A Low-Volume, Multiplexed Microfluidic Viscometer for Protein Viscosity and Stability Characterization

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Abstract

Since their introduction almost 40 years ago, protein therapeutics have become an exciting alternative to small molecule drugs due to their versatility, biocompatibility, and site-specific delivery. However, the manufacturing process for these therapeutics is still complex and typically involves batch-based operations. Throughout development, fill, storage, and delivery, viscosity is an important biophysical parameter that needs to be controlled. Currently, the gold standard in viscosity measurements is the mechanical rheometer that operates by applying a mechanical shear force to a sample fluid and measuring deformation/resistance. While these conventional methods produce accurate results with high reproducibility, they are not suited for measuring viscosity of biologics due to their large sample volume requirements, time consuming measurements with no option for multiplexing, and low resolution especially when dealing with low viscosity solutions or solvents. Here we demonstrate an integrated high throughput, low volume viscometer system—the NeoVISC™—that utilizes microfluidic technology to generate highly accurate and multiplexed viscosity measurements to quantify protein quality at various formulation and manufacturing stages. We highlight some of the ways in which such a system can be used to measure not only relative viscosity but also intrinsic viscosity, which can give the user a unique perspective into the state and conformation of the therapeutic.

Methods

A multi-height microfluidic cartridge was fabricated in plastic (COC) using injection molding. A sample volume of 30μL was pipetted into each of the inlet reservoirs on the cartridge (up to 10), which interface with individual piezo-electric pressure regulators through a custom-built airtight manifold. Each fluid sample was simultaneously driven with an independent ramping pressure profile to produce an automated viscosity vs. shear rate plot. At the beginning of each measurement, the software first tracks fluid movement under a constant pressure to approximate fluid viscosity, η. This information is used to apply an appropriate pressure ramp for the given fluid. In this way, a full range of fluid viscosities can be processed in a single cartridge simultaneously.

The viscosity determination utilizes the relationship between pressure drop and flow rate across a rectangular slit-based channel. Viscosity was found using the following equation:

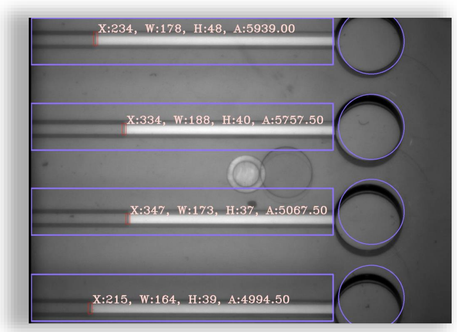
$$\eta = \frac{\tau_w}{\dot{\gamma}}$$

where $\dot{\gamma}$ is the shear rate and τ_w is the shear stress given by $\tau_w = \frac{\Delta P\, wh}{2L(w+h)}$. Here, *ΔP* is the pressure drop applied by the pressure controller, and *w*, *h*, and *L* are the width, height, and length of the resistive channel, respectively[1]. The apparent shear rate, $\dot{\gamma_0}$, is given by $\dot{\gamma_0} = \frac{6Q}{wh^2}$ where *Q* is the fluid flow rate[1]. In order to obtain the true wall shear rate, we correct for the non-parabolic velocity profile of non-Newtonian fluids using the WRM (Weissenberg Rabinowitch Mooney)[2] equation:

$$\dot{\gamma} = \frac{\dot{\gamma_0}}{3}\left(2 + \frac{d(ln\,\dot{\gamma_0})}{d(ln\,\tau_w)}\right)$$

Measurement Technique

An integrated image processing software tracks the leading edge of the fluid-air interface using a Gaussian blur to detect energy changes. The software automatically fits a curve to the distance vs. time data and differentiates to obtain velocity at each pressure, which is converted to flow rate by multiplying by the cross-sectional area of the channel. Shear stress is calculated for each input pressure and plotted against apparent shear rate. The relationship between apparent shear rate and shear stress is used to quantify true shear rate and true viscosity for a range of pressure points.



Software Tracking

Determination of Intrinsic Viscosity

Four BSA solutions ranging in concentration from 10 to 200 mg/mL were prepared in phosphate buffered saline (PBS). Solutions were stirred gently for one hour at 4°C and brought to 25°C. Viscosity of all four solutions was quantified with the NeoVISC™ system (n=3). To induce protein denaturation, the solutions were heated to 60°C and brought to room temperature. Viscosity of the four denatured solutions was then determined (n=3). The intrinsic viscosity of native and denatured BSA was found using the following equation:

$$[\eta] = \lim_{c\to 0} \frac{\eta - \eta_0}{\eta_0\, c}$$

where *η* is the sample viscosity at a particular concentration *c*, and η_0 is the solvent viscosity[3,4]. In the above equation, the $(\eta\text{-}\eta_0)/\eta_0$ term is known as the reduced viscosity, and intrinsic viscosity is found by extrapolating reduced viscosity to a protein concentration of zero.

Determination of Hydrodynamic Radius

From intrinsic viscosity, hydrodynamic radius was determined using the Einstein viscosity relation:

$$V = \frac{2[\eta]M}{5N}$$

where *M* is the molecular weight of the protein, *N* is Avogadro's number, and *V* is the effective diameter of a protein particle assuming the particle is spherical[3,4]. Hydrodynamic radius was then determined from the equation for the volume of sphere:

$$R_h = \left(\frac{3\pi}{4}V\right)^{1/3}$$

The hydrodynamic radii of native and denatured protein were subsequently compared to quantify the conformational change caused by heat-induced protein deformation.

Results

In order to validate our system, we first performed measurements on well-characterized Newtonian solutions and compared against values from literature (Fig. 1). Next we compared results obtained with non-Newtonian solutions (Fig. 2). As can be seen, both results show excellent correlation with standard techniques. Native and denatured BSA demonstrated Newtonian behavior across a broad range of shear rates (Fig. 3). Viscosity of native BSA measured on the NeoVISC correlates well with data obtained by Sharma et. al. as well as the Russel et. al. colloidal rheology model $\frac{\eta(\phi)}{\eta_0} = 1 + 2.5\phi + s\phi^2 + O(\phi^3)$, where *s* depends on the specific interactions of the dispersion[5,6] (Fig. 4). From the subsequent intrinsic viscosity data, we observe that BSA subjected to high temperature denaturation exhibited higher intrinsic viscosity and a larger hydrodynamic radius than the protein in its native state (4.09nm compared to 3.57nm for untreated BSA), indicating chain elongation and aggregation. Utilizing viscosity measurements offered a simple quantitative approach to assessing protein quality.

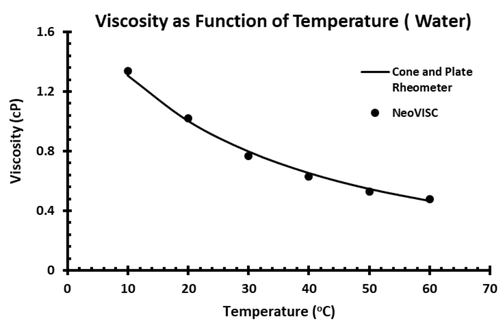


Figure 1. Comparison of NeoVISC™ to Conventional Rheometer

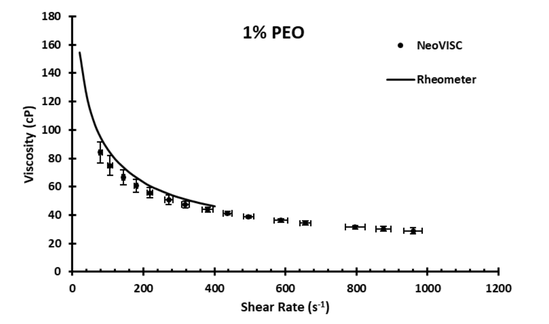


Figure 2. True Viscosity as Function of Shear Rate for Non-Newtonian Fluid

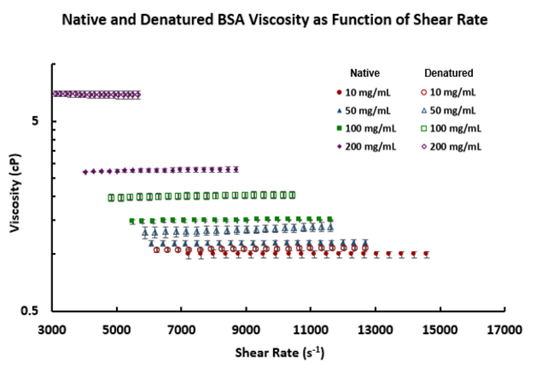


Figure 3. Newtonian Behavior of Native and Denatured BSA Solutions

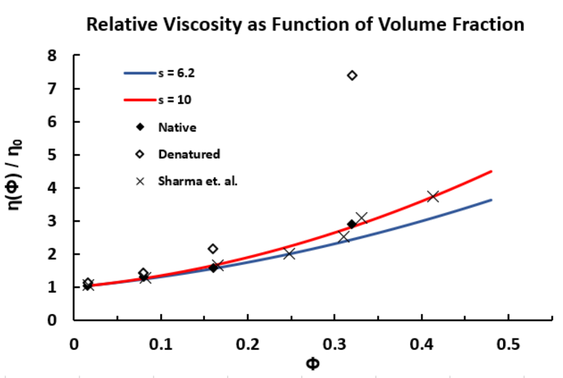


Figure 4. Comparison of NeoVISC BSA Viscosity to Colloidal Rheology Models

Conclusion

The present study demonstrates the ability of the NeoVISC™ to quickly measure protein viscosity over a wide range of concentrations and detect changes in conformation to assess stability at each stage of manufacturing and storage. The NeoVISC™ system generates highly accurate results, minimizing sample volume and maximizing throughput with 10 concurrent measurements. Within minutes, a custom shear rate sweep is generated for 10 samples simultaneously, and a disposable cartridge eliminates the need for time-intensive cleaning protocols. The NeoVISC™ facilitates rapid viscosity testing to assess protein concentration, aggregation, and denaturation at each step of the biotherapeutic development process.

References

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