RheoSense, Inc.
SimplyPrecise™

Application Note: Protein Unfolding or Denaturation Study with VROC ®

Application

This application note addresses measuring viscosity with *m*-VROC™ viscometer. VROC®, **V**iscometer-**R**heometer-**o**n-a-**C**hip, is a micron scale viscosity sensor chip for small sample applications.

Viscosity is an important parameter when optimizing injectable drugs. Monoclonal antibody (mAb) is recognized as an important therapeutic biologics; it targets disease with fewer side effects. For therapeutic efficacy, its concentration must be maintained at a high level; however, viscosity increases when mAb concentration is increased.

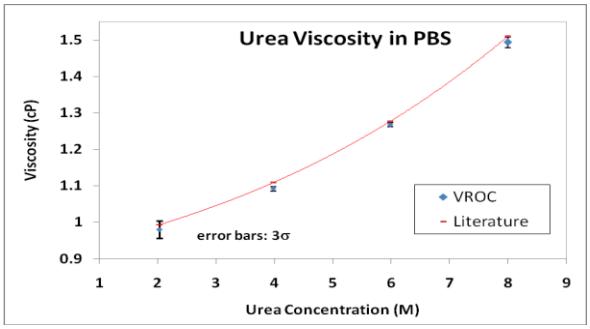
When treating patients, higher viscosities in injections cause major problems. For drug administration, it is difficult to inject high viscosity medicine into the patient without causing physical pain. This makes mAb formulation challenging for drug stability must be achieved with the lowest viscosity values possible.

Viscosity is an effective tool to monitor the denaturation, unfolding, and/or stability of drugs. As protein molecules unfold or aggregate, viscosity increases.

In this application note, protein molecules are unfolded with the addition of Urea. The extent of unfolding is probed and studied with data that is directly related to viscosity.

The Viscosity of Urea Solution in PBS as a Function of Concentration

Urea is a small molecule that is synthesized in the body of many organisms. It is known to interact with proteins and subsequently unfold molecules. In this study, viscosities of the urea solution at varying concentrations were measured with VROC® at 25±0.1°C and compared with the values measured with the glass capillary viscometer in the literature[1]. As illustrated below, the results show a strong consensus.



The Urea concentration is measured in Molarity

[1] K. Kawahara and C. Tanford, "Viscosity and Density of Aqueous Solutions of Urea and Guanidine Hydrochloride," J. Biological Chemistry, Vol. 241, No. 13, 3228 (1966).

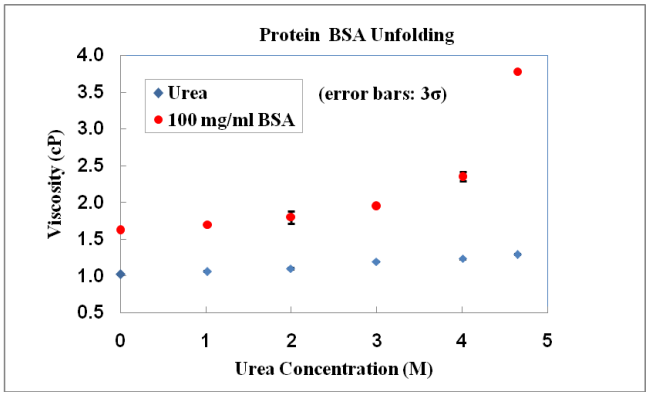
Unfolding Protein Molecules and the Effect on Viscosity

Urea is a known denaturant for protein solutions. During denaturation, protein unfolds and loses its native tertiary structure as well as drug efficacy. Urea interacts with protein molecules and the protein molecules unfold. As the protein molecules unfold, the size of molecules increases as does the hydrodynamic radius. The increase in the hydrodynamic radius results in the increase of viscosity.

To investigate the unfolding, BSA solutions (purchased from Sigma-Aldrich) were prepared in PBS. Series of BSA solutions were prepared with increased concentrations of urea, while the concentration of BSA remained constant at 100 mg/ml.

The following graph clearly shows that the viscosity of BSA (Bovine Serum Albumin) solution increased as the urea concentration increased.

Due to the unfolding BSA molecules, the level of viscosity exceeded the contribution from the increased concentrations of urea. The increase of viscosity became more dramatic when the urea concentration was greater than 3 M.



As the urea increased in concentration, more BSA molecules unfolded, which resulted in a larger volume fraction occupied with molecules.

When the volume fraction of unfolded molecules increased, viscosity increased. This result is consistent with the micro-rheology work by Tu and Breedveld[2].

Light scattering is another technique, which was employed to monitor protein aggregation. However, that technique is limited to using a very dilute concentration; otherwise, complex particle interaction must be introduced. A concentration of 100 mg/ml is probably too high for the light scattering technique. However, the detection range of VROC® is not limited to low concentrations.

Conclusions

① VROC® has high resolution for distinguishing small increases in viscosity, a powerful tool for quantitative research.

② VROC® can be used to investigate drug stability without diluting the solution.

If you have questions or need more information, please contact us.

Main Office — (925) 866 3808

Email — Info@RheoSense.com

[2] R. Tu and V. Breedveld, "Microrheological detection of protein unfolding," Physical Review., E72, 041914-1, 2005.