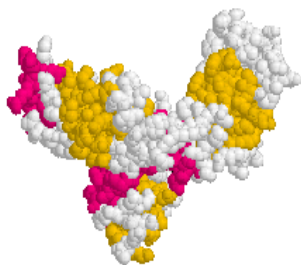


Application Note: Viscosity measurement of a γ -globulin from Bovine Blood

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.

Gamma globulin, also called immunoglobulin, is a class of blood plasma protein that is noted for including antibodies. Because of this important trait, Intravenous immunoglobulin has been administered to treat immunity related illnesses, including Kawasaki.



Structure of Immunoglobulin

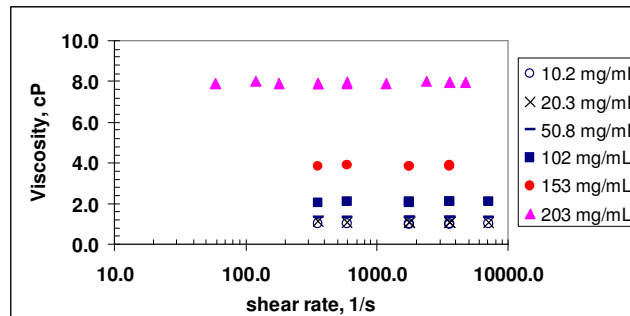
For this study, gamma globulin was tested for viscosity as a function of concentration and shear rates. The concentration dependence of the gamma globulin solution is compared with that of bovine serum albumin solution. The concentration of gamma globulin ranged from 10 mg/mL to 203 mg/mL. The low-range of concentration was measured to demonstrate the superior resolution of VROC technology.

Sample Preparation

Gamma globulin from bovine blood was acquired from Sigma-Aldrich, which was dissolved in 0.01 mM PBS (phosphate buffered saline) at 203 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: 10.1, 20.3, 50.8, 101.7, 152.5, and 203 mg/mL. All measurements were performed at room temperature.

Results

Viscosity vs. Shear Rates



Symbol	Sample description
▲	203 mg/mL
●	153 mg/mL
■	102 mg/mL
-	50.8 mg/mL
x	20.3 mg/mL
○	10.2 mg/mL

Tested shear rates ranged from 60 s⁻¹ to 7140 s⁻¹.

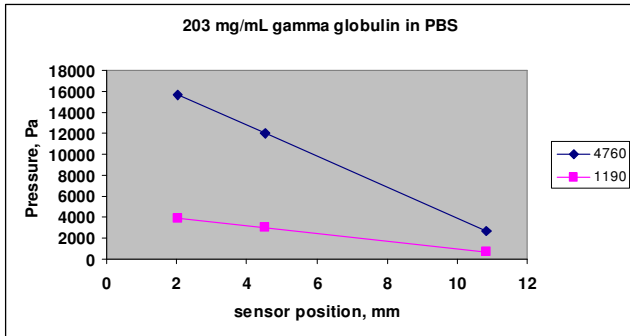
Tested shear stress ranged from 4.7 dyne/cm² to 380 dyne/cm².

Residence time of the protein solution ranged from 270 ms to 22.5 s.

The graph above shows that the viscosity is independent of shear rate. This indicates that *all* gamma globulin solutions are Newtonian, even at the high concentration of 203 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 in the graph below are straight. The pressure values vs. positions along the flow channel suggest there is no abnormality in the flow.

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

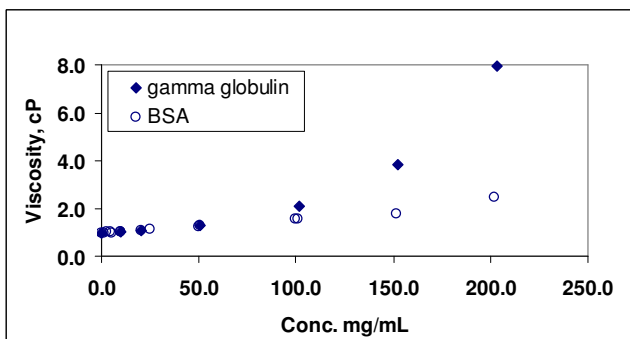


Symbol	Shear rate, 1/s
■	4760
■	1180

Shear force is known to induce the denaturation of protein¹. However, despite a long history and a large body of experimental work, the question of whether a high shear flow will denature a globular protein remains unresolved². Should the denaturation occur inside the chip flow channel, a non-linear pressure profile could have been observed. Denatured protein is known to increase the viscosity.

Viscosity vs. Concentration

The viscosity of each gamma globulin 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 of gamma globulin increases monotonically with concentration. The viscosity values of the gamma globulin solutions are shown in the table below. Viscosity at zero concentration is the measurement of PBS only.

Concentration, mg/mL	Viscosity, cP
203	7.937
153	3.858
102	2.084
50.8	1.306
20.3	1.077
10.2	0.998
0.00	0.934

For comparison, the viscosity of BSA (bovine serum albumin) solution was also plotted in the graph. As can be seen, viscosity of gamma globulin increases at a faster rate with concentration than that of BSA. This difference may be due to the fact that the gamma globulin molecule is larger than the BSA molecule.

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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¹ Creighton, T.E. 1992, Protein folding. W. H. Freeman, New York.

²Jaspe, J. and Hagen, S.J., “Do Protein Molecules Unfold in a Simple Shear Flow?,” Biophysical J. (91), 3415-3424, 2006.