RHEOSENSE. INC.

MULTI POINT VISCOSITY MEASUREMENTS OF PROTEINS

A complete guide to understanding what information can be obtained if more than a single point viscosity measurement is performed.

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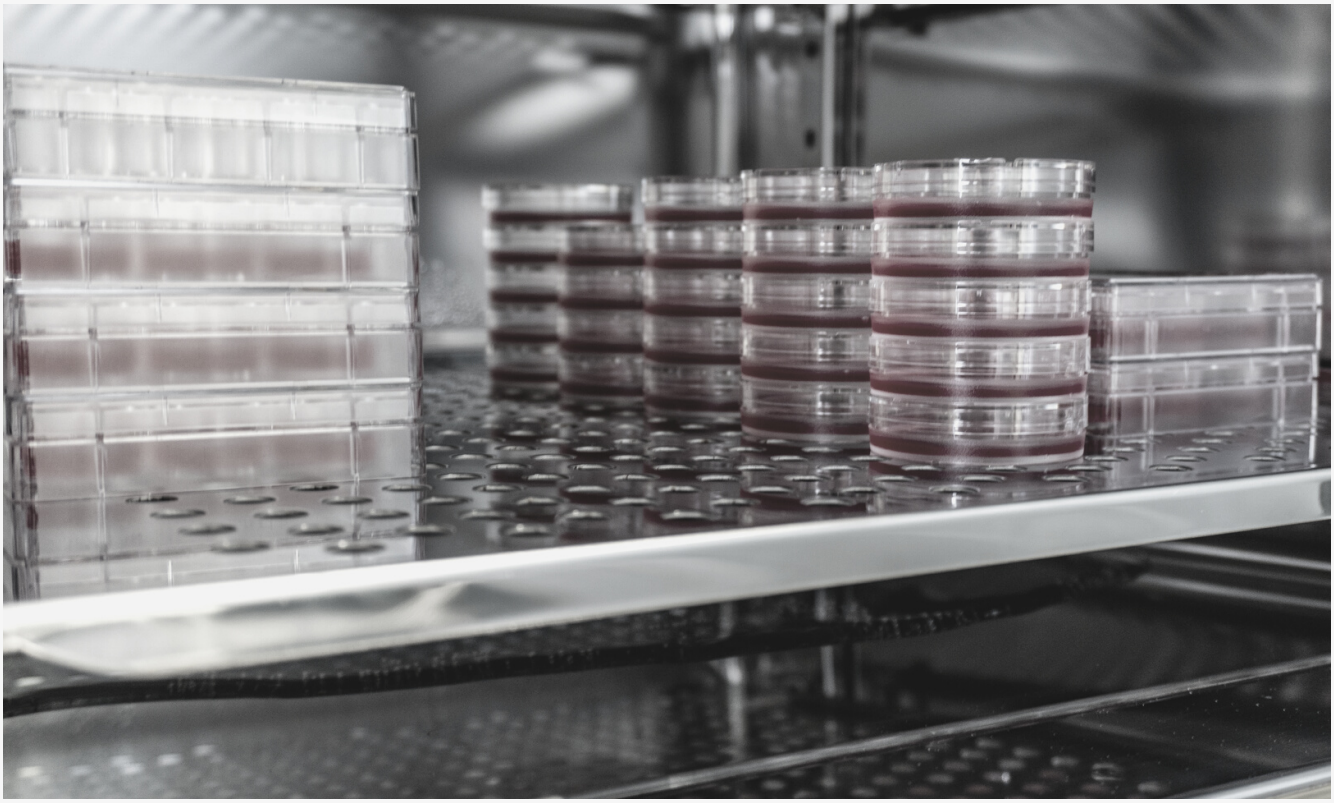
CONCENTRATED PROTEIN SOLUTIONS

CONCENTRATED PROTEIN SOLUTIONS ARE WIDELY USED IN BIOPHARMACEUTICAL RESEARCH AND DEVELOPMENT. PROTEIN-BASED DRUGS ARE THE FASTEST GROWING CLASS OF DRUGS FOR THE TREATMENT OF MANY DISEASES IN HUMANS, RANGING FROM CANCER TO ALZHEIMER'S DISEASE.

CONCENTRATED PROTEIN SOLUTIONS ARE COMPLEX FLUIDS CONTAINING PROTEINS, BUT ALSO SURFACTANTS, STABILIZERS, AND SALTS. THESE COMPONENTS ALL INTERACT WITH EACH OTHER AND CAN INFLUENCE SOLUTION VISCOSITY. VISCOSITY CONTROL FOR CONCENTRATED PROTEIN SOLUTIONS IS IMPORTANT FOR THE MANUFACTURABILITY AND DRUG DELIVERY ROUTES OF MANY PROTEIN THERAPEUTICS OF THE PHARMACEUTICAL INDUSTRY (ZHANG ET AL., 2017). THE RHEOLOGICAL BEHAVIOR OF PROTEIN SOLUTIONS WILL REFLECT DIFFERENCES IN PROTEIN SIZE AS WELL AS THE FORMATION OF COMPLEX STRUCTURES OR NETWORKS RESULTING FROM VARIOUS TYPES OF MOLECULAR INTERACTIONS.

MULTI-POINT VISCOSITY ANALYSIS



CHARACTERIZING CONCENTRATED PROTEIN SOLUTIONS WITH ONLY A SINGLE VISCOSITY VALUE IS INCOMPLETE.

Single point viscosity measurements of complex fluids can suffice for quality control or screening purposes if the shear rate is chosen carefully or if the formulation is confirmed Newtonian. However, it is advantageous to explore the shear rate dependence of protein solutions to understand solution stability, efficacy, and overall performance. Since rheological properties reflect protein formulation, protein-protein interactions, and the resulting microstructure or spatial arrangement of molecules, correlations between buffer composition and the molecular level behavior of the proteins can be made. A thorough viscosity analysis of protein formulations should include both shear rate and temperature variation. This data can be used for practical purposes such as performance prediction at any relevant temperature. It can also be utilized to investigate the component interactions and resulting microstructure.

COMMON MULTI-POINT VISCOSITY MEASUREMENTS

TYPE	APPLICATION
SHEAR RATE SWEEPS	Determine the impact of shear strength on the sample's microstructure with a shear rate sweep
TEMPERATURE SWEEPS	Determine the viscosity of a concentrated protein solution at temperatures relevant to preparation, storage, processing, and delivery
CONCENTRATIONS	Focus on the dilute regime to measure size and interactions between molecules
SCALING ANALYSIS	Extract relevant solution parameters, such as cluster size and gauge magnitude of competing stresses, time scales or length scales

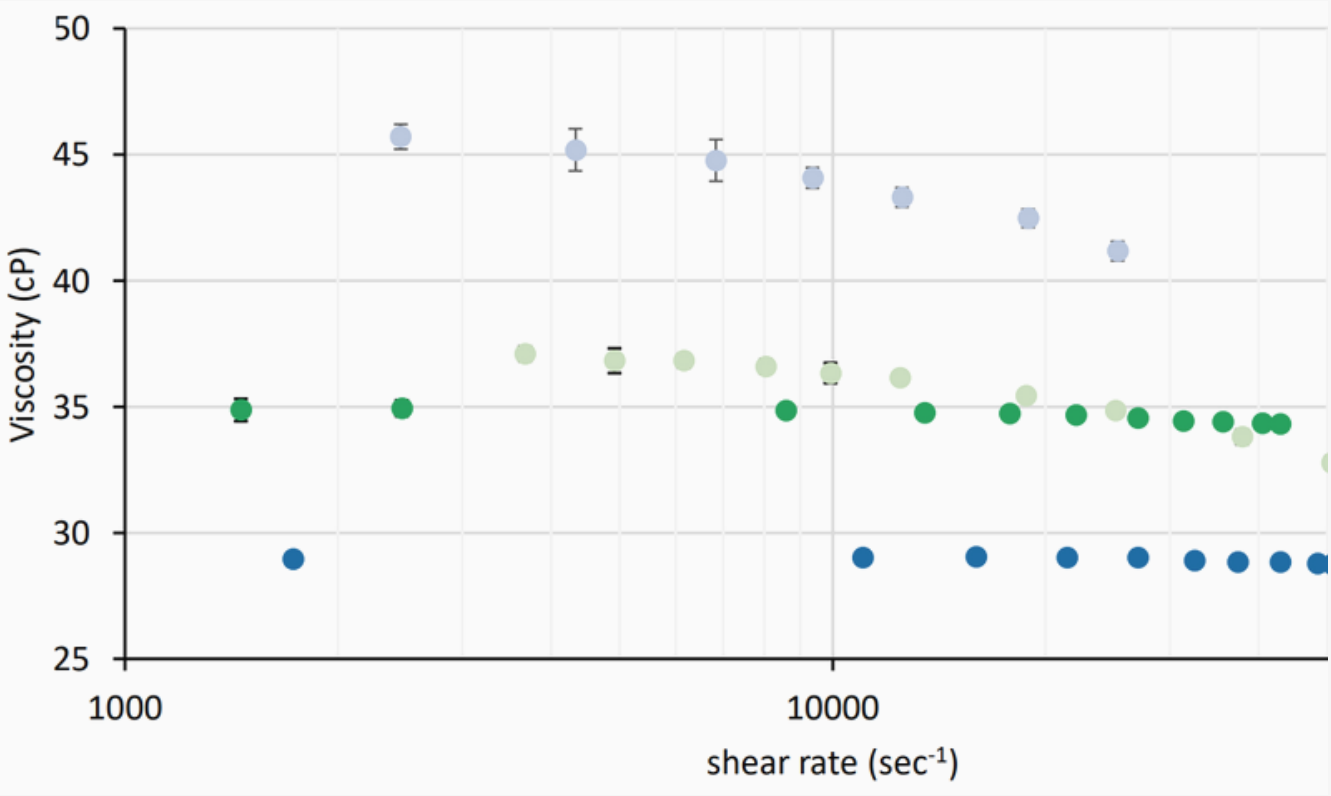
SALT TYPE AND IONIC STRENGTH

INTRODUCING SALTS AT LOW LEVELS CAN INCREASE A PROTEIN'S SOLUBILITY AND IS COMMONLY REFERRED TO AS "SALTING IN".

Both salt type and ionic strength impact the solubility of proteins. The molecular interactions influencing protein solubility are reflected in the formulation viscosity. Introducing salts at low levels can increase protein solubility. However, further increasing the salt content can enhance aggregation by dehydrating the protein. This "salting out" effect is most pronounced with anions that are strongly hydrated. The additional counter ions can also screen the electrostatic repulsion when the pH is not at the isoelectric point and the proteins have a net charge. These changes in the protein-protein interaction (PPI) and resulting structural arrangement will influence the solution viscosity as well as the solubility.

Learn More About Controlling the Viscosity of Concentrated Protein Solutions

SHEAR RATE DEPENDENT VISCOSITY



Changes in protein pair interactions induced by varying buffer composition significantly impact the shear rate dependent viscosity of concentrated protein solutions. Varying shear rate provides information on characteristic times scales of the formulation. Shear rate sweeps offer the opportunity to extract more detailed information about formulation components and microstructure, specifically to determine the onset of non-Newtonian behavior and assess the correlation of increased cluster size with increased plateau viscosity. Even a simple qualitative interpretation can be used to guide formulation development. The first approach to interpreting shear rate sweeps is qualitative. In the case of inherently attractive proteins that form reversible clusters or aggregates, one characteristic time scale of the solution would relate to the diffusion of these clusters. Brownian motion is one mechanism acting to resist the shear and restore equilibrium. Therefore, the critical transition point from Newtonian plateau to shear thinning regime should depend on the diffusion time of the clusters, which is proportional to their size.

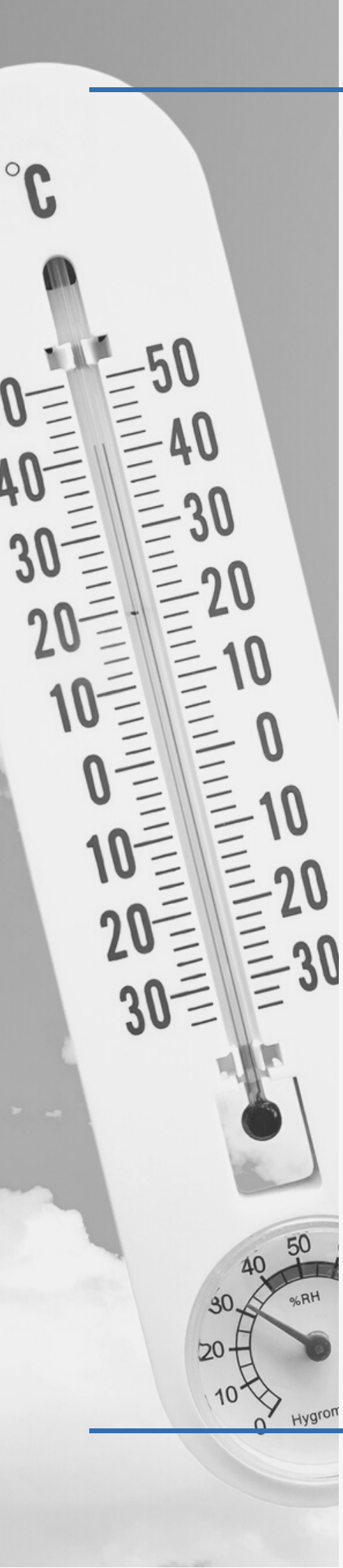
Learn More About Shear Rate Dependent Viscosity of Concentrated Protein Solutions

TEMPERATURE DEPENDENT VISCOSITY

CONCENTRATED PROTEIN SOLUTIONS ARE COMMONLY STORED AT REDUCED TEMPERATURES (E.G. 4˚C) TO PROLONG STABILITY AND ULTIMATELY INTRODUCED INTO THE BODY WHICH HAS AN AVERAGE TEMPERATURE OF 37˚C.

Temperature has a huge impact on viscosity. Water is a good example of this impact. When it gets colder, it starts to freeze and when it gets warmer, it starts to slowly boil. Every fluid sample has it's own characteristics and can display variations at different temperatures including temporary or permanent alteration of the sample. Concentrated protein solutions experience a broad range of temperatures from cold storage to body temperature or even higher during an accelerated aging study. Therefore, measuring viscosity at multiple temperatures relevant to the formulation process, manufacturing, storage, and delivery is critical to predict the ability to handle or process under all relevant conditions. A simple model fit to a few properly selected data points can be used to interpolate to any relevant temperatures related to processing or application.

Learn More About Temperature Dependent Viscosity



SHEAR RATE AND TEMPERATURE VARIATION

ARRHENIUS EQUATION

Typically, as temperature goes up, viscosity will go down. The Arrhenius equation is a formula for the temperature dependence.

$$\eta_o = Ae^{\frac{E_a}{RT}}$$

η_O is the low shear plateau viscosity, A an exponential pre-factor, E_O the activation energy, R the universal gas constant, and T the temperature. The Arrhenius law can be fit to a select number of data points and then used to interpolate as needed to predict behavior under a variety of conditions.

Explore more about Temperature Sweeps

PECLET NUMBER

Peclet number (Pe) is a dimensionless group representing the ratio of diffusion to convection.

$$Pe^* = \frac{\tau_B}{\tau_s} = \frac{6\pi\eta_o L^3\dot{\gamma}}{kT}$$

Pe* represents the ratio of characteristic time scales associated with Brownian motion (τ_B) and the shear flow (τ_s). In this expression k, is the Boltzmann constant, γ is the shear rate, and L is a characteristic length scale, which is equivalent to the hydrodynamic radius for non-attractive proteins mainly present in solution as individual molecules.

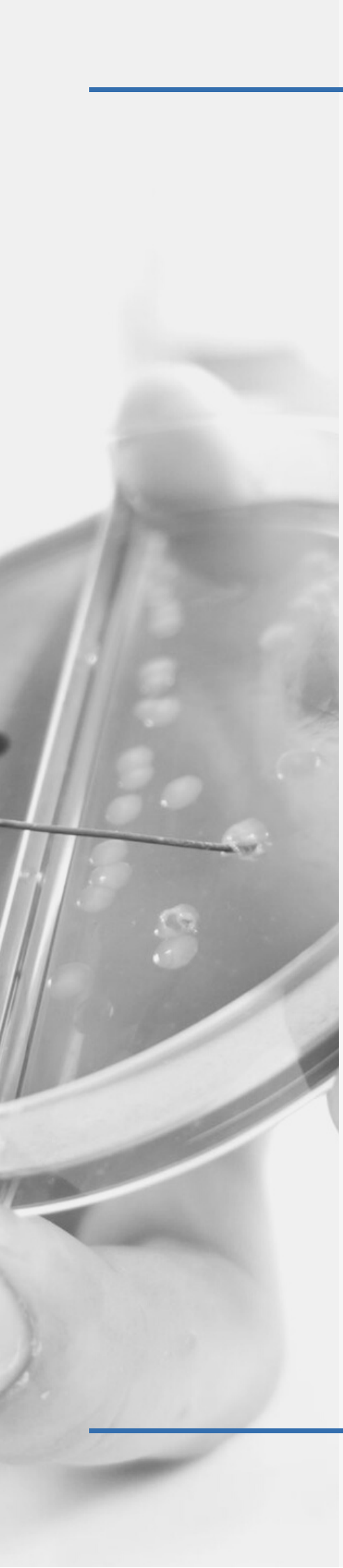
Since Brownian motion is a restorative mechanism maintaining a near equilibrium state and Newtonian plateau, the transition to non-Newtonian behavior occurs at the point where the shear flow begins to dominate over diffusion

Explore more about Shear Rate Sweeps

COLLOIDAL SCALING ANALYSIS

THE SCALING PROCESS ALSO YIELDED A CHARACTERISTIC LENGTH SCALE THOUGHT TO BE ASSOCIATED WITH THE SIZE OF PROTEIN CLUSTERS OR AGGREGATES (GODFRIN ET AL., 2016).

Changes in protein pair interactions induced by varying buffer composition significantly impact the shear rate dependent viscosity of concentrated protein solutions. Varying shear rate provides information on characteristic times scales of the formulation. Shear rate sweeps offer the opportunity to extract more detailed information about formulation components and microstructure, specifically to determine the onset of non-Newtonian behavior and assess the correlation of increased cluster size with increased plateau viscosity. Protein solution data is often interpreted by assuming that the behavior of the molecules is analogous to that of a colloid. Although an imperfect comparison due to the inherent anisotropy and nonuniformity of the soft proteins, it has proven reasonable based on their size and pair interaction magnitude.



COLLOIDAL SCALING OF PROTEIN SOLUTIONS

SHEAR RATE SCALING ANALYSIS

Applying a scaling analysis commonly used for traditional colloidal systems can be used on viscosity data collected for concentrated protein solutions to generate a master curve.

The scaling process also yields a characteristic length scale thought to be associated with the size of protein clusters or aggregates (Godfrin et al., 2016). The observed relationship between low shear plateau viscosity and the estimated cluster size is consistent with protein aggregate formation as the root cause of high viscosity protein formulations. Although the length scale is a relative ranking in the absence of complementary sizing measurements such as scattering techniques, it is a valuable tool to assess the impact of buffer variations on PPI and the resulting extent of aggregation.

TEMPERATURE SCALING ANALYSIS

Applying a scaling analysis to data gathered from shear rate sweeps at multiple temperatures can be used to determine if temperature variation produces changes in protein cluster formation. As temperature is increased, diffusion or thermal motion becomes more rapid. For simple fluids, such as solvents or buffers, this results in a decrease in dynamic viscosity. This trend generally holds true for many complex fluids. Exceptions do exist, such as samples with temperature induced phase separation or self-assembly such as Poloxamers. Temperature variation can also affect aggregation behavior of colloidal systems due to changes in the pair interaction potential. Since the colloidal model is often used as a framework to interpret protein solution behavior, it is of interest to investigate the impact of temperature on the cluster formation of inherently attractive proteins.

Learn More About non-Newtonian Viscosity Analysis of Concentrated Protein Solutions

CONSEQUENCES OF NOT MEASURING VISCOSITY

The long-term stability of pharmaceutical formulations containing proteins or antibodies is a continual concern. In general, these systems are kinetically, rather than thermodynamically stable. Meaning, the changes in these solutions can occur over extended periods of time and are dependent on the component interactions and mobility. Also, the conformation of the individual molecules can adjust with time which can in turn alter the protein-protein interactions (PPI) by hiding or exposing functional groups. Regardless of the scenario, protein formulations are dynamic. Viscosity serves as an ideal indicator of stability since it is highly sensitive to changes in the individual proteins, PPI, and the resulting microstructure.

Intrinsic viscosity is a reliable and sensitive indicator of a solute's molecular interaction with a solvent, and can be used to determine the molecular properties of a sample, such as:

- Molecular weight and size
- Polymerization
- Interaction of molecules
- Degradation
- Branching structure
- Stability of molecules: aggregation, denaturation, or conformational changes of protein molecules
- Protein structure and melting temperatures

RHEOSENSE CAN HELP!

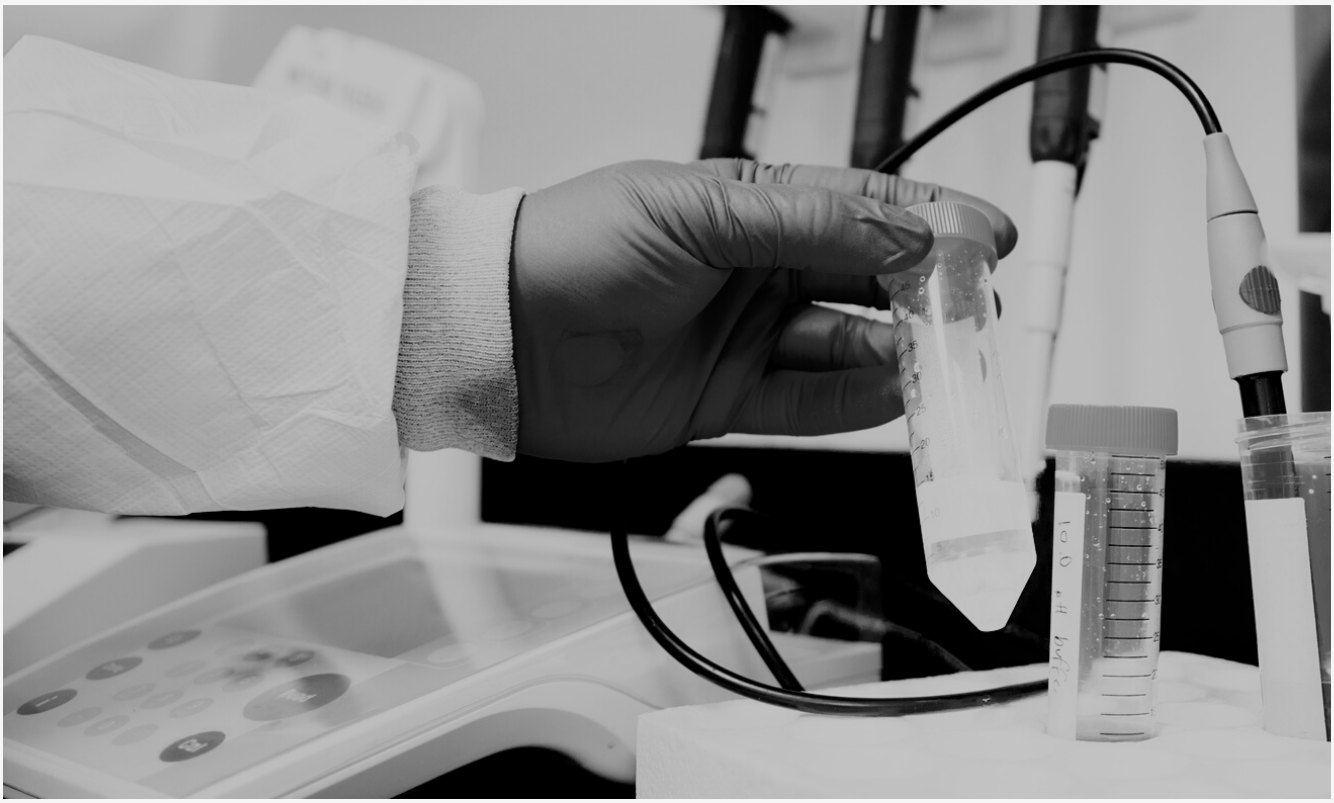
The key to accurately measuring intrinsic viscosity is repeatability. Our VROC powered viscometers offer exceptional repeatability (0.5% of reading), which ensures that you are able to quickly and easily obtain highly accurate (2%), reliable results using small samples. Our new VROC initium one plus is much more than a traditional viscometer; it is a workhorse for your material characterization.

RheoSense offers a variety of viscometers that measure viscosity under high-throughput capabilities under various shear rates. Your research and criteria is a uniquely tailored application, see which VROC® instrument is right for you.

- *microVISC™ - Portable, small sample viscometer*
- *m-VROC® - Small sample, controlled shear rate viscometer*
- *VROC® initium one plus - Fully automatic, small sample viscometer with controlled shear rate with sample retrieval*

CONCLUSION

A THOROUGH VISCOSITY ANALYSIS OF CONCENTRATED PROTEIN FORMULATIONS SHOULD INCLUDE BOTH SHEAR RATE AND TEMPERATURE VARIATION. THE DATA CAN BE USED FOR PRACTICAL PURPOSES SUCH AS PERFORMANCE PREDICTION AT ANY RELEVANT TEMPERATURE. IT CAN ALSO BE UTILIZED TO INVESTIGATE THE COMPONENT INTERACTIONS AND RESULTING MICROSTRUCTURE. SIMPLE ANALYSIS TOOLS SUCH AS MODEL FITTING AND SCALING CAN BE EMPLOYED TO FACILITATE BOTH OBJECTIVES.



Concentrated protein solutions are widely used in biopharmaceutical research and development. Protein-based drugs are the fastest growing class of drugs for the treatment of many diseases in humans,

The components making up concentrated protein solutions all interact with each other and can influence solution viscosity. The rheological behavior of protein solutions will reflect differences in protein size as well as the formation of complex structures or networks resulting from various types of molecular interactions. Viscosity measurements can be used to infer information about protein formulations and probe how formulation changes impact protein solutions.

RHEOSENSE IS WORKING ALONGSIDE BIOPHARMACEUTICAL COMPANIES AND SCIENCE RESEARCH ORGANIZATIONS TO ASSIST AND IMPROVE PROTEIN FORMULATION & DRUG DELIVERY. IF YOU HAVE ANY QUESTIONS OR INQUIRIES, VISIT US AT RHEOSENSE.COM OR EMAIL US AT INFO@RHEOSENSE.COM

CONTACT US!