High-throughput viscosity measurement of biologics formulations

Introduction

One of the major challenges in drug development is the need for rapid formulation of biologic drug candidates. Developed in conjunction with R&D departments across the industry, Unchained Labs addresses this challenge in both vaccine and protein formulation with the freeslate system configured for biologics formulation (Figure 1). The freeslate system provides the opportunity to expand drug developers' experimental design spaces, using less resources and material, in less time than conventional methods.

High concentration protein formulations for biologics can reach viscosities that make them difficult to manufacture, limit delivery options or even require costly delivery methods. Consequently, viscosity is becoming an important consideration during formulation development. To this end, viscosity measurements are a bottleneck. To alleviate this challenge, Unchained Labs developed the automated viscosity station for the early assessment of formulation viscosity. The viscosity station uses a temperature-controlled microcapillary for the measurement of viscosity ranges relevant to biologics formulations (1–20 cP) and can measure viscosities as high as 100 centipoise (cP). Each viscosity measurement only requires 100 µL of sample per measurement. The viscosity station includes an automated, integrated washing system to eliminate any chance of sample-to-sample cross contamination. The viscosity station measures samples at user-specified temperatures between 4-40 °C. Scientists can also test multiple shear rates using the viscosity station. The high-throughput (approximately three minutes per sample) and low sample volume required for the viscosity station enables the practical exploration of a variety of formulation conditions compared to traditional methods.

High-throughput viscosity station

The viscosity station is a miniature capillary viscometer[1]. A positive displacement tip (PDT) pipette, integrated on a freeslate system arm, injects sample through the capillary at a controlled flow rate. The instrument measures the pressure drop across the capillary. For Newtonian liquids, the measured drop in pressure across the capillary directly relates to the solution viscosity (µ) which is calculated using the equation,

$$\mu = \pi R^4 \Delta P / 8LQ$$

where R is the capillary internal diameter, L is the capillary length, ΔP is the change in pressure across the capillary and Q is the flow rate. Two experiments were performed to assess the reproducibility of the viscosity station each with two viscosity standards measured 10 times at a fixed temperature. The results of each standard are shown in Table 1.

	4 °C		40 °C	
Viscosity standard	1 cP	99 cP	0.7 cP	36 cP
Average	0.951	91.9	0.679	36.4
Std dev	0.024	0.038	0.038	0.087
%RSD	2.5	0.8	5.6	0.2
N	10	10	10	10

Table 1: Reproducibility of the viscosity station over a range of viscosities and temperatures.

Measuring the viscosity of Bovine Serum Albumin solutions

Bovine Serum Albumin (BSA) solutions were used to demonstrate the throughput and range of the viscosity station. For this study, BSA was dissolved in two different phosphate buffered saline (PBS) solutions, either containing Tween 80 or containing Tween 80 plus sucrose. Both known to increase the viscosity of solutions. The first solution was 10 mM PBS + 0.6% Tween 80; the other solution was 10 mM PBS + 0.6% Tween 80 + 10% sucrose. BSA was dissolved in the PBS solutions to prepare 200 mg/mL stock solutions. Samples for analysis were made by diluting the stock solutions to 20, 50 and 100 mg/mL BSA with their respective PBS solutions. Measured viscosities of the BSA and stock solutions are presented in Figure 1. Each measurement was made using 100 µL of sample.

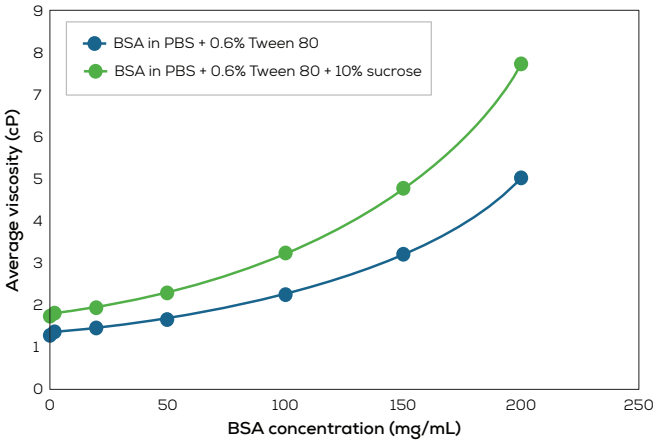

Figure 1: BSA solution viscosity versus concentration results collected using the automated viscosity station.

The standard deviation for each measurement, repeated in triplicate, was usually less than 3% and the maximum standard deviation was 7.4%.

Measuring viscosity of Newtonian and non-Newtonian fluids

The viscosity station can measure the viscosity of Newtonian liquids and non-Newtonian liquids. To characterize non-Newtonian fluids, measurements can be made across a range of shear rates (flow rates). The resultant data can be used to calculate the Weissenberg-Rabinowitsch (WR) correction factor. The WR correction factor can then be used to calculate the actual shear rate from the apparent (Newtonian) shear rate.

An example of using the viscosity station to measure non-Newtonian solutions is the measurement of Refresh Liquigel® eye rewetting drops. Refresh Liquigel is a complex non-Newtonian, shear-thinning liquid. It is designed to have a relatively high viscosity at low shear rates in order to provide good retention on the eye and low viscosity under shear to enable blinking to produce a uniform thin layer of the liquid across the eye. Refresh Tears®, on the other hand, is a Newtonian liquid; the viscosity of this product does not vary substantially with shear rate. The viscosities of Refresh Liquigel and Refresh Tears were measured using flow rates from approximately 1-15 µL/s, which correspond to shear rates from approximately 1,000-15,000 s^{-1}. To analyze the data obtained for the Liquigel product, the WR correction factor was determined and then applied to each measurement.

The actual (corrected) shear rate was used in the viscosity calculation. As expected, the viscosity of the Refresh Tears (Figure 2) did not change appreciably across this range of shear rates. Conversely, the viscosity of the Refresh Liquigel dropped by more than a factor of two across the applied range of shear rates. Using as little as 100 µL of sample, the shear rates of solutions were accurately measured with the viscosity station.

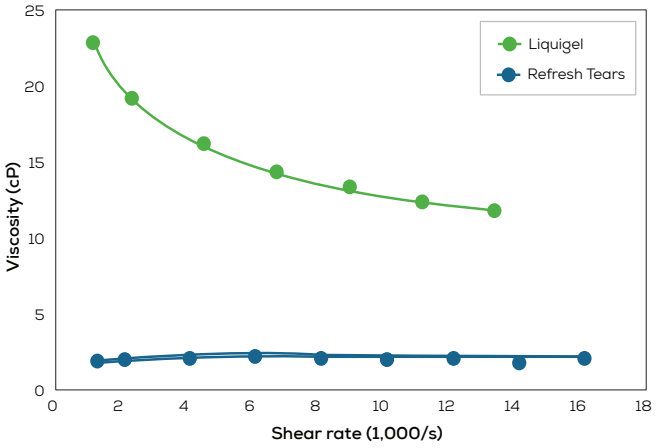

Figure 2: Viscosity measurement of Newtonian and non-Newtonian solutions.

High-throughput viscosity measurement of monoclonal antibody formulations

A set of 24 different monoclonal antibody (mAb) formulations were evaluated in a stability study. The mAb concentration in 12 of the formulations (F01–F12) was 210 mg/mL. The mAb concentrations of the remaining formulations was 50 mg/mL (F13–F22 and F24) or 10 mg/mL (F23). In addition to mAb concentration, the selected formulations also vary widely in pH buffer species, excipient types and excipient levels. The initial viscosity of each formulation was measured (t_0), and six other sets of formulations were stored at 4 °C, 25 °C or 40 °C for 45 and 90 days. At the end of each storage period, the viscosity of each formulation was measured. The viscosity station detected viscosity differences resulting from differences in formulation composition including mAb concentration and excipients. Viscosities of 50 mg/mL formulations (Figure 3) did not change dramatically during the stability study. In some cases, the viscosities of the 210 mg/mL formulations (Figure 4) increased significantly during storage. The viscosities of many 210 mg/mL formulations stored at 40 °C approached or exceeded the instrument measurement range (100 cP). By using the viscosity station for this stability study, we were able to rapidly assess the effect formulation, antibody concentration and storage conditions had on the viscosity of each antibody solution. The integration of the viscosity station into the freeslate systems enables the unattended high-throughput measurement of viscosity in protein solutions and biotherapeutic formulations.

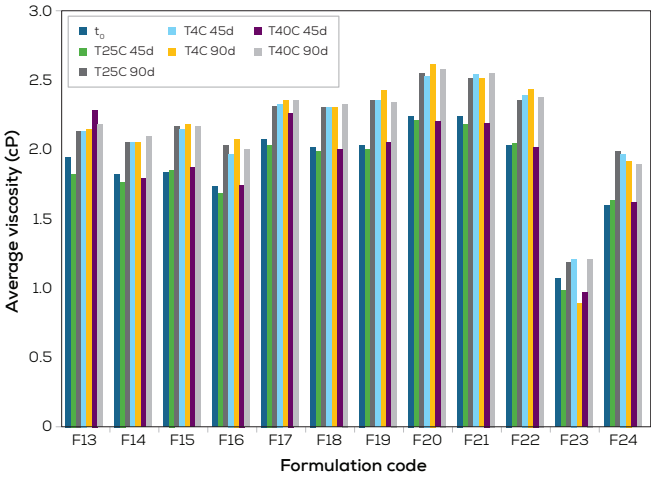

Figure 3: Viscosity measurements during stability testing of 12 mAb formulations at 50 mg/mL (F13–F22 and F24) or 10 mg/mL (F23).

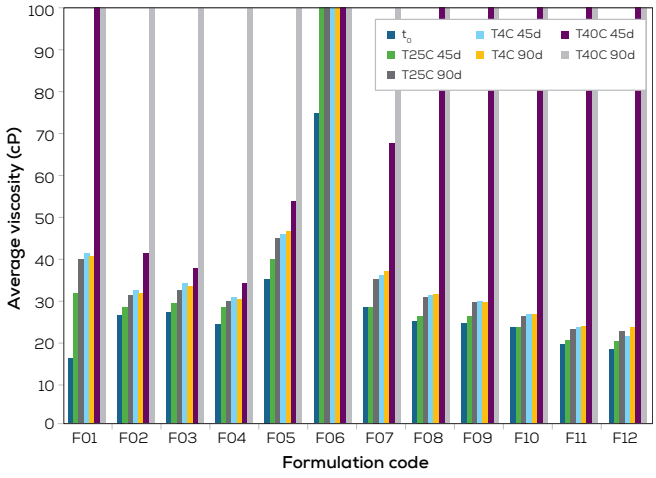

Figure 4: Viscosity measurement of twelve 210 mg/mL mAb formulations during stability testing. Viscosities >100 cP are beyond the performance specifications of the viscosity station and are reported as 100 cP.

Conclusion

Biologics formulations are often high concentration (50–150 mg/mL), and the viscosities of such solutions can pose challenges to drug developers. An integral solution to this challenge is the viscosity station on the freeslate system, which was designed specifically to target the acceleration of formulation development with the following benefits:

- High-throughput viscosity measurements using 100 µL of sample
- Measurement of biologics formulations with viscosities ranging from 1-100 cP
- Viscosity measurements and automatic cleaning in less than three minutes
- Automated cleaning of the capillary between measurements
- Viscosity measurement over a wide temperature range
- Viscosity measurement of Newtonian and non-Newtonian solutions

The freeslate systems configured for biologics formulation enable the automated characterization of an array of candidate formulations. And, when used in conjunction with Lab Execution and Analysis (LEA) software from Unchained Labs, data from off-line analytics (e.g., SEC, cSDS and cIEF), can be associated with a particular formulation for reporting. The combination of the viscosity station on the freeslate system combined with the associated LEA software provide a comprehensive and powerful system for formulation screening and robustness assays of biotherapeutics.

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