

## Application Note: Protein Formulation Stability with VROC®

### *Can protein stability be tested without diluting the protein formulation?*

#### Application

Protein stability is extremely important in protein drug formulation. The shelf life and the efficacy depend on protein stability; depending on the environment, proteins may unfold and aggregate. Most instruments for analyzing protein aggregation are only applicable for low concentration ranges, a few mg/ml.

However, drug efficacy often requires heavy loading of proteins. Measuring viscosity with the VROC® allows studying protein stability at high levels of concentration. The small sample volume requirement of the VROC® allows studying protein formulation stability during an early stage of drug development when materials are limited.

Protein stability is essential for drug efficacy and storage shelf life. When proteins become unstable, molecules unfold and aggregate. Aggregated proteins incur immune response that causes serious side effects, which makes protein stability an integral part of protein formulation.

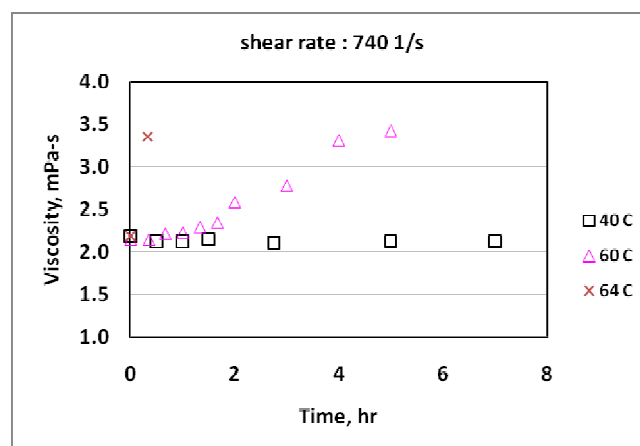
Protein stability depends on temperature, pH, concentration and many other factors. For example, as the temperature increases, protein unfolds and loses tertiary and secondary structures. As unfolding progresses, proteins begin to aggregate, which could lead to the precipitation or formation of a gel-like structure.

Various analytical instruments have been used for the study of aggregation or unfolding. For the protein unfolding, light scattering or circular dichroism techniques were frequently used. Light scattering technique detects the change of the size, whereas dichroism detects protein conformational change. These analytical methods require a dilute concentration for

accuracy. This often requires that samples under investigation are diluted for measurement, which involves altering the environment around the protein molecules. Viscosity is a bulk property and has been used to detect size change of molecules or morphology.

#### Protein Stability Test with VROC®

The following test was performed for bovine  $\gamma$ -globulin in PBS at 100 mg/ml. The solutions were incubated in water bath at different temperatures: 40 °C, 60 °C, and 64 °C. To detect the change in size and stability, the VROC® A chip with 50  $\mu$ m deep flow channel was employed. The results are displayed in the following graph.

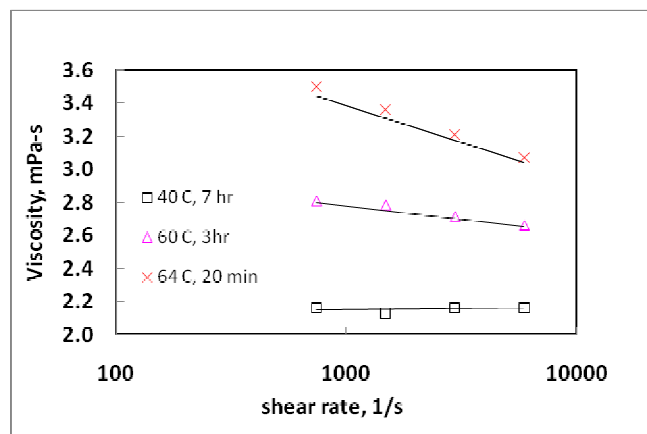


Viscosities of  $\gamma$ -globulin solutions were measured as a function of incubation time. Each incubated sample was tested for viscosity at 25 °C. Viscosity remained the same even after seven hours of incubation at 40 °C. However, when the solution was incubated at 60 °C, viscosity started to increase within 40 minutes. This viscosity increase suggests that proteins unfolded and even aggregated. Viscosity increased immediately after the solution was incubated at 64 °C.

The protein melting temperature is defined at the temperature when 50% of proteins unfold. The

melting temperature is dependent on the time scale, the duration of incubation.

### Non-Newtonian behavior if proteins are aggregated



A notable change with the extent of denaturation or aggregation is that the viscosities of aggregated protein solutions become dependent on the shear rate – shear thinning non-Newtonian.

Shear thinning behavior was observed for the protein solutions incubated for 100 minutes at 60 °C and became obvious after 180 minutes as shown in the graph above. Shear thinning behavior is immediately observed within 20 minutes of incubation at 64 °C. These results imply that shear thinning behavior could be used as a complementary tool for protein stability.

### Conclusions:

1. Proteins denaturation or stability depends on temperature and time. Denaturation can happen immediately or with a significant lag depending on temperature.
2. Aggregated proteins started showing shear thinning behavior. The trend is the first time to be reported.
3. VROC<sup>®</sup> has shown that small increase in viscosity due to unfolding or denaturation can be detected.
4. VROC<sup>®</sup> is a useful tool to investigate drug stability without the requirement of sample dilution.

If you have questions or need more information, please contact us.

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