# Measuring viscosity with Honeybun just got cooler

## Introduction

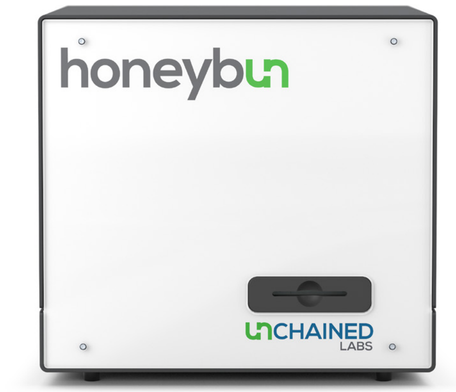
Biologics are cool, but they also need to be kept cold. That can cause problems, especially when you try to increase their concentrations for subcuteaneous delivery. High viscosities of these formulations at low temperatures can plague you anywhere from discovery to manufacturing to administration.

Viscosity is a critical quality attribute at every stage of the development pipeline. In discovery and early development, high viscosity indicates protein molecules are interacting either with each other or with solvents and excipients. Thus, viscosity can be a bellwether of stability issues, like aggregation and denaturation, even when stored at 4 °C. As a biologic moves into later development stages, its manufacturability is an added focus beyond stability considerations.

Highly viscous, high concentration protein solutions are prone to membrane fouling and surface adsorption, resulting in lost material and lower yields.[1] They also flow more slowly through tubing and pumps during filtration steps. High viscosity solutions require higher pressures during fill/finish, which can stress and damage shear-sensitive proteins. Lastly, if a high concentration biologic is supposed to be self-injected subcutaneously by patients, you need to know the temperature at which its viscosity gets below comfortable injectibility limits, usually cited to be about 20 cP.[2]

Taking into account how important viscosity is as a metric, it's surprising that no one gets as much viscosity information as they need. Classic techniques require hours of hands-on time in super slow, one-sample-at-a-time instruments that use up high volumes of sample. Even modern tech uses expensive chips that create a one-by-one bottleneck and need lots of cleaning and calibration.

A

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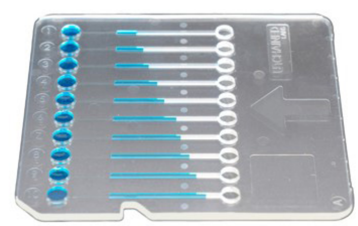

Figure 1: Honeybun (**A**) is the only rapid microvolume viscometer and rheometer for proteins, vaccines, viral vectors and injectables. Honeybun and its consumable Bun (**B**) read up to 10 samples in minutes from 4–45 °C with no more than 35 µL of sample.

Honeybun (Figure 1A) is the only rapid microvolume viscometer and rheometer that combines low sample volume requirements (35 µL in default and 15 µL in low volume mode) with the ability to run up to 10 samples at any temperature from 4–45 °C in its disposable consumable, the Bun (Figure 1B). For low viscosity samples (≤10 cP), these 10 measurements are done in under a minute, while high viscosity samples take less than 10 minutes.

Using Honeybun couldn't be any easier. Simply pipette your analyte into the Bun, insert it into Honeybun, and get your super accurate and precise viscosity results. Honeybun's dynamic range (0.5–150 cP) is factory-calibrated and covers the needs of the overwhelming majority of biological therapeutics.

## Methods

Solutions of 0, 70, 75, 77, and 78% weight/weight (w/w) glycerol in deionized water were prepared and stored at room temperature until they were measured in 20 replicates across 2 Buns at 4 °C in default mode (35 μL) on Honeybun. Solutions of 170 mg/mL monoclonal antibody (mAb) 1 & 2 in 10 mM histidine were prepared from 26 mg/ml stock solutions using Unagi and the concentration, hydrodynamic diameter, and polydispersity were verified using Stunner. The viscosities of mAb 1 & 2 were measured at five temperatures between 4 and 40 °C in Honeybun in default and low volume (15 μL) modes, each in triplicate. All results are depicted as means plus or minus one standard deviation.

## Results

Most viscometers and rheometers on the market require significant amounts of hands-on time and can only measure one sample at a time. Honeybun delivers reliable viscosity results from 35 μL across its entire dynamic range from 0.5–150 cP down to 4 °C, as shown by the measurements of the viscosity of water and 4 different solution of glycerol with their predicted viscosities (Figure 2).[3]

Water has a predicted viscosity of 1.567 cP at 4 °C; Honeybun measured this value as 1.56 cP± 2.2% (Table 1).[3] A solution of 78% (w/w) glycerol in water has an expected viscosity of 150.0 cP and a measured viscosity in Honeybun of 157.4 cP ± 3.7%.

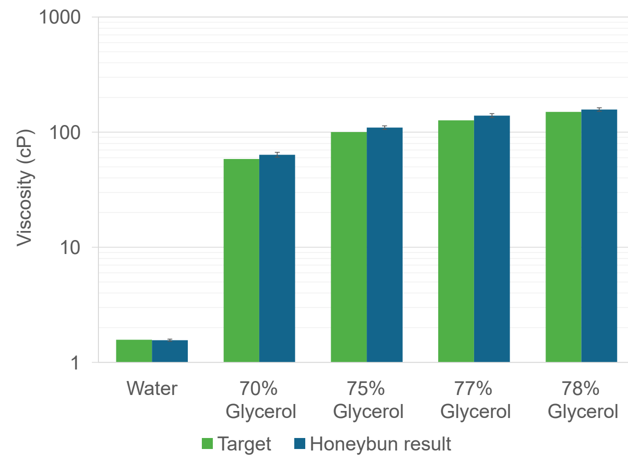


Figure 2: The viscosities of water and 70, 75, 77 and 78% (w/w) glycerol in water were measured in 20 replicates each in default mode on Honeybun at 4 °C. Note the graph is in log scale.

Subcutaneous delivery of high concentration biologics is made more difficult by their high viscosity, but highly viscous solutions also pose a challenge to manufacturability. Without process optimization, your development pipeline can clog just like your tubing. Honeybun is equipped with fine-tuned temperature controls, allowing viscosity measurements of monoclonal antibody formulations from 0.5–150 cP and from 4–45 °C.

The viscosities of mAb1 and mAb2 were determined in triplicate at 5 temperatures between 4 and 40 °C (Figure 3). Both antibody solutions were at a concentration of 170 mg/mL in 10 mM histidine. The viscosities measured in low volume and default mode were indistinguishable for the same mAb at each temperature.

| Sample | Target viscosity (cP) | Honeybun viscosity (cP) |
|---|---|---|
| Water | 1.567 | 1.56 ± 0.03 |
| 70% (w/w) glycerol | 58.54 | 63.70 ± 3.0 |
| 75% (w/w) glycerol | 100.0 | 109.74 ± 3.71 |
| 77% (w/w) glycerol | 126.6 | 139.36 ± 5.64 |
| 78% (w/w) glycerol | 150.0 | 157.4 ± 5.8 |

Table 1: Predicted ("Target") viscosities of water and 5 aqueous glycerol samples at 4 °C.[3] Mean plus or minus the standard deviation of Honeybun viscosity measurements, recorded in default mode.

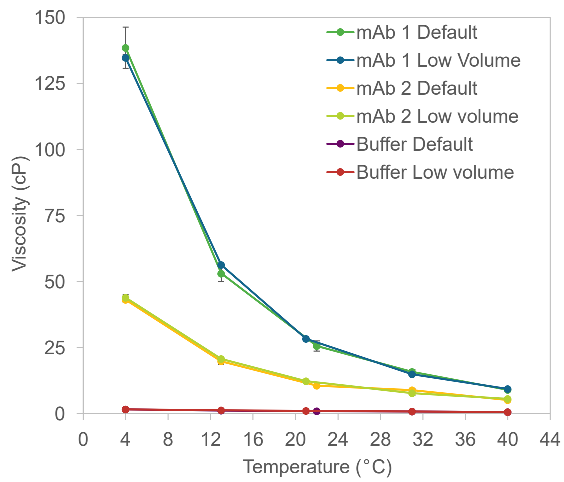


Figure 3: Triplicate measurements of 170 mg/ml mAb1 & 2 in 10 mM histidine and histidine buffer alone as a function of temperature between 4 and 40 °C, in default and low volume modes of Honeybun. The viscosity of all samples decreased non-linearly with increasing temperature.

Increasing temperatures caused the viscosity of both mAbs to decrease approximately logarithmically. The viscosity of 10 mM histidine solution without antibodies remained similar to that of water throughout .

Despite being in the same formulation, mAb1 was consistently higher viscosity than mAb2, indicating stronger protein-protein interactions and the possibility of colloidal aggregation during long-term storage.[4] A commonly cited upper limit for comfortable subcutaneous injection is 20 cP, which mAb1 would likely reach at approximately 28 °C; mAb2 would likely reach this threshold at just about 13 °C.

## Conclusion

The viscosity of biologics is hard to predict and rarely linear with concentration or with temperature. To keep things flowing through the development pipeline, Honeybun delivers straight-forward, fast, low volume measurements from 0.5–150 cP on up to 10 samples at a time. Accuracy, reliability, and precision are no problem at all for Honeybun. In a few minutes you can get viscosity data from 4–45 °C to see if a protein is stable and how it will behave, be that straight from the fridge or at body temperature. Fast to set up and run, low volume, hands-free, cleaning-free, and hassle-free – Honeybun checks all the boxes to deliver as much viscosity data as you need.

## References

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Rev A