UNCHAINED LABS

Application Note

Relative Viscosity Measurement using the UNit

Characterizing the viscosity of biologic formulations is a critical part of formulation development as the viscosity can have an impact on bioprocessing as well as final administration of the biologic. The UNit with the optional 445 nm laser enables the relative viscosity measurement and retains the 266 and the 473 nm lasers, thus retaining its capability to simultaneously measure conformational stability and aggregation propensity of samples. In this application note, we demonstrate the use of the UNit with the optional 445 laser in combination with a fluorescent molecular rotor to screen relative viscosities of samples in a high throughput manner.

Key benefits of the relative viscosity assay using the UNit:

- Works for samples with viscosities up to 50 cP
- Runs 48 samples in 10 minutes
- Uses only 9 µL of sample
- Same sample can be used to measure simultaneous T_m and T_{agg} using intrinsic fluorescence on the UNit with the standard 266 and 473 nm lasers
- Optimal for screening formulations with consistent protein concentrations

CCVJ (9-(2-Carboxy-2-cyanovinyl)julolidine) is a known molecular rotor whose fluorescence characteristics depend on the viscosity of its environment. CCVJ can be excited using the optional 445 nm laser in the UNit with a typical fluorescence emission centered at 500 nm. In solutions with low viscosity, the excited state of the dye can easily convert into a twisted intramolecular charge transfer (TICT) state, which is lower in energy than the excited state. From the TICT state, the dye goes back to the ground state non-radiatively reducing the fluorescence intensity around the 500 nm peak. In solutions with high viscosity, formation of the TICT state is sterically hindered and as a result, the fluorescence emission around the 500 nm peak is increased. Thus the fluorescence intensity of CCVJ increases with increasing solution viscosity and can be used to determine relative viscosity of samples.

CCVJ is known to interact with proteins forming a protein-dye complex which can increase the CCVJ fluorescence. Due to this reason, it is strongly recommended that performing this application on the UNit be used for screening relative viscosities of formulations that have a constant protein concentration across all samples.

Experimental set-up and data analysis

Materials

The UNit with optional 445 nm laser
UNis and silicone seals
UNi Frames
UNi Filling Tool
CCVJ (9-(2-Carboxy-2-cyanovinyl)julolidine) (Sigma Aldrich #72301)
DMF (anhydrous N,N-dimethylformamide) (Sigma Aldrich #227056)

Methods

A stock solution of CCVJ of at least 5 mM was prepared in anhydrous DMF and stored at 4 °C protected from light for up to one month. Typically, a final CCVJ concentration of 100 µM in the samples is recommended, although lower or higher concentrations may be used depending on the samples and signal levels.

The ***Determine the relative viscosity of samples*** application under the ***Applications*** section of the UNit Client software is used to set up relative viscosity

measurements. The application invokes an isothermal method at 30 °C, wherein the 266 nm and the 473 nm lasers are disabled and only the 445 nm laser is enabled. It is strongly recommended, and is set as a default in the Client software, that at least five repeat measurements be performed on each well. It is also recommended that the fluorescence intensity of the samples be tested in the Manual Configuration mode and exposure time adjusted if necessary before starting the run to ensure that there is no signal saturation.

Data analysis is performed in the Advanced Analysis mode of the UNit Analysis software. The differences in fluorescence intensity between samples are parametrized by integrating the area underneath the fluorescence emission curves between 460 and 650 nm and then taking the median value of the repeat measurements from each well as a measure of the relative viscosity of the sample.

Example data

Relative viscosity measurements over a wide dynamic range

Several solutions of varying viscosities were prepared by mixing different ratios of glycerol and water. Increasing fluorescence signal is observed with increasing glycerol concentrations, i.e., increasing viscosity (**Figure 1A**). A plot of the integrated intensities against the theoretically calculated viscosities of the glycerol-water mixtures (**Figure 1B**) shows that the integrated fluorescence on the UNit increases as expected with the calculated solution viscosity up to 50 cP, which is the typical range of interest for biologic formulation development.

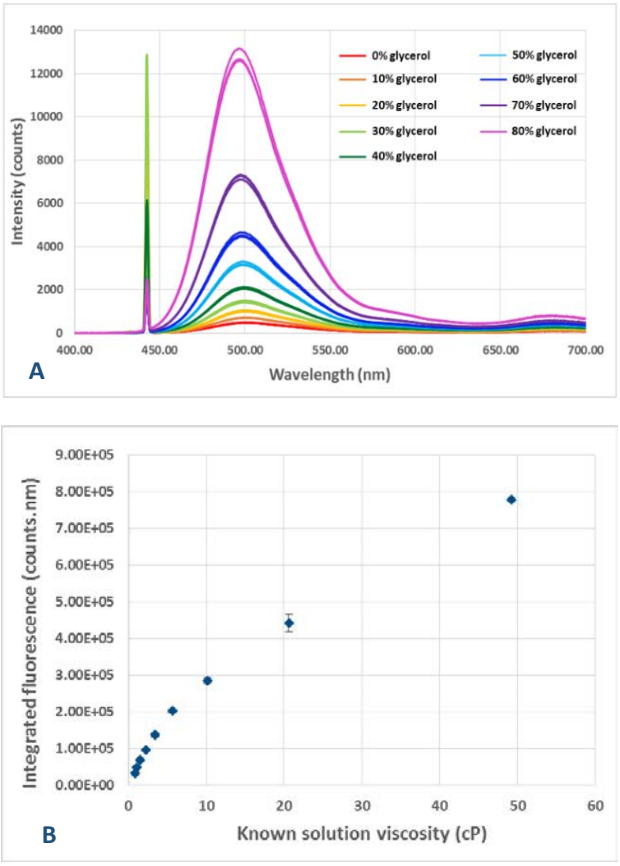

Figure 1: UNit assay data for various glycerol-water mixtures. (A) Fluorescence emission curves measured on the UNit 445 nm for various mixtures of glycerol and water show increasing glycerol concentrations correlating to increasing viscosity. (B) Integrated fluorescence emission intensities measured on the UNit for various viscosities show that increased integrated fluorescence directly correlates to increased viscosity.

UNit measurements correlate well with orthogonal viscosity measurements

250 kDa molecular weight dextran was used to prepare solutions over a wide concentration range and measured both with an m-VROC viscometer and UNit with the optional 445 nm laser to compare the two methods of quantifying relative viscosity. The data obtained from the UNit assay correlate well with orthogonally measured viscosities (**Figure 2**).

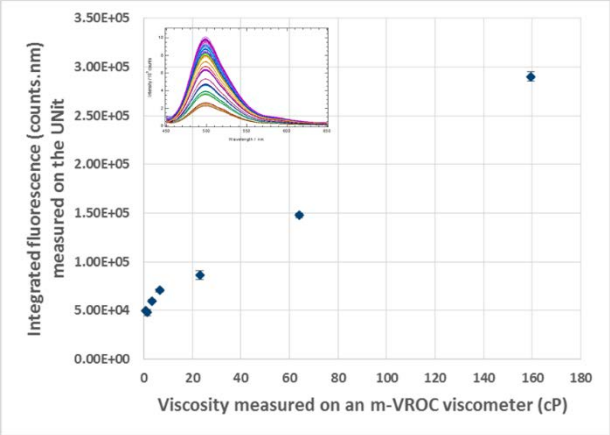

Figure 2: Good agreement between the viscosity measured on an m-VROC viscometer and the relative viscosity (via integrated fluorescence) measured on the UNit for 250 kDa dextran solutions at different concentrations.

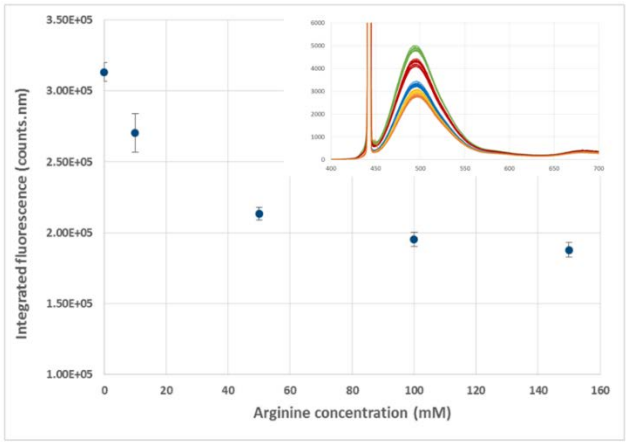

Figure 3: Increasing levels of arginine lead to decreased relative viscosity in human IgG solutions.

Rapid screening of formulation additives: effect of arginine and salts on protein solution viscosity

The following examples demonstrate the application of the UNit assay to rapidly screen the relative viscosities of IgG solutions in the presence of various additives.

Arginine is known to reduce the viscosity of protein solutions by altering protein-protein interactions. To test the effect of arginine additives on protein formulation viscosity, different amounts of arginine were added to a 100 mg/mL human IgG solution, and the relative viscosity of the solutions measured using CCVJ fluorescence on the UNit with the 445 nm laser. As expected, the viscosity of the formulation decreases with increasing arginine amounts until the effect begins to saturate at a concentration of 150 mM arginine (**Figure 3**).

Potassium iodide (KI) is a chaotrope that is known to reduce viscosity. The relative viscosity of 10 mg/mL poly IgG solutions with different concentrations of KI measured using the UNit assay (**Figure 4**) show that increasing the KI concentration had a marked difference on the relative viscosity. To further study the effect of additives on viscosity, sodium salts of different anions were added to samples of 10 mg/mL poly IgG. The pH and ionic strength of the solutions was kept constant. These results can be seen in **Figure 4**, and the order of the salts from left to right matches the order of the Hofmeister series.

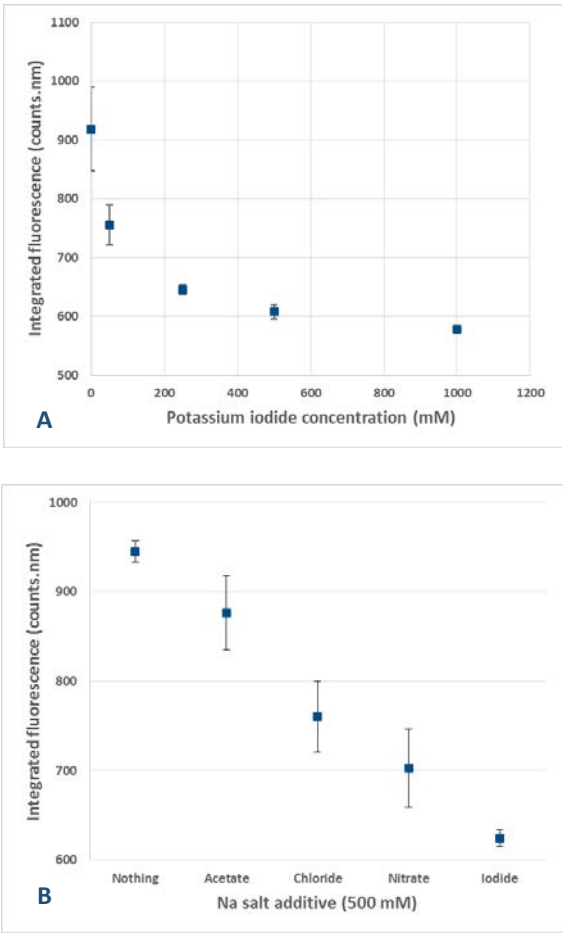

Figure 4: Effect of salts on the viscosity of protein solutions. (A) Relative viscosity of 10 mg/mL poly IgG samples of different potassium iodide concentrations shows that increased concentrations of potassium iodide correlate to a decrease in relative viscosity. (B) Relative viscosity of 10 mg/mL poly IgG samples with different salts from the Hofmeister series. The extent to which salts reduce the protein solution viscosity matches the order of the salt anions in the Hofmeister series.

Conclusion

- The relative viscosity assay can be used to rapidly screen relative viscosity for 48 samples in 10 minutes
- Requires 9 µL sample volume
- The same UNis can be used to determine conformational stability and aggregation propensity of samples, minimizing sample preparation time and maximizing the amount of information obtained from very low sample volumes
- Best suited to screen different formulations with consistent protein concentrations
- The assay utilizes the UNit with the optional 445 nm laser and CCVJ fluorescence

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