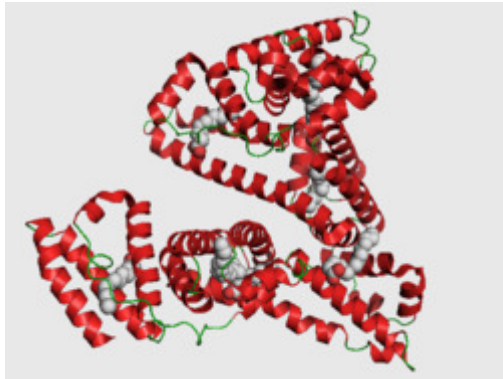


Application Note: Viscosity measurement of a model protein solution of BSA

Application

This application note addresses measuring viscosity with the *m*-VROC® system. VROC® is a viscometer-rheometer-on-a-chip, which is a micron scale viscosity sensor chip for small sample. Micron scale geometry enables measuring viscosity at shear rates beyond the limits of conventional technology.

Albumin is the most abundant plasma protein in human or other animals.



Structure of Human Serum Albumin

BSA solution has been tested with numerous analytical instruments. Significant data is available, which can be referenced for a detailed comparison of measurement performance.

For this study, a model protein, BSA (Bovine Serum Albumin), was tested for viscosity as a function of concentration and shear rates. While albumin concentration in plasma is approximately 50 mg/mL, higher concentrations were modeled for a more concentrated protein loading in delivery. The measured concentration of BSA varied from 1.2 mg/mL to 404 mg/mL. The low-range of concentration was measured to demonstrate the superior resolution of VROC technology.

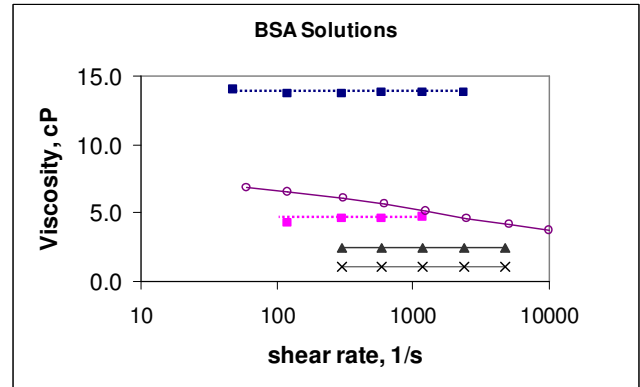
Sample Preparation

BSA was acquired from Sigma-Aldrich, which was dissolved in 0.01 mM PBS (phosphate buffered saline) at 404 mg/mL as a stock solution. The sample was not filtered prior to testing as PBS was filtered separately. PBS was used to dilute samples of the stock solution to less concentrated solutions—1.2, 2.4, 4.8, 10.1, 20.2, 50.4, 100.8, 151.7, 202, 300, and 404 mg/mL.

The polyethylene oxide solution was prepared by dissolving a polymer from Sigma-Aldrich in de-ionized water at 0.32wt% (or 3.2 mg/mL). The nominal molecular weight of the polymer is 2 million Da.

Results

Viscosity vs. Shear Rates



Symbol	Sample description
■	404 mg/mL
■	300 mg/mL
▲	202 mg/mL
x	20.2 mg/mL
○	PEO 0.32 %

Tested shear rates ranged from 47.0 s⁻¹ to 4700 s⁻¹.

Tested shear stress ranged from 5 dyne/cm² to 274 dyne/cm².

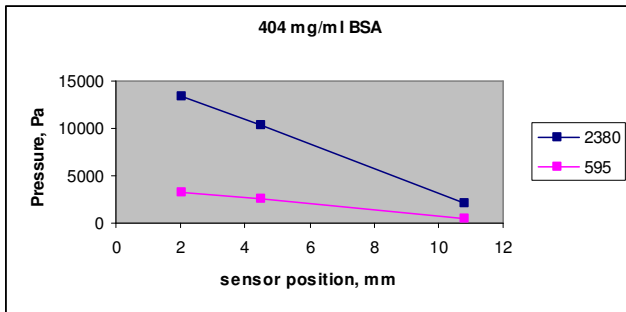
The graph above shows that the viscosity is independent of shear rate. This indicates that *all* BSA solutions are Newtonian, even at the high concentrations of 404 mg/mL. The viscosity values are the same whether shear rate was increased or decreased. This suggests there is no shear history effect on the protein structures.

Note that the pressure profiles are straight. The pressure values vs. positions along the flow channel suggest there is no abnormality in the flow.

Also note the Newtonian behavior of the highly concentrated BSA solution, which is particularly interesting when compared to the shear thinning behavior of high molecular weight PEO solution. The PEO solution shows a slight yet clear shear thinning behavior, even when the polymer concentration is

VROC® – A MEMS Device for Newtonian and Non-Newtonian Viscosities

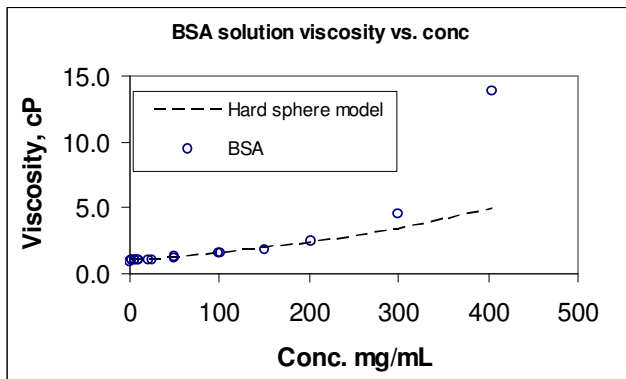
0.32 wt% (3.2 mg/mL). Higher molecular weight of the PEO polymer possesses longer relaxation time.



Symbol	Shear rate, 1/s
■	2380
■	595

Viscosity vs. Concentration

The viscosity of each BSA solution was calculated by averaging the viscosity at different shear rates. The viscosity was then plotted against concentration as shown in the graph below. The standard deviation is less than 1% of the average values.



The viscosity monotonically increases until the concentration reaches 250 mg/mL. The viscosity increases dramatically at higher concentrations. In the graph, the measured viscosity values are compared to theoretical values, which were calculated using the equation shown below.

It is assumed that the natural BSA molecule is a hard sphere in the PBS solution. The viscosity of the hard sphere suspension varies as a quadratic function of volume fraction with the following coefficients¹.

$$\eta = \eta_s (1 + 2.5\phi + 6.2\phi^2)$$

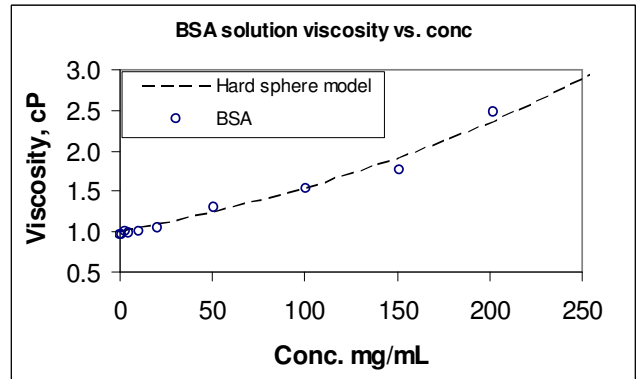
ϕ is the volume fraction occupied by the protein.

¹ A. Einstein, Investigations on the Theory of Brownian Movement (Dover, New York, 1956).

η and η_s are the measured viscosity and the solvent viscosity.

The radius of the folded BSA protein hard sphere is assumed 3.42 nm, as calculated by Tu and Breedveld².

As shown in the comparison below, the model clearly describes the viscosity behavior of the protein of concentrations up to 200 mg/mL. Difference between the measured value and the model calculation is 5% at BSA concentration of 200 mg/mL.



Summary

- VROC® is demonstrated as an effective viscosity measurement tool for proteins
- No solvent evaporation
- High accuracy and high resolution
- The flow channel depth can be adjusted to simulate 28 and 30 gauge needle flow of protein solutions in which protein drugs experience high shear rates

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

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² R. S. Tu and V. Breedveld, "Microrheological detection of protein unfolding," Phy. Rev. E. 72, 041914 (2005).