Let it flow: automated low volume viscosity measurements with Junior

Introduction

Biologic drug products typically need to be manufacturable and deliverable at high concentrations. High concentration monoclonal antibody (mAb) formulations are often highly viscous, making the manufacture and delivery of these formulations potentially problematic. Measuring the viscosity of a biologic formulation at its intended final concentration can help to more fully characterize the developability of the biologic drug product.

Routine methods to determine viscosity tend to require extensive hands-on time and large sample volume, which limits the number of formulations that can be tested in early formulation development. A Big Kahuna or Junior equipped with a viscosity station is an automated, low volume method to analyze the viscosity of biologic formulations (Figure 1). The viscosity station is a micro-capillary viscometer that measures the change in pressure when a sample is injected into the capillary to calculate the viscosity of Newtonian and non-Newtonian fluids, shear rate, and shear stress. Each measurement requires 100 µL of sample and the capillary is automatically cleaned between measurements to prevent cross contamination.

The viscometer temperature and injection flow rate are user-defined, allowing for flexibility in experimental design and a large viscosity range (up to 100 cP). With minimal hands-on time and low volume requirements, Junior with a viscosity station enables viscosity analysis on more samples to assess the developability of more biologic and formulation candidates.

In this application note, Junior was used to measure the viscosity of glycerol standards with known viscosities and a concentration series of a mAb with unknown viscosities.

Figure 1: A Big Kahuna or a Junior (pictured) with a viscosity station automates low volume, high-throughput viscosity measurements.

Viscosity Station

The Big Kahuna and Junior viscosity station is a micro-capillary viscometer that uses the following equation to calculate viscosity (µ).

$$\mu = \frac{\pi R^4 \Delta P}{8LQ}$$

The capillary length (L) is known and injection flow rate (Q) is set by the user. The apparent inner radius (R) of the capillary is determined during calibration of the viscometer. When a sample is injected into the viscometer, the change in pressure (ΔP) is measured and used to calculate viscosity.

To calibrate the viscometer, solutions with known viscosities are used and injection flow rate is set by the user. With viscosity, capillary length, and injection flow rate known, the apparent inner radius can be calculated by measuring the change in pressure when a sample is injected into the capillary. Once the apparent inner radius of a capillary is determined, calibration does not need to be repeated.

Methods

Results from Junior with an installed viscosity station and a cooled storage bay were compared to those from a *micro*VISC™ viscometer

(Rheosense). The viscosity station temperature and the cooled storage bay were set to 20 °C. The viscosity station injection flow rate was set to 15 µL/s, and 100 µL was used per measurement. The capillary was cleaned between each measurement.

To calibrate the viscosity station, glycerol standards of known viscosities were used. Each standard was measured in triplicate. Calibration determined the apparent inner radius of the capillary, which was then used for all subsequent measurements.

The *micro*VISC does not control temperature but does record ambient temperature, therefore samples were analyzed at room temperature (about 24 °C). Volume required for the *micro*VISC varied between samples and measurements. For all samples, approximately 400 µL filled the *micro*VISC pipette, and between 16.6 µL and 154.9 µL of sample was used per measurement.

The viscosities of glycerol standards of 0, 10, 20, 30, 40, 50, 60, 65, and 70% (w/w) were measured in triplicate by Junior and the *micro*VISC. The measured viscosities from each viscometer were compared to published viscosities of each glycerol standard[1] to determine the accuracy of the viscosity station relative to the *micro*VISC. The nine glycerol standards were measured on Junior in triplicate in 2.5 hours.

A stock mAb was prepared in 10 mM histidine, pH 6.0 and 0.1% PS80. A concentration series from 1-150 mg/mL at unknown viscosities was prepared. Each sample was measured in triplicate on Junior and on the *micro*VISC. The total run time to measure eight samples on Junior in triplicate was 2.25 hours.

The LEA software suite was used for experimental design, execution, and analysis of results. The experimental designs were created in Library Studio with Design Creator and were executed in Automation Studio. Average viscosity and standard deviation for each sample was calculated in Excel using the LEA Analysis Add-in. Hands-on time to design each experiment in Library Studio was less than 5 minutes, time to start an experiment in Automation Studio was less than 2 minutes.

Results

Glycerol standards with known viscosities at 20 °C were measured on the viscosity station and the *micro*VISC, and compared to published viscosities (Figure 2). Measurements from the viscosity

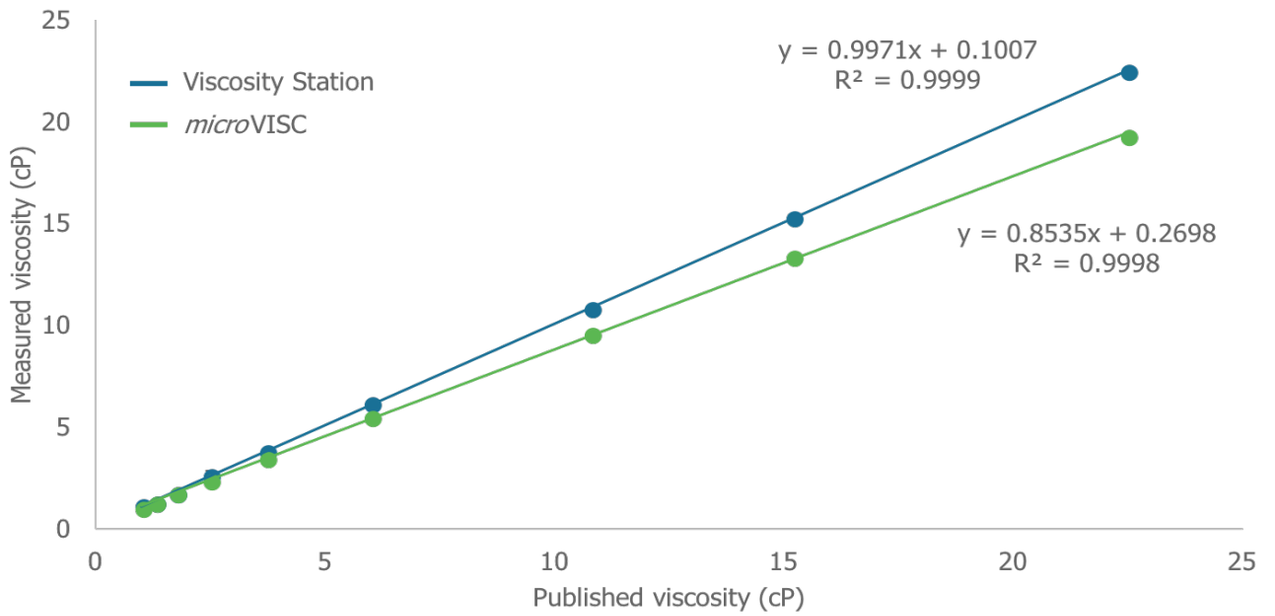

Figure 2: Viscosity station measurements (blue) are highly reproducible, and accurate when compared to published viscosities for glycerol standards. The *micro*VISC measurements (green) are also reproducible, but less accurate without temperature control.

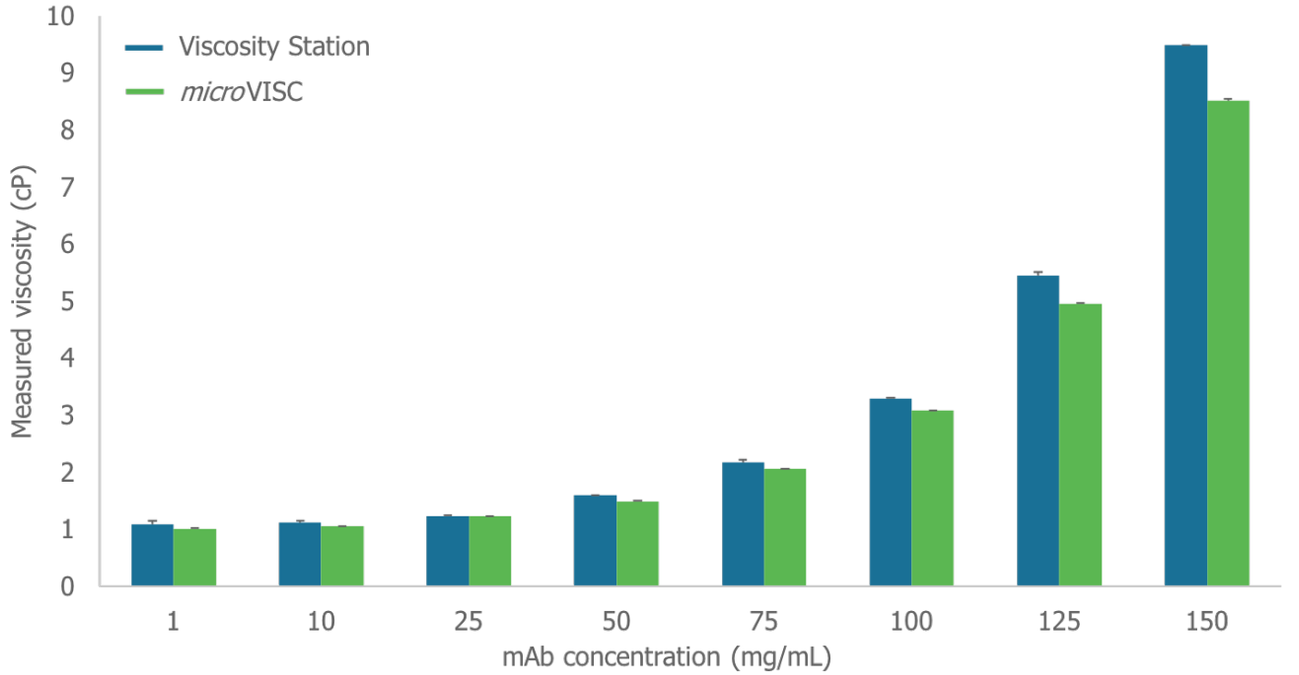

Figure 3: Viscosity of a stock mAb increases exponentially with mAb concentration.

station matched published values with a slope of nearly one (m = 0.9971) and a high correlation (R^2 = 0.9999). The Junior viscosity station showed high accuracy and reproducibility with measurements of the glycerol standards (Table 1). The *micro*VISC also had a high correlation (R^2 = 0.9998) but a lower slope (m=0.8535), corresponding to measured values lower than both the published values and viscosity station measurements. Viscosity is known to be highly dependent on temperature, so the lack of temperature control on the *micro*VISC is likely the reason for the discrepancy.

Percent Glycerol	Measured viscosity (cP)	Published viscosity (cP)
0	1.17 ± 0.03	1.005
10	1.31 ± 0.10	1.31
20	1.74 ± 0.05	1.76
30	2.64 ± 0.20	2.50
40	3.83 ± 0.08	3.72
50	6.17 ± 0.03	6.00
60	10.84 ± 0.05	10.8
65	15.31 ± 0.13	15.2
70	22.49 ± 0.10	22.5

Table 1: Viscosity station measurements are highly reproducible and comparable to published viscosities of glycerol standards.

After confirming accuracy and reproducibility, samples with unknown viscosities were analyzed. The mAb concentration series was measured on the viscosity station on Junior and on the *micro*VISC, with the expectation that viscosity would increase exponentially with concentration. Results from an automated analysis with the viscosity station on Junior and a manual analysis with the *micro*VISC show this exponential increase in viscosity (Figure 3), with a maximum viscosity for this molecule of 9.49 cP at 150 mg/mL (Table 2). The low standard deviations of the viscosity station measurements (Table 2) indicate high reproducibility of the viscosity station on Junior. The measurement discrepancies with the *micro*VISC are likely due to temperature differences between the two systems' measurements.

Conclusion

Automated, low volume viscosity measurements on Junior or Big Kahuna can be performed reproducibly and consistently in comparison with published values and other benchtop

mAb conc. (mg/mL)	Measured viscosity (cP)
1	1.08 ± 0.069
10	1.11 ± 0.033
25	1.23 ± 0.021
50	1.59 ± 0.009
75	2.17 ± 0.056
100	3.28 ± 0.022
125	5.45 ± 0.062
150	9.49 ± 0.002

Table 2: Viscosity station measurements of a mAb concentration series are highly reproducible.

instruments. The viscosity station on Junior allows for user-defined temperature and injection flow rate control for flexibility in experimental design and a large viscosity range (up to 100 cP). With only 100 µL required per measurement, sample can be conserved for use with other analytic measurements, many of which can be integrated into the same Big Kahuna or Junior system.

1. Segur, J.B. and Oberstar, H.E. Viscosity of Glycerol and Its Aqueous Solutions. *Industrial and Engineering Chemistry*. September 1951.

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