at different concentrations and pH.

Results

SNR measurements showed adsorbed mass increased when solution pH was closer to the isoelectric point of the mAb alongside larger adsorbed mass for the whole antibody compared to the individual fragments Figure 1, 2 and 3. Increasing concentration increased layer thickness and density of layer.

Conclusion

The adsorption of COE-3 and its individual fragments was successful with no measurable loss of SS the interface. Altering concentration and pH affected adsorbed mass. Comparison between FC and Fab with the whole antibody can shed light on the driving forces behind adsorption. Further work with surfactants such as polysorbate80 will look to investigate how excipients can reduce adsorption via competitive adsorption or increased stabilisation of monomer in solution.

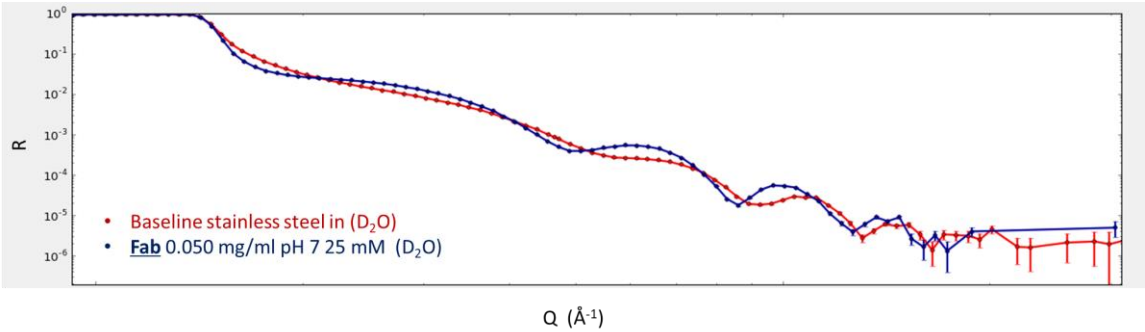

Figure 1 SNR measurement of 0.050mg/ml Fab in pH 7 25 mM phosphate buffer (blue) adsorbed to sputter coated stainless steel (red)

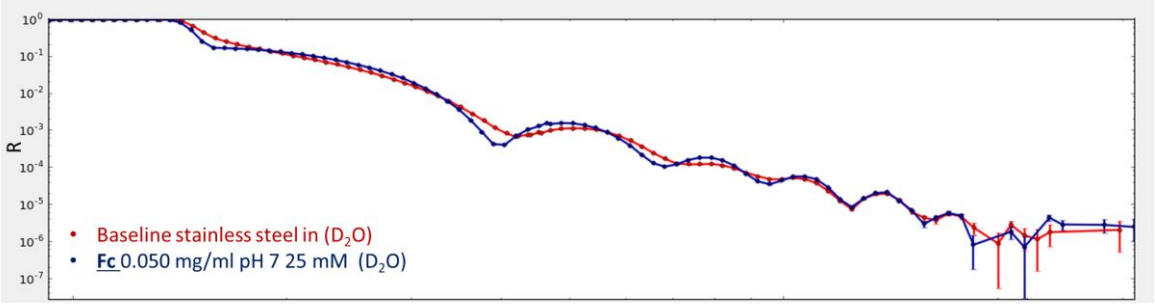

Figure 2 SNR measurement of 0.050mg/ml Fc in pH 7 25 mM phosphate buffer (blue) adsorbed to sputter coated stainless steel (red)

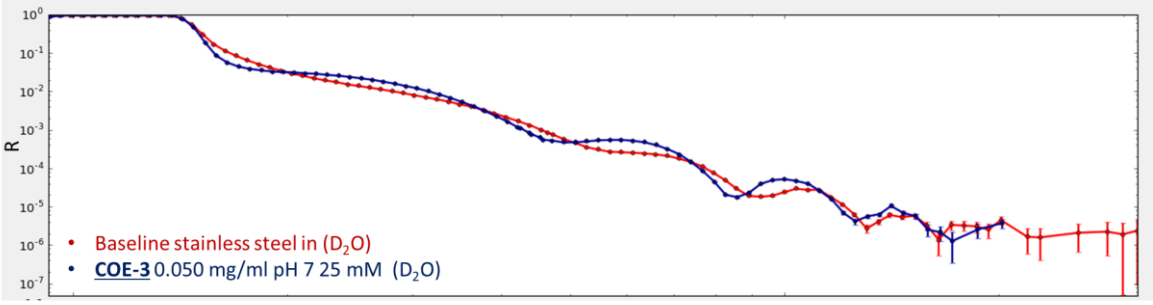

Figure 3 SNR measurement of 0.050mg/ml COE-3 in pH 7 25 mM phosphate buffer (blue) adsorbed to sputter coated stainless steel (red)

1. Hollowell, P.; Li, Z.; Hu, X.; Ruane, S.; Kalonia, C.; van der Walle, C.F.; Lu, J.R. Recent Advances in Studying Interfacial Adsorption of Bioengineered Monoclonal Antibodies. *Molecules* **2020**, *25*, 2047, doi:10.3390/molecules25092047.