Experimental report

05/09/2022

Proposal: 8-04-923 **Council:** 4/2021

Title: Tunable equilibrium monoclonal antibody nanocluster dispersions at high concentrations

Research area: Biology

This proposal is a new proposal

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Samples: IgG1 monoclonal antibodies + trehalose in D2O

Instrument	Requested days	Allocated days	From	To
D11	1	1	12/09/2021	13/09/2021
IN16B	3	3	07/09/2021	10/09/2021

Abstract:

The sugar trehalose constitutes a frequently employed and compliant additive of drug formulations, including monoclonal antibody (mAb) formulations. It has been shown that trehalose can induce colloidally stable and reversible nanoclusters in mAb solutions [Borwankar et al., Soft Matter 9, 1766 (2013)]. The resulting mAb nanocluster suspensions are syringeable even at mAb concentrations of at least 200mg/ml.

Here we aim to fundamentally understand the mechanism underlying the formation of these clusters by probing in particular the regime of small clusters at the transition from a monomeric system to a system containing large clusters. We will employ neutron backscattering to determine the hydrodynamic size of these clusters unambiguously via their self-diffusion and to simultaneously obtain new information on the mAb internal dynamics. Additionally, we will employ SANS to characterize the time-averaged structure of the clusters up to the scale of at least 50nm.

This research being part of an InnovaXN PhD project, we have already embarked on successful experiments employing mAbs by Lonza AG using Rheology, DLS, SAXS (ID02, exp.MD-1284), and a test on BATS.

Experimental report - 8-04-923 (IN16B and D11, 07-09/09 and 23-24/09/2021)
Tunable equilibrium monoclonal antibody nanocluster dispersions at high concentrations

Scientific background. Antibody proteins are essential in the immune response of mammals and are, hence, generally important for therapeutics. Antibody injections are used in various therapies. For pharmaceutical applications, highly concentrated monoclonal antibody (mAb) solutions (up to 200 mg/ml) are required to obtain a significant therapeutic effect whilst keeping the injected volumes sufficiently small. The phase behavior and viscosity of highly concentrated mAb solutions is influenced by protein-protein-interaction and, thus, by mAb structure and physicochemical properties, as well as solution conditions (pH, buffer, ionic strength), and temperature. mAb solutions with viscosities > 20 mPa*s are considered difficult to inject (due to the high injection forces required) [1], limiting their market access and potential therapeutic use. The conflicting requirements of minimizing the injection volumes and limiting injectable viscosities render pharmaceutical research on dense aqueous antibody solutions essential. On the microscopic level, reversible cluster formation has been found to be at the origin of the large viscosities of some monoclonal antibody solutions. To minimize the viscosity of mAb solutions, the interactions between the mAbs can be modified, e.g. by employing different additives and/or by changing the storage/transport temperatures. Here, we investigate highly concentrated solutions of therapeutically relevant mAbs provided by our industrial partner Lonza AG using QENS and SANS at different temperatures in order to understand how temperature affects their clustering behavior. This experiment is a continuation of the work started with exp. 8-04-908, where pure mAb solutions were investigated. In this present case, trehalose was employed as an additive and its effects on clustering have been studied.

Experimental results. (a) SANS. Example SANS data are shown in Fig. 1. A fit based on a sticky hard sphere model was performed for mAbs AMS-106 and AMS-59 (solid black line in Fig. 1a). Based on the fit, the reduced 2nd virial coefficients (B_2/B_2^{HS}) were calculated (Fig. 2).

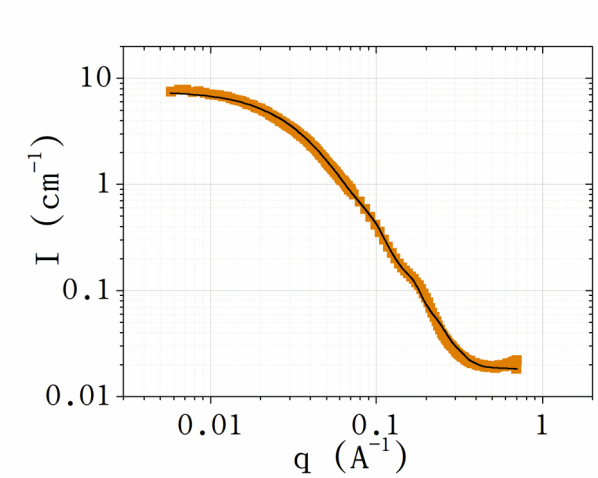

Figure 1: *SANS data set on the mAb AMS-106 at 21 °C. The black solid line represents a sticky hard sphere fit (see IGOR manual by S. Kline) with axes lengths of 58 and 7 A.*

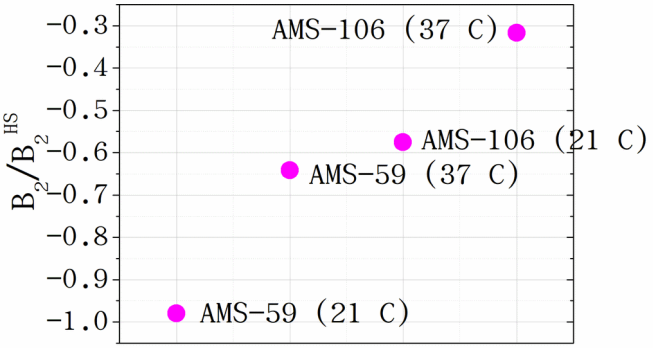

Figure 2: *B_2/B_2^{HS} values for AMS-106 and AMS-59. The more negative the B_2/B_2^{HS} value, the stronger the attractive interactions between mAb molecules are.*

The current status of the SANS data analysis indicates that the mAb interactions become less attractive with increasing temperature. This may imply that higher temperatures would prevent clustering. We intend to deepen our understanding of the molecular interactions of these mAbs by measuring a concentration series using SAXS on ID02 (foreseen for Dec 2022).

(b) QENS. Spectra for the samples were collected at 280, 295 and 310 K (see Figs. 3-4 for examples). Deuterated solutions of three different antibodies were measured in the presence of

trehalose; highly concentrated polyclonal Ig solutions were also measured as a reference. The samples of interest were: AMS-38, AMS-59 and AMS-106 in a concentration of 80 mg/ml + 10 and 20 mg/ml of trehalose. Data are being analysed by following the framework already used in a previous work from our group [2], where PEG instead of trehalose has been employed as an additive.

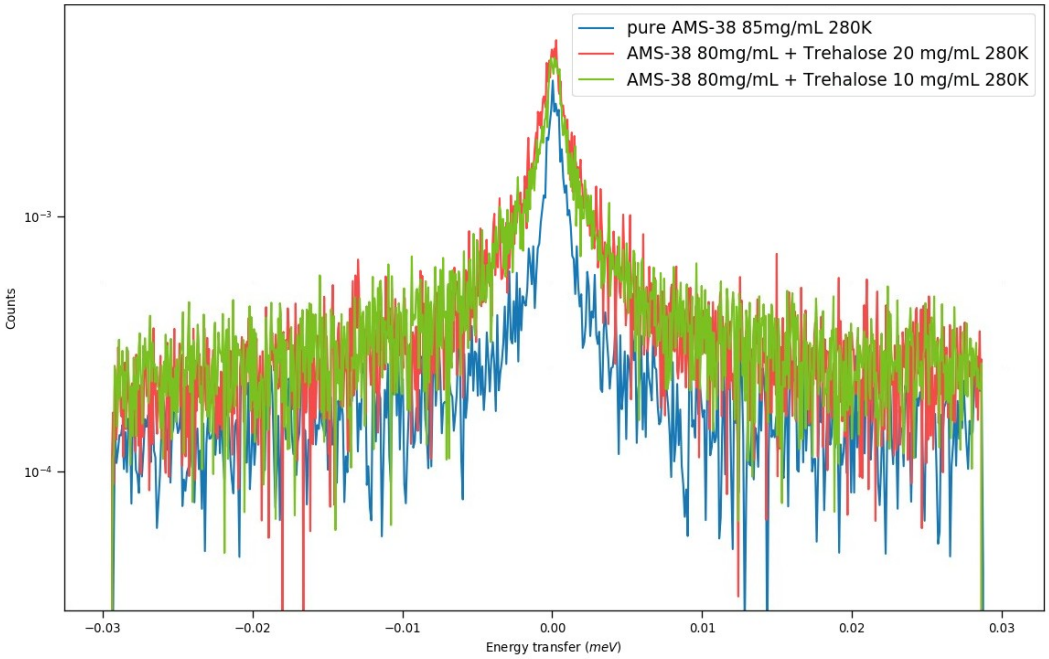

Figure 3: *Measured spectra at q=1.03 Å^{-1} of antibody AMS-38 deuterated solutions at 280 K. The blue line refers to pure antibody solution (data from previous experiment 8-04-908, always using IN16B), while the other two are the spectra for the samples with trehalose as an additive. The red line refers to AMS-38 solution with 20 mg/ml of trehalose, while the green one is for AMS-38 solution with 10 mg/ml of trehalose. The small difference in the apparent baseline level in the spectra for the two different trehalose concentrations, as well as in the absence of trehalose indicates that the contribution of trehalose to this apparent background is sufficiently small and manageable in the data analysis. Errorbars are hidden for a better reading of the plot.*

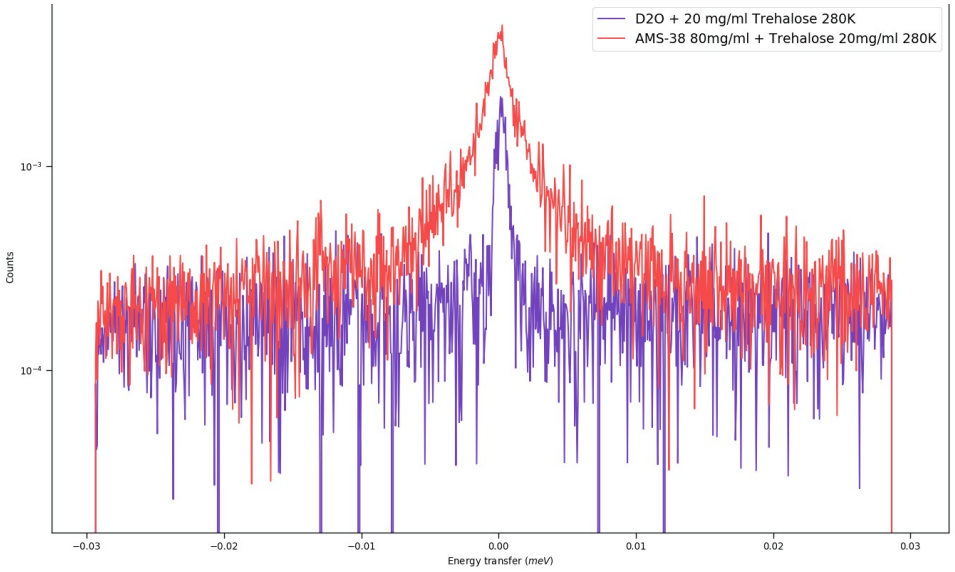

Figure 4: *Measured spectra at q=1.03 Å^{-1} of antibody AMS-38 deuterated solution with 20 mg/ml of trehalose at 280 K (red) and of the buffer formed by D2O with 20 mg/ml of trehalose.*

Conclusions. Combining QENS and SANS allows us to determine the molecular interactions of our mAb samples as well as the resulting dynamic processes.

References

[1] S. Jolles and J. Sleasman. Advances in Therapy 28.7 (2011)
[2] Girelli, A., Beck, C., Bäuerle, F. et al. *Molecular flexibility of antibodies preserved even in dense phase after macroscopic phase separation.* Mol. Pharmaceutics 2021, 18, 11, 4162–4169