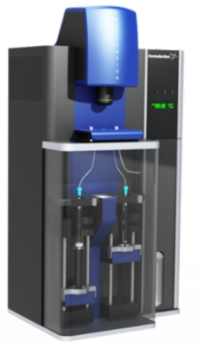
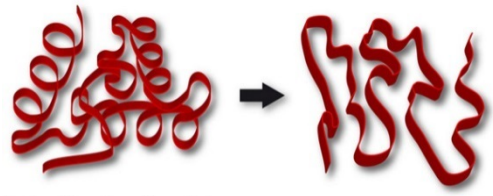
Quantifying protein unfolding inhibition with viscosity

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.

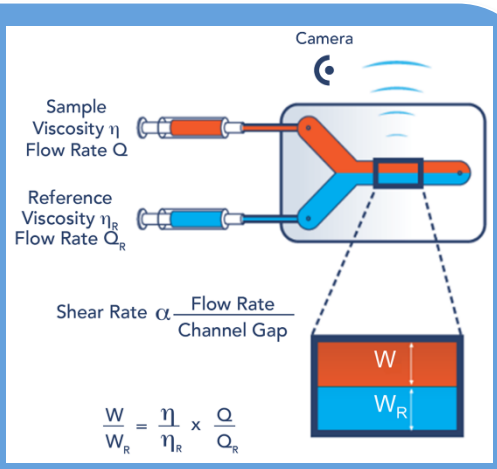


Active (functional) protein — Denatured protein

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 RHEO uses a co-flow microfluidic principle to measure viscosity of various products. A sample and viscosity standard are introduced together in the microfluidic channel (typically 2.2mm X 150µm) where they undergo strong confinement. Applied shear rate is simply adjusted by a computer-controlled syringe pump. Under these conditions, the interface position is related to the viscosity ratio between the sample and the reference. Images of the resulting laminar flow are acquired thanks to an integrated camera and the viscosity of the sample is automatically extracted as a function of shear rate and plotted directly in the software giving a resulting rheological curve.



Experimental results

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). FLUIDICAM RHEO works with a visual acquisition system allowing to observe the product directly during the measurement (below an example of acquired image).