Formulaction
Smart scientific analysis

PHARMACEUTICAL

Quantifying protein denaturation inhibition with viscosity

Introduction

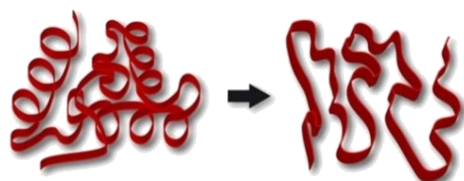
The advent of protein-based drugs brings along new challenges in term of stability and formulation. Indeed, proteins have a strong tendency to denature and/or aggregate, depending on parameters such as temperature, shear, solvent composition, etc. This instability affects the shelf life and can alter the drug efficiency. Moreover, their high molecular weight, associated to a low permeability, prevents oral administration, meaning protein-based drugs often have to be injected. In term of formulation, injecting implies a low viscosity and a small volume, so a high protein concentration

KEY **BENEFITS**

FAST

SMALL SAMPLE VOLUME

ACCURATE



Various compounds may be considered as additives in order to prevent protein denaturation. Most commonly studied are: Arginine and Histidine, susceptible of stabilizing protein structures and so preventing increase of viscosity.

Reminder on the technique

FLUIDICAM uses a co-flow microfluidic principle to measure viscosity. Sample and reference standard are pushed together to the microfluidic channel (typically 2.2mm X 150µm) under controlled flow rates. In this laminar flow, interface position between sample and reference relates the viscosity ratio between the two to the flow rates.

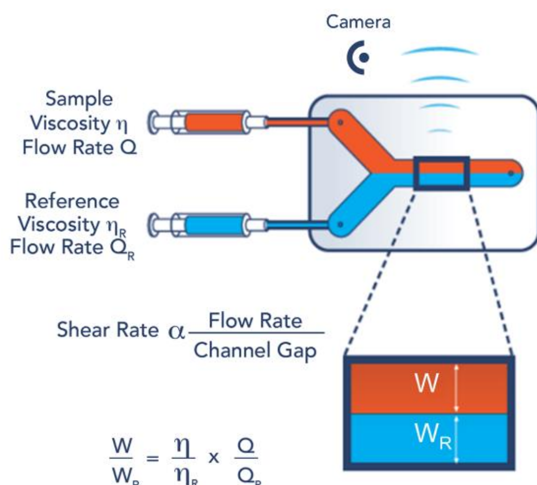
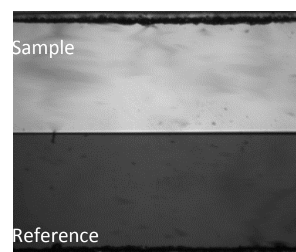


Fig. 1: Fluidicam measuring principle

Images acquired during the measurement allow to calculate the position of the interface and plot directly an interactive flow curve.



Method

Several BSA solutions were prepared in this study, two concentrations of additives were considered (50 and 200mM), and two natural protein solutions one in water and the other in PBS – phosphate buffer solution. In order to determine the influence of additive on protein unfolding and thus protection efficiency, the viscosity was measured after subjecting the solutions to high temperatures (60°C for 4h and 25°C for 48h).

Protein protection from denaturation

Viscosity of BSA solutions, prepared in water or in PBS, was measured after storing the solutions at high temperature. BSA - Water solution presents the highest viscosity and most pronounced shear thinning behavior compared to other solutions. Arginine 50mM and Histidine 200mM additives have the lowest viscosity and kept Newtonian behavior of native protein

BSA in PBS and BSA - Histidine 50mM are still liquid solutions but start to show shear thinning behavior – indication of the beginning of denaturation

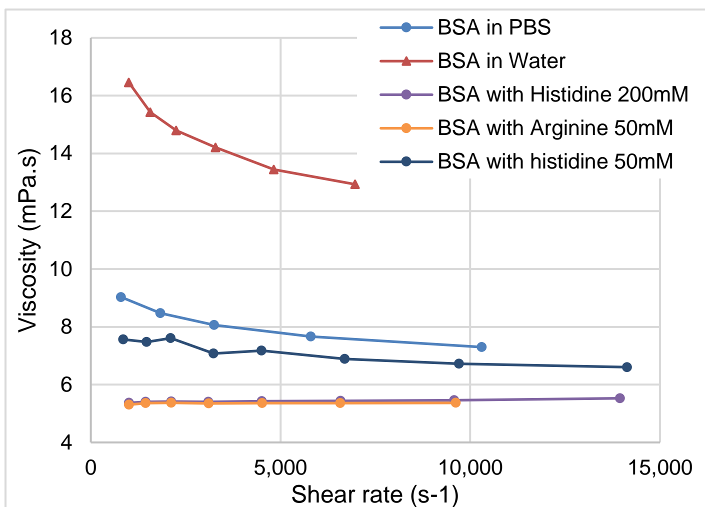


Fig 2. Viscosity of BSA solutions with different protection additives after thermal stress.

As compared to freshly prepared BSA solution, both solutions with no additives are more viscous. PBS however, seem to provide additional protection to the protein. It is from one part the pH control and NaCl ions that help to avoid unfolding.

On the other hand, the viscosities of BSA with Arginine (50mM) and Histidine (200mM) are closest to the initial value of freshly prepared protein. Histidine's protection is greater at higher concentrations, surprisingly Arginine with only 50mM has provided good protection

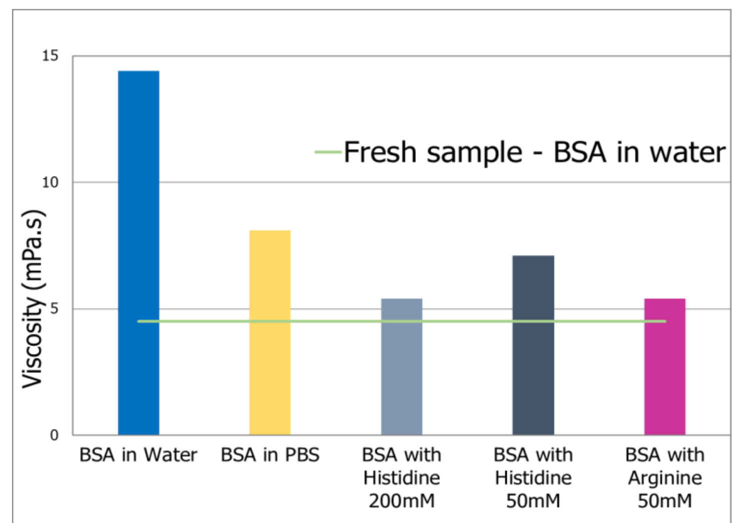


Fig 3. Viscosity of BSA as a function of additive concentration.

CONCLUSION

FLUIDICAM RHEO allows high precision viscosity measurements. Working with microfluidic chips allows to achieve high shear rates and consume only a small sample volume. The feature is especially attractive for pharmaceutical industry, where the drug availability is limited by the quantity and price. We have shown in less than 5 minutes (time for one flow curve) the ability of Histidine and Arginine to prevent protein unfolding, using only a couple of mL of the sample.