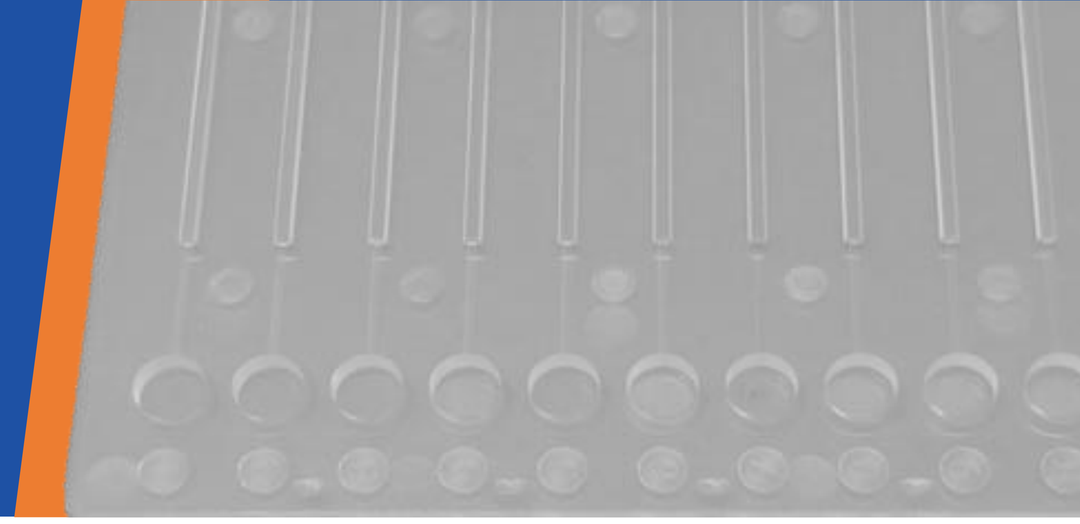
Effect of Protein Adsorption on Low-Volume Viscosity Measurements

Lisa Lyon[1], Brennan Larkin[1], Yadir Guerrero[1], Deepak Solomon[1]
[1]Neofluidics, 6650 Lusk Blvd, Suite B101 & B102, San Diego, CA 92121

CONTACT INFORMATION: info@neofluidics.com



Abstract

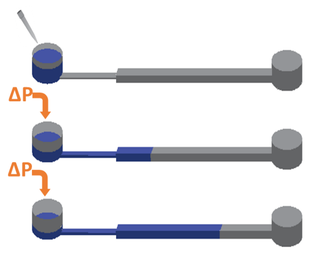
When employing biologics for therapeutic use, highly concentrated solutions (>200mg/mL) are desired in order to limit the volume delivered to the patient. However, the viscosity of these highly concentrated solutions frequently approaches or surpasses the limit of what is safe to inject.[1] Therefore, viscosity measurements are critical during the biotherapeutic development process to determine viable protein concentrations. Current viscometers on the market have several limitations that hinder their usability for biotherapeutic viscosity measurements especially in early discovery. In addition to being high-volume, low-throughput, and expensive, conventional rheometers create a protein-air interface that results in an interfacial protein film. Such a film introduces a viscosity artifact that causes the viscosity values to be higher than what is actually present in the bulk solution. To address the drawbacks of current technology, we have developed a multiplexed microfluidic viscometer platform—the NeoVISC—that utilizes low volumes (<40 µL), can run 10 samples simultaneously, and does not have interfacial protein adsorption effects even at volumes <20 µL. We used 300 mg/mL bovine gamma globulin in dilute PBS to compare viscosity measurements taken in our device to those produced with a conventional cone-and-plate rheometer. Additionally, to emphasize the influence of protein-air interface at low volumes, we also tested our device with a lower sample volume (18 µL) to quantify the effect of the air-liquid interface on bulk viscosity measurements.

Methods

A microfluidic cartridge made of cyclic olefin copolymer (COC) was manufactured using injection molding. Each cartridge contains ten straight microfluidic channels, where a low aspect ratio (h/w) channel is connected downstream to a larger aspect ratio channel. The dimensions of the low aspect ratio channel (small channel) are designed such that its hydrodynamic resistance is at least three orders of magnitude greater than the hydrodynamic resistance of the large aspect ratio channel (large channel). Two fluid wells are present on either side of the microfluidic channel for sample loading and waste, respectively. To operate the device, fluid sample is pipetted into each of the 10 inlet reservoirs. The cartridge is then placed in the instrument. For each measurement, the fluid flow through the channels is driven by an independent piezo electric pressure controller, and the velocity of the fluid is correspondingly tracked in the large channel to determine viscosity using an energy-based image detection algorithm. As fluid moves through the channels, the applied pressure ramps up dynamically, such that viscosity can be quantified at a full range of shear rates in less than 3 minutes in a single run..

Measurement Principle

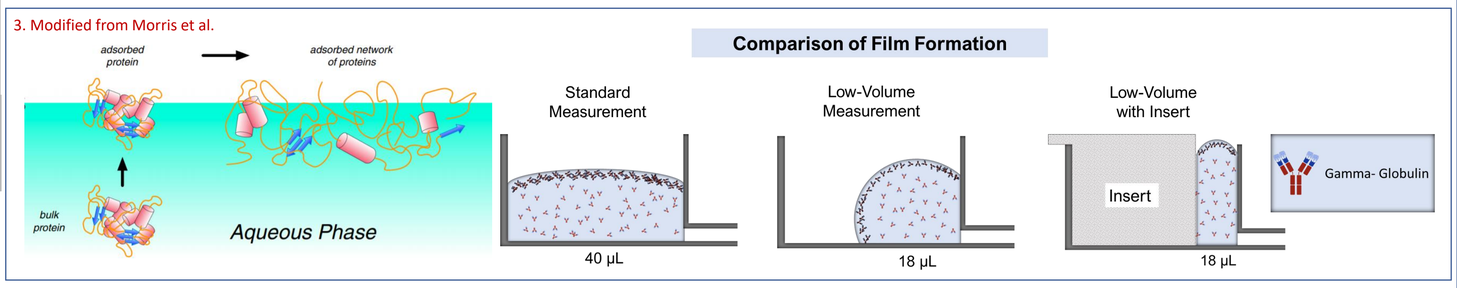
1	Viscosity is calculated as function of shear stress and shear rate using Newtons law of viscosity.	$\mu = \frac{\tau_w}{\dot{\gamma}}$
2	The Shear stress is controlled using input pressure.	$\tau_w = \frac{\Delta P\ wh}{2L(w+h)}$
3	Apparent shear rate is then calculated using the measured flow rate.	$\dot{\gamma_0} = \frac{6Q}{wh^2}$
4	Shear rate is then corrected based on the WRM[2] relation to account for non-Newtonian flow profiles.	$\dot{\gamma} = \frac{\dot{\gamma_0}}{3}\left(2 + \frac{d(\ln\dot{\gamma_0})}{d(\ln\tau_w)}\right)$



Viscosity Determination

The protein solution was prepared by dissolving bovine gamma globulin in 0.01mM PBS and gently stirring at 4ºC for 1 hour. In each case, the solution was pipetted into the inlet, left to sit for 3 minutes to allow for protein film formation, and measured in the NeoVISC. Results were compared to assess the effects of the air-fluid interface on the measured protein viscosities.

3. Modified from Morris et al.



Results

To validate our microfluidic viscometer against conventional viscometers on the market, we first performed measurements on well-characterized Newtonian (Fig. 1) and non-Newtonian (Fig. 2) fluids and compared the results with those obtained on a conventional cone-and-plate rheometer (Brookfield,Middleboro,MA). In both cases, our system demonstrates an excellent correlation to standard techniques. As a further validation, high concentrations of bovine serum albumin (BSA) were prepared and compared to another microfluidic viscometer on the market (Rheosense, San Ramon, CA) (Fig. 3). The viscosity measurements of 300mg/ml gamma-globulin using 40 µL of protein solution were significantly lower than those that used 18 µL. We hypothesized that with smaller sample volume, there was increased film formation at the liquid-air interface relative to bulk volume, resulting in a significant increase in viscosity of the corresponding protein solution. This was indeed the case as evidenced in Fig. 4. To address this issue, we designed a custom insert to reduce the surface area of the protein-air interface. We found that this insert corrects for the artifact in bulk viscosity measurements introduced by the film formation at the interface (Fig. 4).

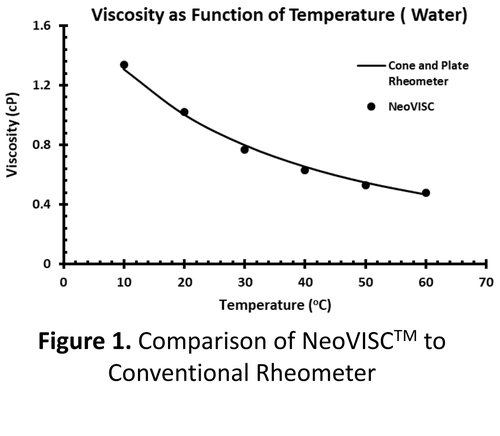


Figure 1. Comparison of NeoVISC™ to Conventional Rheometer

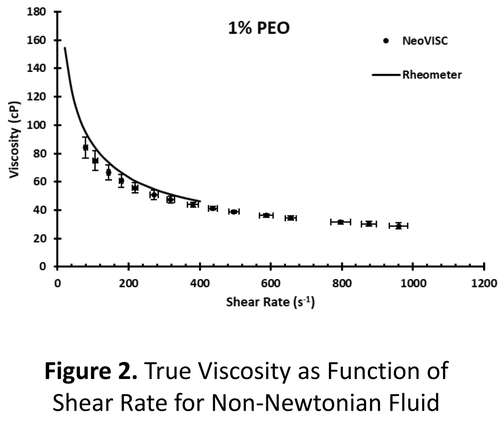


Figure 2. True Viscosity as Function of Shear Rate for Non-Newtonian Fluid

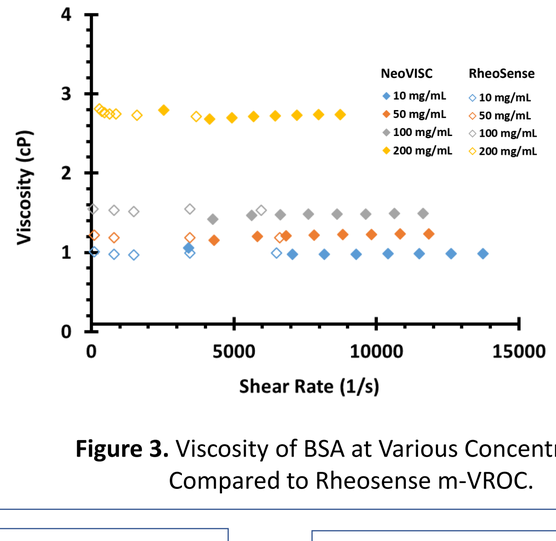


Figure 3. Viscosity of BSA at Various Concentrations Compared to Rheosense m-VROC.

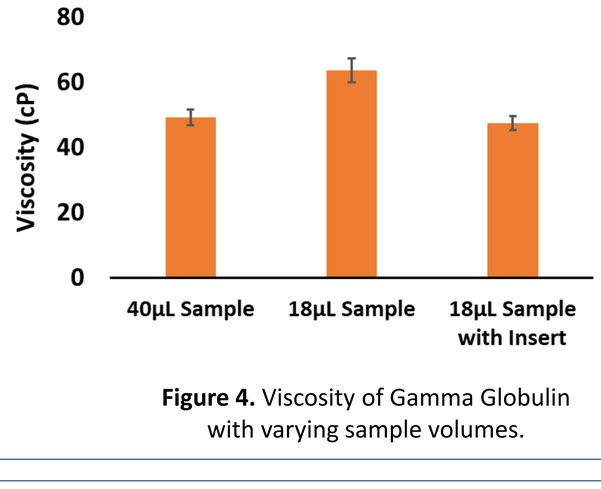


Figure 4. Viscosity of Gamma Globulin with varying sample volumes.

Conclusion

The current work demonstrates the NeoVISC's ability to accurately measure protein viscosity without any interfacial adsorption effects even at small volumes. The NeoVISC platform offers highly reproducible, low-volume, and multiplexed true viscosity measurements. Additionally, the system does not require complicated protocols or cumbersome cleaning procedures. We demonstrate that a custom viscosity vs. shear rate plot can be generated in under five minutes for up to 10 samples in parallel. The NeoVISC thus facilitates rapid and reliable viscosity testing for biotherapeutics and other protein solutions, which is difficult to attain using conventional rheometers. The NeoVisc thus opens the possibility for high-throughput viscosity studies of biologics even at early stages of discovery

References

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