A Tale of Two Proteins

Key Words: High concentration Protein Formulation, Viscosity Modifiers, Excipients, Protein Stability, Automated Viscosity Measurements, Rheology Modifiers

Goal: The goal of this application note is to demonstrate that proteins can be differentiated with automated viscosity measurements. The molecular interactions and solution microstructure can be inferred by probing viscosity across multiple shear rates. Lastly, the impact of rheological modifiers like arginine-HCl on the viscosity of protein solutions can be assessed and correlated to the structure and electrostatic interactions.

Introduction

Viscosity is a measure of the molecular interactions within a fluid. Concentrated protein solutions are complex fluids consisting of various characteristic length scales and component interactions. Therefore, viscosity measurements can be used to infer information about protein formulations. The rheological behavior will reflect differences in protein size as well as the formation of complex structures or networks resulting from various types of molecular interactions. The initial plateau viscosity value can be used to infer thermodynamic information, which encapsulates Brownian motion, electrostatics, and attractions of various origins. Measuring over a range of shear rates provides information on the interaction strength and the extent of structure formation which can be determined by the onset of shear-thinning in the fluid. The process of shear thinning is a result of fluid structure disruption that manifests as a decrease in viscosity as shear rate increases.

Viscosity measurements can also probe how formulation changes impact protein solutions. A commonly used rheological modifier in protein formulation is the amino acid arginine. Arginine is a charged amino acid at neutral pH, with a side chain comprised of a three-carbon chain ending with a charged guanidyl group. It has been speculated that arginine stabilizes proteins, specifically antibodies, in solution, which manifests as a reduced viscosity when compared to formulations lacking arginine. In this application note we show that arginine reduces the viscosity of a model antibody-like protein bovine gamma globulin (BγG), while curiously increases the viscosity of a model globular protein bovine serum albumin (BSA). These results demonstrate that viscosity is a useful tool that can probe and characterize both proteins and excipients used in formulation science.
Experiment

250 mgmL\(^{-1}\) Bovine gamma globulin (B\(\gamma\)G) and 325 mgmL\(^{-1}\) Bovine Serum Albumin (BSA) were dissolved in two phosphate buffer saline (PBS) solutions: 1X PBS and 1X PBS + 225 mM Arginine-HCl (Arg-HCl). The viscosity of each solution was measured on the VROC\(^\text{®}\) initium with a B05 (depth = 50 µm, \(P_{\text{max}} = 42\) kPa) and an E02 chip (depth = 20 µm, \(P_{\text{max}} = 1600\) kPa) at 25 °C at shear rates between 1,000 to 50,000 sec\(^{-1}\). The shear rates explored depended on the viscosity of each protein sample. The loaded volume for each sample was approximately 70 µL and 5 measurements were made for each with the retrieval feature activated.

Data and Analysis

The viscosity as a function of shear rate for each protein formulation is depicted in Figure 1.

![Figure 1. Shear rate sweep from 1,000 to 50,000 sec\(^{-1}\). Viscosity (cP) plotted as a function of shear rate (sec\(^{-1}\)) for 250 mgmL\(^{-1}\) B\(\gamma\)G and 325 mgmL\(^{-1}\) solubilized in 1X PBS and 1X PBS + 225 mM Arginine-HCl. Error bars correspond to 3 standard deviations.](image-url)

The B\(\gamma\)G containing formulations exhibited non-Newtonian behavior, which was consistent with our previous observations. As the shear rate increased, the viscosity of the formulations decreased, which is known as “shear-thinning.” In contrast, the BSA formulations exhibited Newtonian behavior. The viscosity of the solutions was independent of shear rate over the range tested. Interestingly, the inclusion of arginine-HCl into the formulations altered their rheology in
opposite directions. For the ByG formulations, arginine-HCl decreased viscosity by 19.5%, but for the BSA formulations, including arginine-HCl increased viscosity by 8.4%.

The stark differences in rheology between the protein formulations can be explained by differences in the total volume occupied by the primary molecules and aggregates and their interactions in solution. ByG is larger than BSA (150 kDa versus 66.5 kDa) and readily forms dimers in solution because of attractive intermolecular forces (Da Vela et al. 2017). Although we could not reach shear rates that induced shear thinning in the BSA solutions with the instrument used in this study, the onset of non-Newtonian behavior for ByG was observed at ≤10,000 sec⁻¹. This result is consistent with previous work demonstrating the larger ByG molecules are prone to cluster formation (Da Vela et al. 2017). The higher plateau viscosities measured for the ByG solutions can be attributed to a potentially higher effective volume fraction and stronger interparticle interactions including attractions. Lastly, viscosity measurements indicated that arginine-HCl, a known rheology modifier of proteins, affected the two proteins differently. These differences can be attributed to the intermolecular interactions between the proteins in solution. The exact mechanism of how arginine interacts with proteins is still debated. Some speculate that the arginine’s charged guanidyl group electrostatically screens active groups within proteins, while simultaneously able to interact with hydrophobic groups with its three-carbon aliphatic chain. These interactions result in a reduced viscosity in the case of metastable ByG, but an increased viscosity in the more electrostatically stabilized BSA (S. Yadav et al. 2011 and P.S. Sarangapani et al. 2013).

**Concluding Remarks**

Proteins are important biological molecules that can be powerful drug candidates. Differences in the protein size as well as protein-protein interactions can be detected using automated viscosity measurements. Examining the lower shear regions of a shear rate sweep can yield information about the thermodynamic interactions within a fluid, while information about fluid structure can be examined at higher shear rates to observe shear thinning behavior. Furthermore, intermolecular interactions can be probed by introducing excipients like arginine-HCl and quantifying how the rheological properties change with formulation alterations.

**Reference**

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