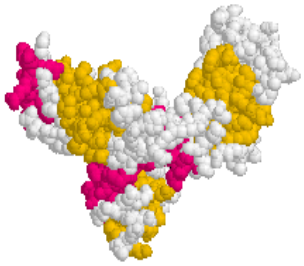
RheoSense, Inc.
SimplyPrecise™

Application

This application note addresses measuring viscosity with the Lab-VROC system. VROC® is a **V**iscometer-**R**heometer-**o**n-a-**C**hip, which is a micron scale viscosity sensor chip for small sample applications. 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 disease.



*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.

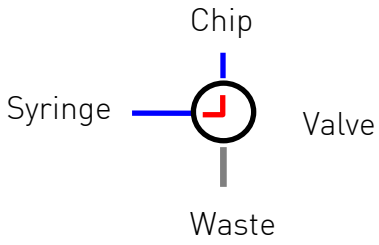
Sample Preparation

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.

Measurement Procedure

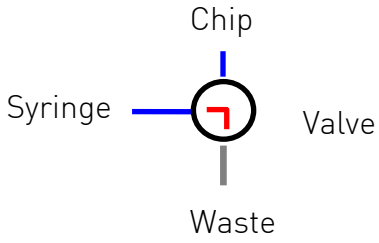
1. Load the sample into a syringe.
2. Mount the syringe to the pump.

The syringe and A05 VROC® chip were connected through a switching valve. An A05 is a chip with a 50 µm flow channel depth, designed for a sensitive and accurate measurement of the low viscosity range (0.2 – 100 cP).



① Measure the viscosity as a function of shear rates. This was accomplished by running the shear rate sweep

② After the measurement, empty the system. The valve was switched to the waste-syringe position before loading the next sample into the syringe.



1. To ensure accuracy, load the new sample and remove air bubbles.
2. To remove the air bubbles, a small portion of the test sample was pumped through the waste output of the valve.

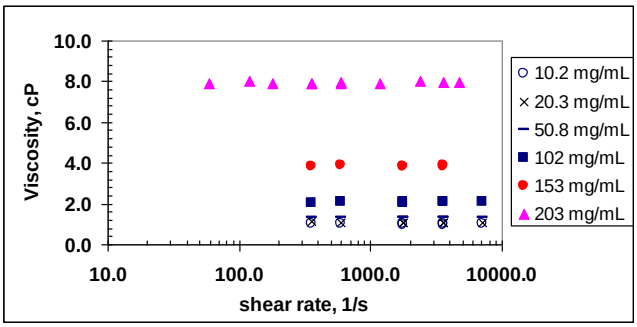
① Set the valve to the syringe-chip position and run the shear rate sweep program for viscosity measurement.

② Between test samples, clean the chip with a detergent such as Aquet solution.

The Aquet solution is proven to effectively remove trapped air bubbles inside of the chip. This cleaning process does not affect measurement accuracy.

Results

Viscosity vs. Shear Rates

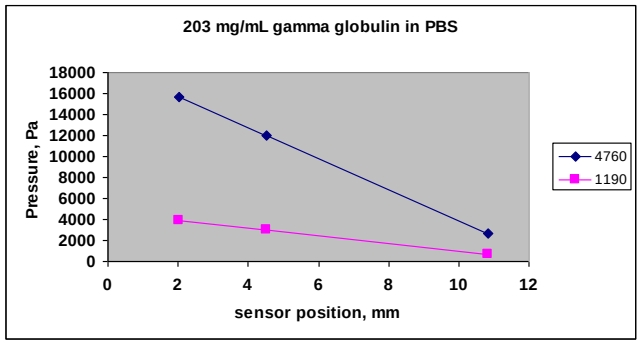


Symbol	Sample Description
▲	203 mg/mL
●	153 mg/mL
■	102 mg/mL
-	50.8 mg/mL
x	20.3 mg/mL
o	10.2 mg/mL

- Tested shear rates ranged from 60 s^{-1} to 7140 s^{-1}.
- Tested shear stress ranges from 4.7 dyne/cm^2 to 380 dyne/cm^2.
- Residence time of 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 abnormally in the flow.

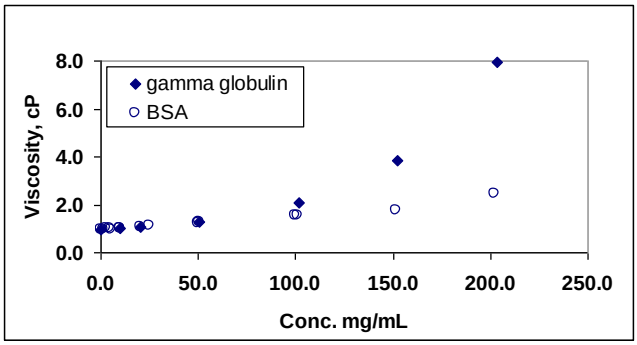


Symbol	Shear rate, 1/s
■	4760
■	1180

Shear force is known to induce the denaturation of protein[1]. 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 unresolved[2]. Should the denaturation occur inside the chip flow channel, a non-linear pressure profile could be observed. Denatured proteins are 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.



[1] Creighton, T.E. 1992, Protein folding. W. H. Freeman, New York.
[2] Jaspe, J. and Hagen, S.J., "Do Protein Molecules Unfold in a Simple Shear Flow?," Biophysical J. (91), 3415-3424, 2006.

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

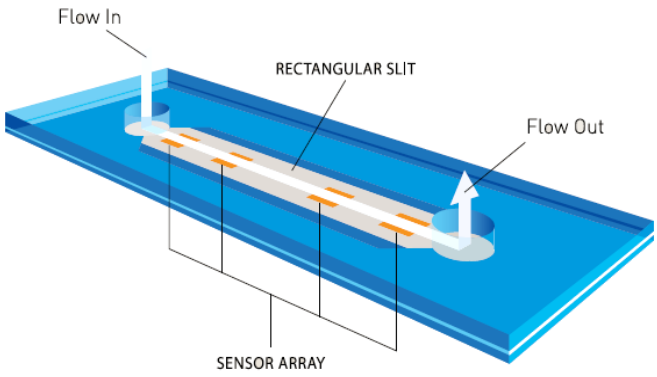
- VROC® is demonstrated as the effective viscosity measurement tool for proteins
- No solvent evaporation
- High accuracy and high resolution
- The flow channel depth can be adjusted to simulate 28 and 30 gauge needle flow of protein solutions in which protein drugs experience high shear rates

Principle of Viscosity Measurement with VROC®

VROC® measures viscosity from the pressure drop as test liquid flows through a rectangular slit; this is a well known scientific application (K. Walters, Rheometry[1]).

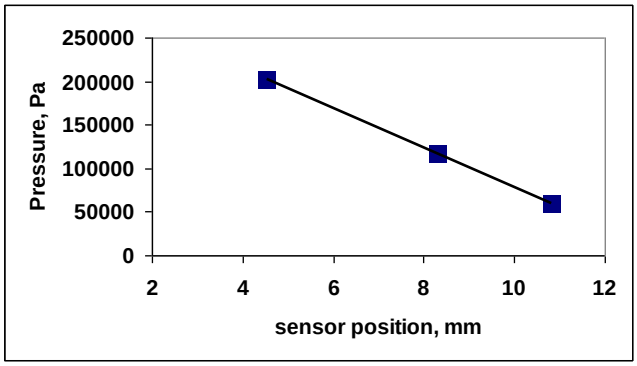
Physical Structure

The VROC® chip consists of a rectangular slit that is formed with glass and a monolithic Si pressure sensor array. The width of the rectangular slit is far greater than the depth of the slit — the edges of the slit are a negligible contribution to the pressure drop.



Usage

When the test sample is pumped to flow through the slit channel, the monolithic pressure sensor array measure pressure at separate locations. As previously described, the flow disturbance is negligible.



Data was obtained for Newtonian Glycerol at 1,220 s^{-1} using a C Chip.

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

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