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www.RheoSense.com ◦ info@RheoSense.com ◦ (925) 866-3808

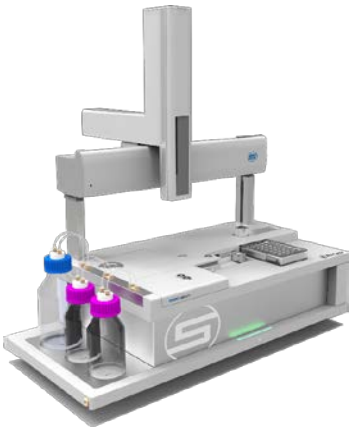
Optimization of Protein Formulation Complete Viscosity Characterization of γ-Globulin and Serum Albumin Solutions – Viscosity Fingerprinting and Analysis

Optimization of candidate monoclonal antibodies (mAb) is an essential step in the development of therapeutic formulations. Injectability is a key property required to assess the safety and efficacy of candidate mAb's. Viscosity of a formulation is the sole parameter that dictates injectability and is thus critically important to characterize during the development process. Highly accurate and reliable viscosity measurements are required during the optimization process.

Since very limited volumes of candidate formulations are normally available early in the development cycle, the ability to accurately and reliably measure viscosity in small samples is vital to success of the development program. In this note, we demonstrate that such accurate and repeatable viscosity measurements of model globular proteins are obtained with 0.5% RSD (relative standard deviation) or better for viscosity fingerprinting using the RheoSense VROC® initium.

Using a simple model, accurate estimates of viscosities of globular protein solutions over a wide concentration range are possible using only a clouding factor and the intrinsic viscosity measured at dilute concentrations. The model estimates the viscosities of bovine γ-Globulin and serum albumin solutions in PBS buffer very well. Measurement of viscosity with VROC® initium facilitates automated, high throughput collection of valuable viscosity data from multiple samples in each run.

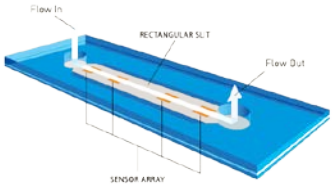
Material: Bovine γ-Globulin from Sigma Aldrich (Lot # SLBO4091V) was used as a model mAb. 10 mM Phosphate Buffered Saline was prepared by dissolving dry power from Sigma Aldrich (Lot 039K8200) in one liter of deionized water. Bovine serum albumin (BSA) obtained from Sigma Aldrich (Lot 060M1708V) was also used for this study.



VROC® initium offers unattended shear viscosity measurements with the smallest sample volume requirement and a wide dynamic operation range. High accuracy and repeatability makes it ideal for R&D and QC applications.

- Automated sample loading and cleaning
- 40 vial rack and 96 well plate compatible
- Smallest sample volume (12 µL)
- Accuracy: 2% of reading
- Repeatability: <0.5% of reading
- Shear Viscosity range: 0.2 – 1,000 mPa-s
- Shear Rate range: 0.5 -80,000 s^{-1}
- Temperature control: 4-70 °C

VROC® Technology and Principle of Operation



RheoSense's *Viscometer-Rheometer-on-a-Chip* (VROC®) combines microfluidic and MEMS technology to measure viscosity. As the test fluid is pumped through the channel at a controlled shear rate the MEMS pressure sensor array captures the pressure drop, which is proportional to the shear stress at the wall. The viscosity of the test fluid is obtained as the ratio of shear stress to shear rate.

2420 Camino Ramon, Suite 240 San Ramon, CA 94583
P: (925) 866-3801 F: (925) 866-3804

www.RheoSense.com ◦ info@RheoSense.com ◦ (925) 866-3808

Sample Preparation: Bovine γ-Globulin and serum albumin were measured and dissolved into PBS buffer at different concentrations.

Measurement: VROC® initium was used to test γ-Globulin solutions in PBS buffer using a measurement protocol that takes 12 measurements at a flow rate of 600 µL/min (or 11,786 1/s) with sample retrieval at 25 °C. γ-Globulin solutions at concentrations higher than 150 mg/mL were tested at a flow rate of 300 µL/min. The first four data points of each measurement were discarded due to possible air entrapment in the leading volume of solution. 100 µL of each sample was used, although the measurement protocol also works well with 50 µL sample volumes. For all measurements, %RSD is smaller than 0.5%.

Table 1 summarizes the intrinsic viscosity values and hydrodynamic radii of the γ-Globulin and BSA solution, which are used to calculate volume fraction. Tables 2 and 3 summarize the measured data of γ-Globulin and BSA solutions.

Table 1. Measured intrinsic viscosities and hydrodynamic radii of γ-Globulin and BSA solution.

	γ-Globulin	BSA
[η], mL/mg	0.0061	0.0042
Hydrodynamic radius, R_h (nm)	5.26	3.53
K_H	1.922	1.003

Results: Measured viscosities of γ-Globulin and BSA solutions are summarized in the tables below.

Table 2. Viscosities of γ-Globulin solutions in PBS buffer

C, mg/ mL	ϕ (vol. fraction)	η, mPa-s	%RSD	η_r	η_r, estimate
0	0.000	0.907	0.42	1.000	1.000
10.18	0.026	0.971	0.26	1.071	1.069
17.35	0.044	1.024	0.31	1.128	1.123
26.00	0.066	1.093	0.25	1.205	1.196
34.66	0.088	1.178	0.20	1.298	1.277
42.64	0.109	1.261	0.22	1.390	1.362
50.61	0.129	1.360	0.23	1.499	1.457
57.71	0.147	1.467	0.10	1.617	1.552
65.17	0.166	1.584	0.12	1.746	1.664
73.90	0.188	1.741	0.15	1.919	1.814
84.68	0.216	1.981	0.30	2.183	2.034
101.7	0.259	2.452	0.14	2.702	2.485
120.3	0.306	3.175	0.13	3.499	3.199
130.3	0.339	3.876	0.06	4.271	3.917
152.7	0.389	5.300	0.11	5.841	5.554
173.4	0.441	7.835	0.12	8.635	8.783

In the table, η_r, η_{sp}/C, and ϕ are calculated using the following equations:

$$\eta_r = \frac{\eta(solution)}{\eta(solvent)}, \quad \frac{\eta_{sp}}{C} = \frac{\eta_r - 1}{C}, \text{ and } \phi = \frac{4\pi}{3} R_h^{\,3} \frac{CN_a}{M}$$

Where R_h is the hydrodynamic radius, C is concentration, N_a is Avogadro's number, and M is the molecular weight. The hydrodynamic radius is calculated from the intrinsic viscosity, [η] using the following equation for an apparent spherical shape:

$$R_h = \sqrt[3]{\frac{3[\eta]M}{10\pi N_a}}$$

Table 3. Viscosities of BSA solutions in PBS buffer

C, mg/ mL	ϕ (vol. fraction)	η, mPa-s	%RSD	η_r	η_r, estimate
0	0.000	0.91	0.56	1.000	1.000
9.60	0.016	0.947	0.36	1.041	1.041
19.03	0.032	0.99	0.43	1.088	1.085
28.08	0.047	1.032	0.35	1.134	1.129
38.42	0.064	1.08	0.38	1.187	1.183
51.67	0.087	1.147	0.19	1.260	1.257
58.42	0.098	1.189	0.48	1.307	1.298
67.22	0.113	1.261	4.25	1.386	1.354
78.67	0.132	1.318	0.93	1.448	1.432
88.93	0.149	1.389	0.38	1.526	1.508
99.77	0.167	1.463	0.27	1.608	1.595
120.8	0.202	1.638	0.39	1.800	1.785
137.1	0.230	1.791	0.16	1.968	1.956
157.5	0.264	2.01	0.79	2.209	2.203
173.3	0.291	2.203	0.07	2.421	2.428
199.5	0.334	2.567	0.19	2.821	2.877

As shown in the Tables, relative standard deviations of almost all measurements are less than 0.5%, suggesting that viscosity measurements are highly repeatable. Smaller RSD values were typically achieved for higher viscosity solutions.

Figure 1 below shows the measured relative viscosity versus protein concentration. As expected, viscosity increases rapidly as concentration increases.

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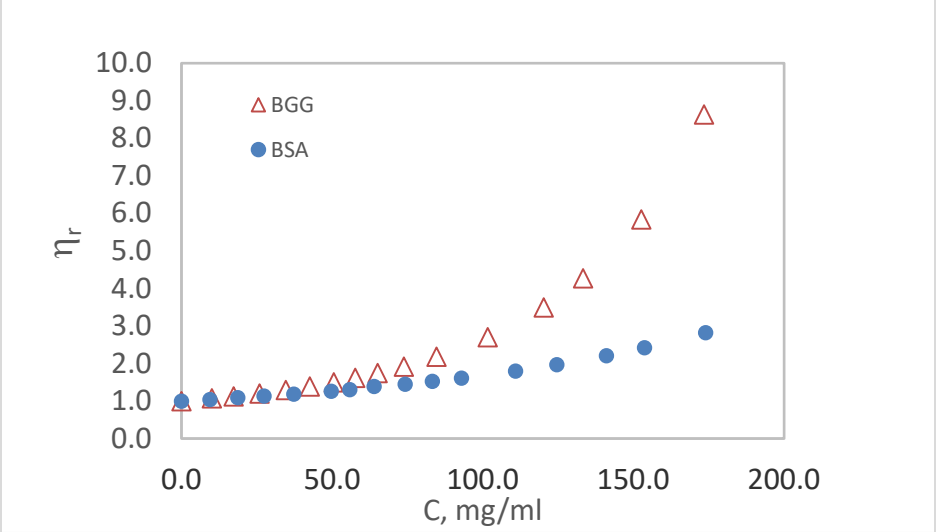

Figure 1. Relative viscosity of γ-Globulin and BSA solutions in PBS buffer.

Alternatively, relative viscosity can be plotted against the volume fraction (ϕ) of the protein molecules in the solution. In Figure 2, relative viscosities of γ-Globulin and BSA solutions are plotted against the volume fraction and compared with Batchelor's estimation of viscosities based on hard spheres with hydrodynamic interaction.

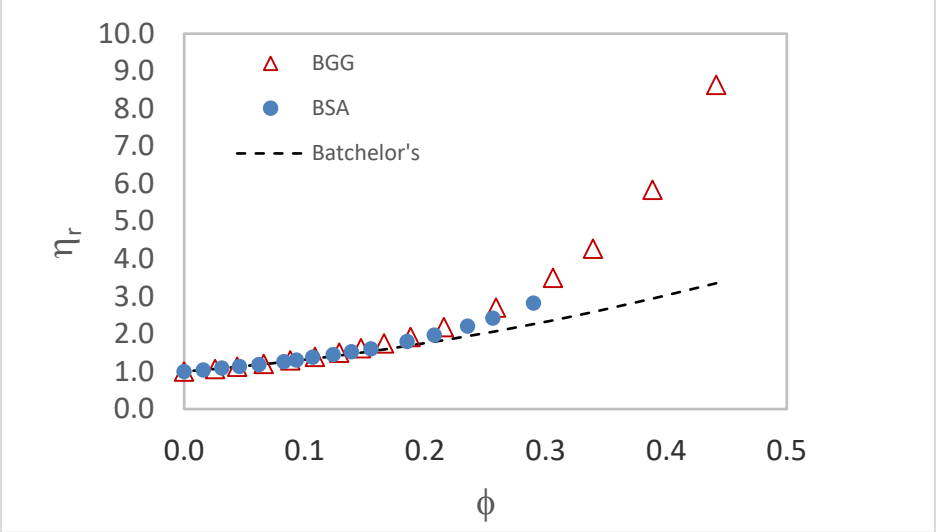

Figure 2. Relative viscosity vs. volume fraction of protein molecules.

As shown, BSA solution viscosity agrees well with the Batchelor's prediction up to a volume fraction of 0.3, whereas viscosity of γ-Globulin solution deviates from the prediction at a lower volume fraction of ~0.1. The discrepancy between γ–Globulin solution and Batchelor's prediction [Reference 1] motivates an attempt to correlate the measured data with another model in the appendix.

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Conclusions:

VROC® initium measures viscosity of protein solutions accurately and with high repeatability. RSD is typically below 0.5%. Unattended viscosity measurement of many samples increases throughput for efficient optimization of formulation. Each data set was acquired overnight during an automated run.

Reference 1: Batchelor, G. K. (1977). The effect of Brownian motion on the bulk stress in a suspension of spherical particles. *J. Fluid Mech*. 83, 97-117.

If you have questions regarding viscosity measurement or need more information about VROC initium or other applications, please contact us:

Main Office — 1 925 866 3801; Information — info@RheoSense.com Sales — Sales@RheoSense.com

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Appendix:
To characterize the interaction between protein molecules and medium through hydrodynamic radius, the intrinsic viscosity is measured for protein solutions within a dilute concentration regime. However, one can extend the same concept to intermediate concentrations. When additional protein molecules are added to an existing protein solution at concentration C, what happens to viscosity? Does the solution viscosity increase at different rate? To explore this question, apparent intrinsic viscosity, $[\eta]_c$ is defined [Reference 2]:

$$[\eta]_c = \frac{1}{\eta}\frac{d\eta}{dC} = \frac{1}{\eta_r}\frac{d\eta_r}{dC}$$

The apparent intrinsic viscosity quantifies how viscosity increases with increased concentration over the viscosity of the solution at the concentration of interest. For C = 0, the equation coincides with the intrinsic viscosity, which quantifies how much the viscosity increases with addition of proteins over the viscosity of solvent.
To calculate the apparent intrinsic viscosity for γ-Globulin and BSA solutions, 6^{th} polynomial regression was applied to obtain the best fit curves of measured viscosities in Tables 2 and 3. The curves were then differentiated to calculate the slope, dη/dC. From the slope and viscosity value at each concentration, the apparent intrinsic viscosity is calculated and plotted in Figure A1 and A2 for γ-Globulin and BSA solutions.

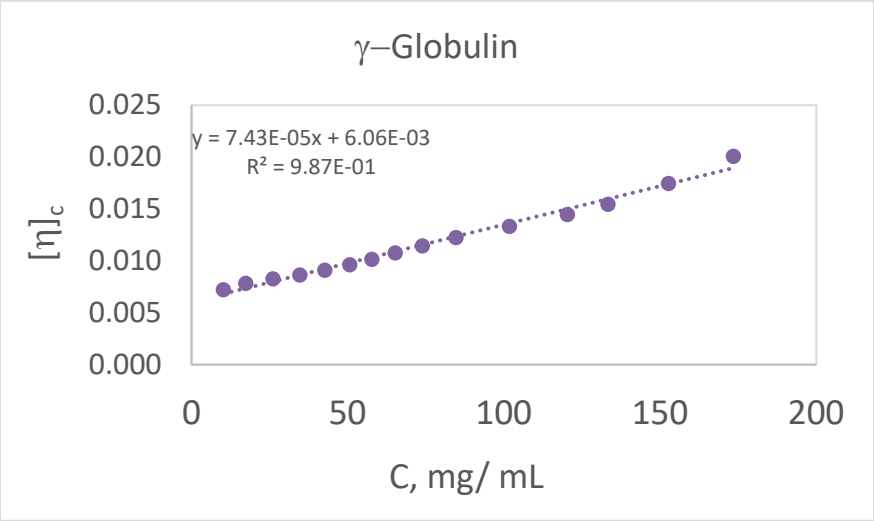

Figure A1. The apparent intrinsic viscosity vs. C for γ-Globulin solutions

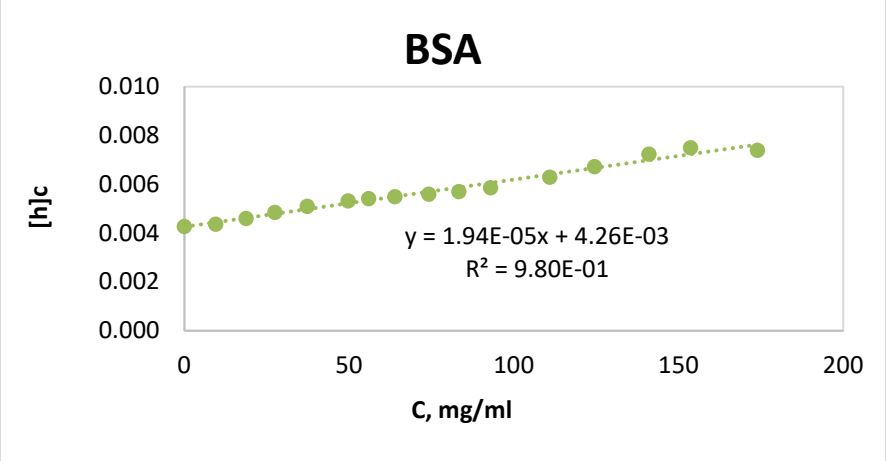

Figure A2. The apparent intrinsic viscosity vs. C for BSA solutions.

As shown in the graphs, the apparent intrinsic viscosity is proportional to the first order of the concentration within measurement error, suggesting that viscosity is following an exponential function of concentration as follows:

$$\eta_r = \exp(C(k_1 C + k_2))$$

The exponential function of C can be converted into the Ross and Minton equation, which approximates the viscosity behavior of concentrated *non-interacting* spherical particles where primary contribution to solution viscosity is from the excluded volume [Reference 3]:

$$\eta_r = \exp\left(\frac{[\eta]C}{1-\frac{k}{\nu}[\eta]C}\right)$$

Where k denotes the self-crowding factor and v is the Simha shape parameter. By applying the viscosity data in Tables 2 and 3, one can calculate the best fit value of k/v. Table A1 summarizes calculated best fit values for γ-Globulin and BSA solutions.

Table A1. Parameter (k/v) values of γ-Globulin and BSA solutions in PBS buffer.

	γ-Globulin solutions	BSA solutions
k/v	0.441	0.415

Figures A3 and A4 compare the measurement data with calculated values based on the Ross and Minton equation using the parameter values in Table A1 for γ-Globulin and BSA solutions.

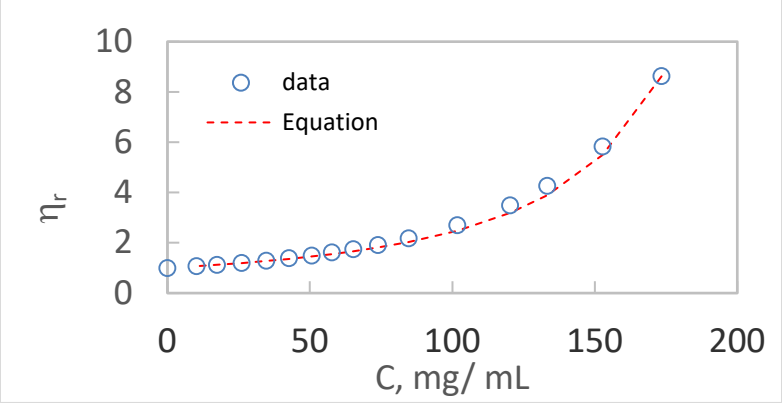

Figure A3. Comparison of the measured data with calculated values using the equation for γ-Globulin solutions.

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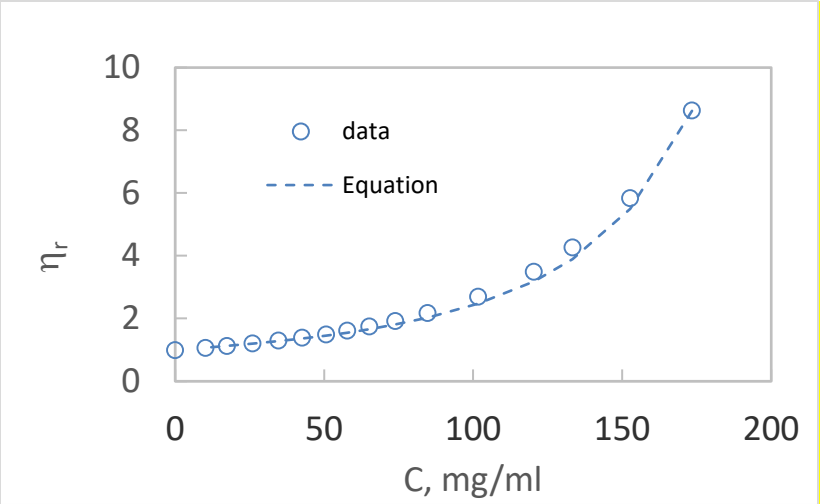

Figure A4. Comparison of the measured data with calculated values using the equation for BSA solutions.
For both protein solutions, the Ross and Minton equation estimates viscosity of concentrated protein solutions well, as shown in the η_r estimate columns in Tables 2 and 3. Provided that k/v parameter values are known a priori, this approach can accurately predict viscosity of concentrated protein solutions.

Reference 2: Weissenberg, S. G. et al. Viscosity of dilute and moderately concentrated polymer solutions. J. Research of the National Bureau of Standards, Vol. 47, No. 4, October 1951, 298-314
Reference 3: Ross P.D. and Minton AP. Hard Quasispherical model for the viscosity of hemoglobin solutions. Biochem Biophys Res Commun. 1977; 76:971-6.

Get more information about VROC® initium from our product webinars:
http://www.rheosense.com/viscosity-webinars

Contact Information

If you have questions, please contact us:
Main office — 1 925 866 3801
Information — info@RheoSense.com
Sales — sales@RheoSense.com