ESRF	**Experiment title:** Influence of antibody type, temperature and additives on the viscosity of monoclonal antibody therapeutics	**Experiment number:** MD-1368
Beamline: ID02	**Date of experiment:** from: 02/12/2022 to: 05/12/2022	**Date of report:**
Shifts: 9	**Local contact(s):** Lauren Matthews (email: lauren.matthews@esrf.fr)	*Received at ESRF:*

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Report:

The aim of the beamtime was to investigate protein-protein interactions in pharmaceutically relevant solutions of several monoclonal antibodies (mAbs), produced and characterized at Lonza, in order to understand whether and how the antibody type, the temperature and the presence or the absence of additives can influence their macroscopic viscosity. The data collected are complementing existing neutron spectroscopy measurements. During the beamtime allocated, we investigated different antibody solutions by changing systematically the protein concentration, the additives as well as the temperature. For seven different monoclonal antibodies, samples were measured in 20 mM His-HCl buffered solutions at pH 6.0 at concentrations $c_p = 1,\ 2,\ 10,\ 5,\ 20,\ 60\,\frac{\text{mg}}{\text{ml}}$ at $T = 7,\ 22,\ 37\,^{\circ}\text{C}$. Polyclonal IgG samples were measured under the same conditions as reference for comparison to previously published results (Da Vela, *et al.*, JPC B 121 (2017) 5759). To test the influence of different excipients, samples with the same protein concentrations containing respectively $20\,\frac{\text{mg}}{\text{ml}}$ ($\simeq$ 58 mM) of trehalose and 150 mM NaCl were measured in addition. For some mAbs, samples with higher protein concentrations were also measured, if the concentration of the initial stock solution was high enough; for two mAbs, concentrations up to 250-300 $\frac{\text{mg}}{\text{ml}}$ were reached. One of the mAbs showed an upper critical solution temperature (UCST); to avoid phase separation in the flow cell, these samples were measured in capillaries with a temperature-controlled sample holder. Also one mAb in a gelly state was measured in capillaries, along with some solutions with the highest

concentrations, which are quite viscous and likely to stick to the flow through cell. For these samples, buffers were also measured in capillaries. To reduce the waiting time for temperature equilibration, capillary measurements were carried out while the flow through cell changed temperature and vice versa. In Figures 1, 2 and 3, the temperature variation for $c_p = 60\,\frac{\text{mg}}{\text{ml}}$, the change in protein concentration for one mAb (AMS-095) and the influence of different excipients (trehalose and NaCl) on the same mAb at $c_p = 60\,\frac{\text{mg}}{\text{ml}}$ are shown, respectively. Form factor contributions are clearly visible at high q (Figure 2), while the effects of temperature variations and different additives on the solutions become remarkable in the low-q region, where lower intensities indicate less attraction between proteins at higher temperatures (Figure 1) and in the presence of trehalose. Figure 4 compares different mAb solutions at $c_p = 60\,\frac{\text{mg}}{\text{ml}}$ at $T = 37°\text{C}$ showing how mAb type also plays an important role in tuning the intensity of intermolecular interactions.

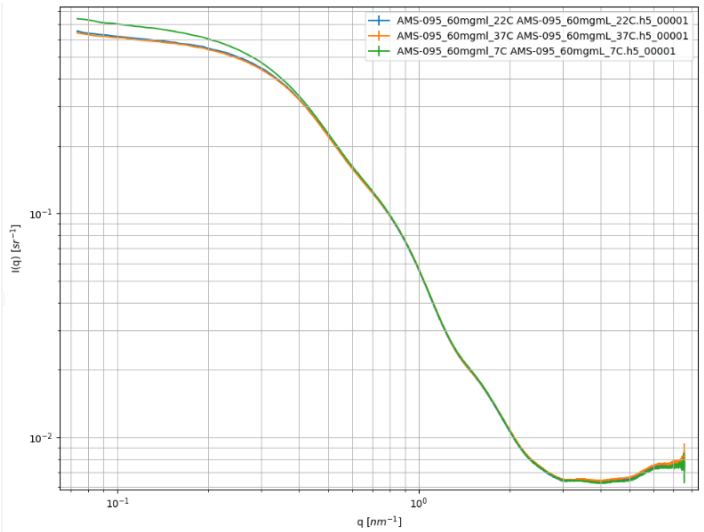

Figure 1: Temperature dependence of mAb AMS-095 at $c_p = 60\,\frac{\text{mg}}{\text{ml}}$.

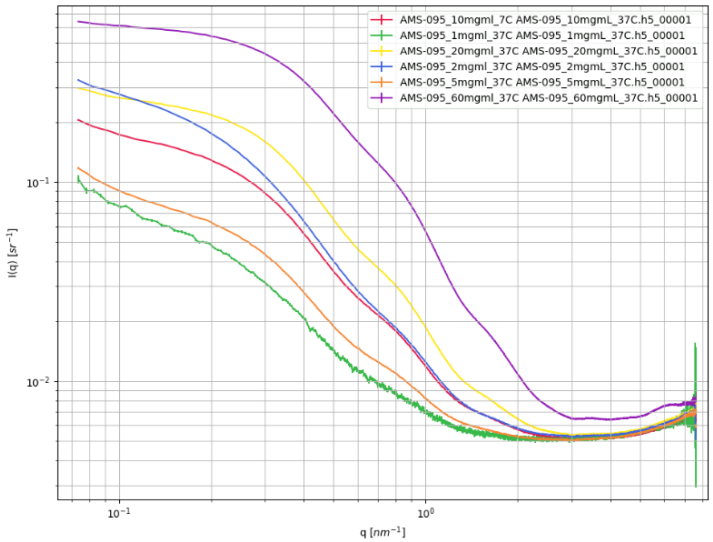

Figure 2: Concentration series (1-60 $\frac{\text{mg}}{\text{ml}}$) of AMS-095 at $T = 37°\text{C}$.

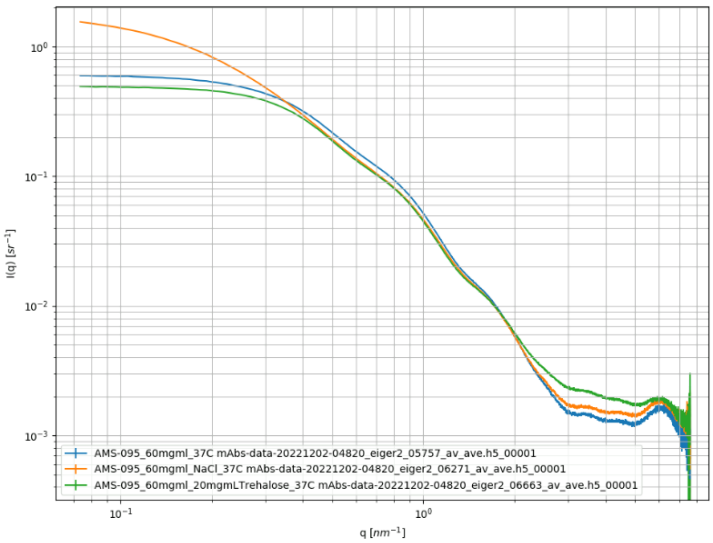

Figure 3: Influence of NaCl and trehalose on AMS-095 at $c_p = 20\,\frac{\text{mg}}{\text{ml}}$ at T=37°C.

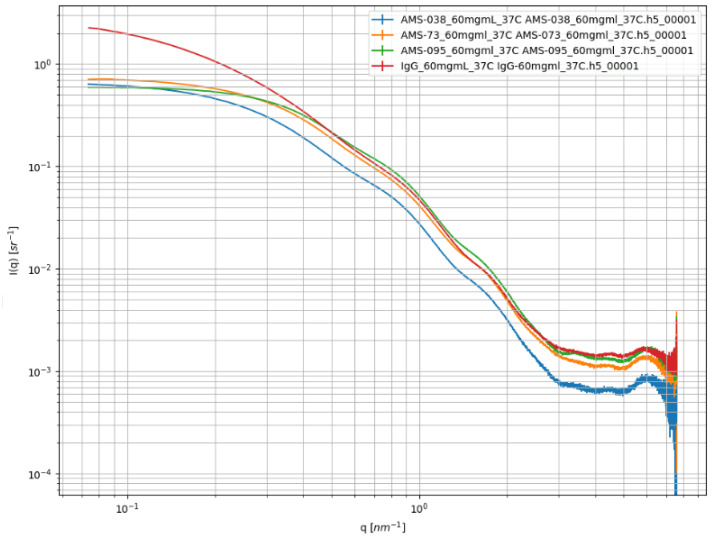

Figure 4: Comparison of different mAbs without excipient at T=37°C, $c_p = 60\,\frac{\text{mg}}{\text{ml}}$.