

## Application Note: Protein Formulation Stability with VROC® – Sucrose Effect

Can protein stability be tested without diluting the protein formulation? How about the effect of addition of Sucrose?

### Application

Protein stability is important for 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 protein loading. Measuring viscosity with the [VROC®](#) allows studying protein stability at high levels of concentration. The small sample volume requirement of the [VROC®](#) enables studying protein formulation stability during an early stage of drug development when materials are limited.

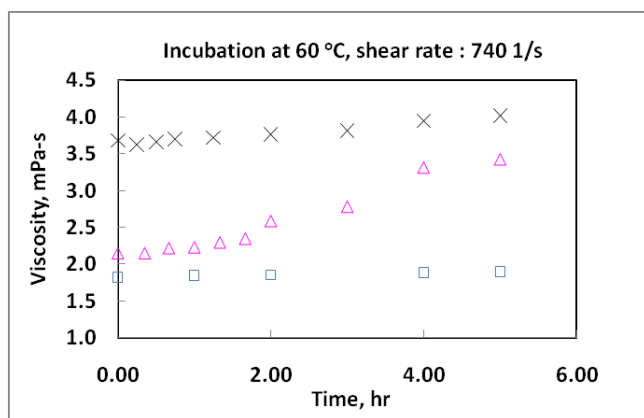
Protein stability is essential for drug efficacy and storage shelf life. When proteins become unstable, molecules unfold and aggregate. Aggregated proteins incur immune responses that cause serious side effects, which makes protein stability an integral part of protein formulation. Protein stability depends on temperature, pH, concentration, excipients (sucrose, etc.) and many other factors. For example, as the temperature increases, protein unfolds and loses secondary and tertiary 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 to study aggregation or unfolding. For 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® for Addition of Sucrose

Sucrose is known to increase the stability of Immune Globulin of Carimune® NF. To explore the effect of the addition of sucrose to bovine  $\gamma$ -globulin solution in PBS (pH = 7.4), two solutions were prepared. One solution consists of 100 mg/ml bovine  $\gamma$ -globulin in PBS; another solution consists of 100 mg  $\gamma$ -globulin in 1 ml PBS with 0.25 g of Sucrose. The solutions were incubated in water bath at 60 °C.

The change in the stability was monitored by measuring the viscosity of the samples. To detect a change in stability, a [VROC®](#) A05 chip with 50  $\mu$ m deep flow channel was employed. The results are illustrated in the following graph.



△	100 mg $\gamma$ -globulin in 1 ml PBS
X	100 mg $\gamma$ -globulin in 1 ml PBS and 0.25 g sucrose
□	1 g Sucrose added to 4 g PBS - control

Viscosities of  $\gamma$ -globulin solutions were measured as a function of incubation time. Each incubated sample was taken out of the bath and tested for viscosity at 25 °C. When the pure gamma globulin solution was incubated at 60 °C, viscosity started to increase within 40 minutes, which was followed by an accelerated viscosity increase.

This viscosity increase suggests that proteins unfolded and even aggregated. However, a very slow increase in viscosity was observed for the solution with sucrose; most likely, protein denaturation was significantly slowed as well. A very slight increase in viscosity was observed for 25 wt% glucose solution in PBS with incubation time due probably in part by water evaporation.

## Conclusions:

- 1 Protein denaturation can happen immediately or with a significant lag depending on temperature and other components.
- 2 Aggregated proteins started showing shear thinning behavior. The trend is the first time to be reported.
- 3 VROC® has shown that small increase in viscosity due to unfolding or denaturation can be detected.
- 4 VROC® 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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