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RESEARCH PAPER

Intermolecular Interactions and the Viscosity of Highly Concentrated Monoclonal Antibody Solutions

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ABSTRACT

Purpose The large increase in viscosity of highly concentrated monoclonal antibody solutions can be challenging for downstream processing, drug formulation, and delivery steps. The objective of this work was to examine the viscosity of highly concentrated solutions of a high purity IgG_1 monoclonal antibody over a wide range of protein concentrations, solution pH, ionic strength, and in the presence / absence of different excipients.

Methods Experiments were performed with an IgG_1 monoclonal antibody provided by Amgen. The steady-state viscosity was evaluated using a Rheometrics strain-controlled rotational rheometer with a concentric cylinder geometry.

Results The viscosity data were well-described by the Mooney equation. The data were analyzed in terms of the antibody virial coefficients obtained from osmotic pressure data evaluated under the same conditions. The viscosity coefficient in the absence of excipients was well correlated with the third osmotic virial coefficient, which has a negative value (corresponding to short range attractive interactions) at the pH and ionic strength examined in this work.

Conclusions These results provide important insights into the effects of intermolecular protein-protein interactions on the behavior of highly concentrated antibody solutions.

KEY WORDS antibody · protein interactions · rheology · virial coefficients · viscosity

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ABBREVIATIONS

BSA Bovine serum albumin
IgG Immunoglobulin G

INTRODUCTION

Monoclonal antibodies are typically delivered in very highly concentrated solutions to achieve the desired dosage (mg antibody per kg patient body weight) in the limited volumes that can be delivered by subcutaneous injection (1). The high viscosity of these solutions can have a significant impact on both the delivery and purification of the monoclonal antibody product (1). There have thus been a number of prior studies focused on understanding the key factors controlling the viscosity of highly concentrated solutions of different monoclonal antibodies.

Liu et al. (2) investigated the effects of salt concentration and solution pH on the viscosity of a humanized IgG_1 monoclonal antibody using both capillary and cone-and-plate rheometers. The viscosity of a dilute (10 g/L) solution behaved like a Newtonian fluid over shear rates between 0.1 and 10,000 s^{-1}, but data with a 200 g/L solution in a 25 mM histidine buffer at pH 6 with 435 mM sucrose showed significant shear-thinning behavior over the entire range of shear rates. Data were obtained in the presence of a surfactant (polysorbate 80), suggesting that the shear-thinning was not due to the air-liquid interface. The viscosity of a 125 g/L solution in a 16 mM histidine buffer with 266 mM sucrose decreased by more than a factor of 8 as the NaCl concentration was increased from 0 to 200 mM due to shielding of the net repulsive electrostatic intermolecular interactions. The viscosity in the absence of NaCl (in the presence of acetate or arginine) showed a sharp maximum around pH 5.8, which was well below the protein isoelectric point. The authors attributed this behavior to the presence of net attractive electrostatic interactions leading to

reversible self-association; this behavior was not seen with other monoclonal antibodies having very similar structure.

Chari et al. (3) examined the viscosity of an IgG_2 antibody using high frequency rheology. In contrast to the results obtained by Liu et al. (2), the viscosity reached its maximum value around the protein isoelectric point (pH 9). Model calculations indicated that the rheological behavior was dominated by long-range net repulsive interactions at low protein concentrations but that short range attractive interactions (e.g., dipole-dipole attraction) became important at high protein concentrations.

Saito et al. (4) obtained data for the viscosity of three humanized IgG_1 monoclonal antibodies over a range of pH using a microfluidic Rheosense viscometer. The viscosity of two antibodies increased monotonically with increasing solution pH (from pH 5 to 8 in a 10 mM acetate or phosphate buffer with 140 mM NaCl), while the viscosity for the third antibody showed a significant decrease with increasing pH. The viscosity data for all 3 antibodies were well correlated with independent measurements of the second virial coefficient (B_2). However, the reduction in viscosity with increasing values of B_2 is exactly opposite of that predicted using available theories for the viscosity of concentrated colloidal dispersions (5), all of which predict that an increase in repulsive interactions (as described by the second virial coefficient) should lead to an increase in the effective size of the colloidal particles and thus an increase in viscosity. Sarangapani et al. (6) discussed the limitations of colloidal descriptions of the viscosity of concentrated protein solutions in the context of the behavior of bovine serum albumin, specifically highlighting the effects of surface hydration, surface charge distribution, and conformational changes.

Yadav et al. (7) examined the effects of charge distribution on the viscosity of a series of monoclonal antibody variants with different surface charge in a histidine buffer at pH 6.0. Antibodies with more non-uniform surface charge had higher viscosity, which the authors attributed to the increased intermolecular attraction between oppositely charged "patches" on different antibodies. The results were analyzed in terms of the second virial coefficient (determined from light scattering measurements), although there was no direct correlation between these parameters. The authors explained the behavior qualitatively in terms of the different effects of intermolecular interactions on self-association and irreversible aggregation, both of which affect the solution viscosity.

Despite the previous work in this area, there are still considerable uncertainties over the key factors controlling the viscosity of highly concentrated solutions of monoclonal antibodies. The objective of this work was to examine the viscosity of highly concentrated solutions of an IgG_1 antibody over a wide range of protein concentrations, solution pH, salt concentrations, and the presence / absence of different excipients. The viscosity results were analyzed in terms of the antibody virial coefficients (both B_2 and B_3 as evaluated from osmotic pressure data) to obtain additional insights into the effects of intermolecular protein-protein interactions on the behavior of these highly concentrated antibody solutions.

MATERIALS AND METHODS

All experiments were performed using a highly purified IgG_1 monoclonal antibody provided by Amgen with molecular weight of 142 kDa and isoelectric point of 8.1. The antibody was stored at −80°C and slowly thawed prior to use. The antibody was placed in the desired buffer by diafiltration through fully retentive Ultracel^{TM} composite regenerated cellulose membranes with 30 kDa nominal molecular weight cutoff (Millipore Corp., Bedford, MA). Data were also obtained by adding sucrose (Sigma, S-2395), L-proline (SPECTRUM, P1434), or arginine-HCl (JT Baker, 2067–06) as excipients of USP grade to the protein sample after diafiltration. Protein solutions were kept at 4°C for up to a week; solutions used for longer periods of time were kept at −30°C. More details on sample preparation were reported previously (8).

The steady-state viscosity (ratio of shear stress to shear rate) was evaluated using a Rheometrics Fluids Spectrometer (RFS II, strain-controlled rotational rheometer) with a concentric cylinder geometry having an inner diameter of 1.65 cm, an outer diameter of 1.71 cm, and height of 1.37 cm. The RFSII has two transducers with different torque sensitivity that provide reliable viscosity measurements from about 1 mPa.s to more than 100 mPa.s.

The rheometer was initially calibrated using a series of Newtonian standards (oils and water) of known viscosity. The sample temperature was maintained at 25 ± 1°C using a circulating water bath surrounding the outer cylinder; the temperature was monitored using a thermocouple connected to the inner cylinder. The sample holder and inner cylinder were thoroughly washed with deionized water and completely dried prior to each experiment. 1 mL of the antibody solution was carefully loaded into the sample holder, taking extra care to avoid any bubble formation. The shear stress was then measured over a range of shear rates from 10 to 1000 s^{-1} with two data points typically taken per decade. Most experiments were performed with continually increasing shear rate; limited data were obtained with decreasing shear rate to verify that there was no hysteresis or shear-induced changes to the protein.

The antibody concentration for each sample was measured using the UV absorbance at 280 nm determined using a NanoDrop spectrophotometer both before and after each experiment. Measurements were typically within 1%, with the largest absolute deviation being 4–5 g/L at the highest protein concentrations (>200 g/L). In addition, size exclusion chromatography with a Superdex 200 column was performed on

select samples to evaluate the fraction of antibody dimers and higher order oligomers. In most cases, the dimer peak was undetectable, and there was no evidence of any higher order oligomers in any of the samples.

The net protein charge in the solutions with different pH and ionic strength was evaluated by electrophoretic light scattering at constant voltage using a Malvern Zetasizer Nano (Worcestershire, UK). The measured electrophoretic mobility, determined from at least 20 runs using 5 repeat measurements, was used to evaluate the protein zeta potential and the net charge assuming that the antibody was a uniformly charged sphere. Additional details were reported previously (8).

RESULTS

Monoclonal Antibody Viscosity

Typical data for the viscosity (η) of the monoclonal antibody solution in a pH 5 acetate buffer with 20 mM NaCl at different antibody concentrations are shown in Fig. 1. In each case, the viscosity was independent of the shear rate over the range from 10 to 1000 s^{-1}, indicating that the antibody solution was Newtonian over this range of conditions. This behavior is consistent with recent data by Castellanos et al. (9), who attributed the non-Newtonian behavior seen in other studies using rotational rheometers to the effects of the air-liquid interface at the top of the cylinder. The RFS II rheometer used in this work has a cylinder length of 1 cm; thus, the air-liquid interface would not be expected to cause a significant perturbation in the measured (average) shear stress at the relatively high shear rates examined in this work. In addition, limited experiments were performed in the presence of 10% Triton X-100, a non-ionic surfactant that has been used previously to minimize interfacial viscosity. The measured viscosity in the presence of the Triton X-100 was within 10% of that for the pure antibody solution under otherwise identical conditions, indicating that the results obtained with the RFS II rheometer reflect the true bulk viscosity of the antibody solution.

There was also no evidence of any hysteresis; data obtained with increasing and decreasing shear rate (not shown) were indistinguishable. The viscosity increases significantly with increasing antibody concentration, going from around 6 mPa.s at an antibody concentration of 140 g/L to 80 mPa.s at 270 g/L.

The effect of the antibody concentration on the relative viscosity, η/η_0 where η_0 is the viscosity of the protein-free buffer, is examined in Fig. 2. The data at pH 5 were obtained in a 5 mM acetate buffer while a 5 mM phosphate buffer was used for the results at pH 6 and 7, both with 20 mM NaCl. The solid and dashed curves are model fits as described in the next section. The viscosity at any given antibody concentration increases with increasing pH, particularly at high protein concentrations. This effect was quite significant, with the viscosity at pH 7 being nearly double that at pH 5.

Figure 3 shows the effect of solution ionic strength, adjusted by the addition of NaCl, on the antibody viscosity in a 5 mM acetate buffer at pH 5. The relative viscosity increases with increasing NaCl concentration at all 3 concentrations,

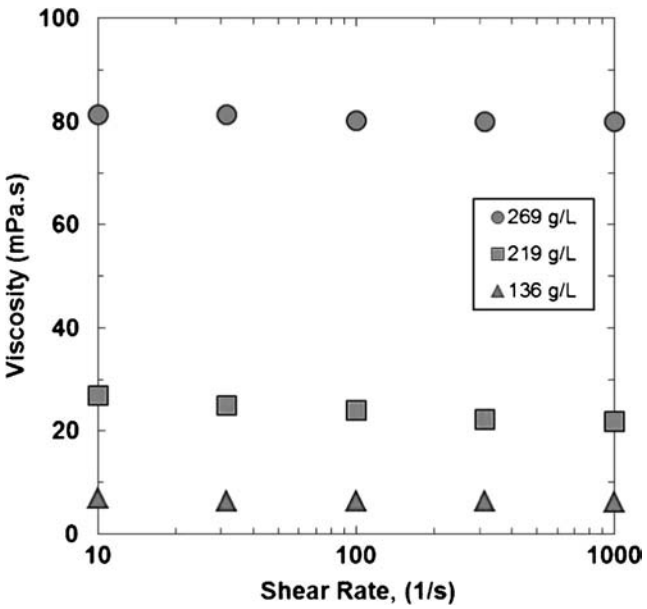

Fig. 1 Viscosity as a function of shear rate for the IgG_1 monoclonal antibody in a 5 mM acetate buffer at pH 5 with 20 mM NaCl.

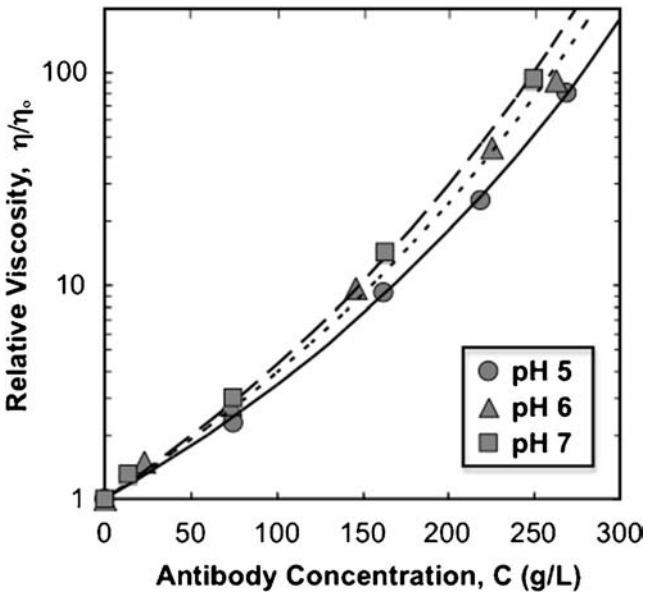

Fig. 2 Relative viscosity as a function of the antibody concentration at several pH values. Data at pH 5 were in an acetate buffer while for pH 6 and 7 a phosphate buffer was used, all with 20 mM NaCl. Solid and dashed curves are model calculations using Eq. (3) with the best fit values of b and $C_{max} = 800$ g/L.

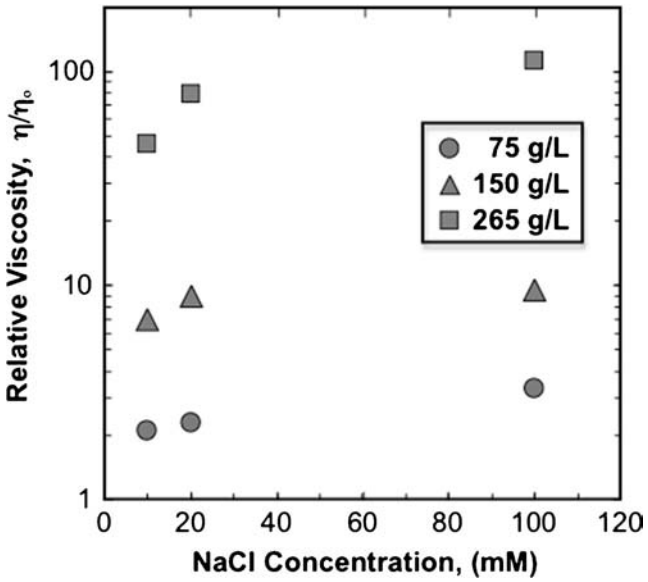

Fig. 3 Relative viscosity of the antibody solution as a function of added NaCl concentration for experiments performed using a 5 mM acetate buffer at pH 5.

suggesting that the shielding of the electrostatic interactions causes the observed increase in viscosity.

The behavior seen in Figs. 2 and 3 is exactly opposite to that predicted by classical models for the rheology of colloidal dispersions (5):

$$\frac{\eta}{\eta_0} = 1 + 2.5\varphi + \left[2.5 + \frac{3}{40}\left(\frac{d_{eff}}{2a}\right)^5\right]\varphi^2 + O(\varphi^3) \qquad (1)$$

where φ is the protein volume fraction (proportional to the protein concentration), a is the protein radius (treated as a sphere), and d_{eff} is the effective diameter of the protein accounting for a square well repulsive potential. Detailed expressions for the higher order term (e.g., of order φ^3) for charged spheres are not currently available. d_{eff} is expected to decrease with increasing pH due to the reduction in net charge of the monoclonal antibody, which varies from $Z=14$ at pH 5 to $Z=6$ at pH 6 and $Z=3$ at pH 7 (as determined from the measured values of the electrophoretic mobility (8)). d_{eff} is also expected to decrease with increasing ionic strength due to the reduction in the thickness of the electrical double layer. These phenomena are discussed in more detail subsequently.

Model Correlations

A number of different theoretical models and empirical correlations have been used in the literature to describe the behavior of concentrated protein solutions. Connolly et al. (10) used a simple exponential model to describe the viscosity for a range of monoclonal antibodies:

$$\frac{\eta}{\eta_0} = \exp(kC) \qquad (2)$$

where k is an empirical coefficient. Although Eq. (2) is qualitatively consistent with the data in Fig. 2, the results at high protein concentrations show significant upward curvature on the semi-log plot. More accurate fits to the data were developed using the Mooney equation (11), which was also used by Ross and Minton (12) to describe the viscosity of concentrated hemoglobin solutions:

$$\frac{\eta}{\eta_0} = \exp\left[\frac{bC}{1-\left(\frac{C}{C_{\max}}\right)}\right] \qquad (3)$$

where b is related to the intrinsic viscosity and C_{max} provides a measure of crowding and steric constraints in the highly concentrated protein solutions. Equation (3) was developed based on an excluded volume model, without any direct consideration of long range (e.g., electrostatic) interactions.

In order to verify the appropriateness of Eq. (3) for describing the viscosity results obtained with the monoclonal antibody solutions, the data in Fig. 2 were re-plotted in Fig. 4 in a linearized form as:

$$\frac{1}{\ln\left(\frac{\eta}{\eta_0}\right)} = \left(\frac{1}{b}\right)\frac{1}{C} - \frac{1}{bC_{\max}} \qquad (4)$$

The data at pH 5 are highly linear when plotted in this fashion, with $r^2>0.99$, with the best fit values of the slope and intercept giving $b=0.0108\pm0.0010$ L/g and $C_{max}=640\pm90$ g/L.

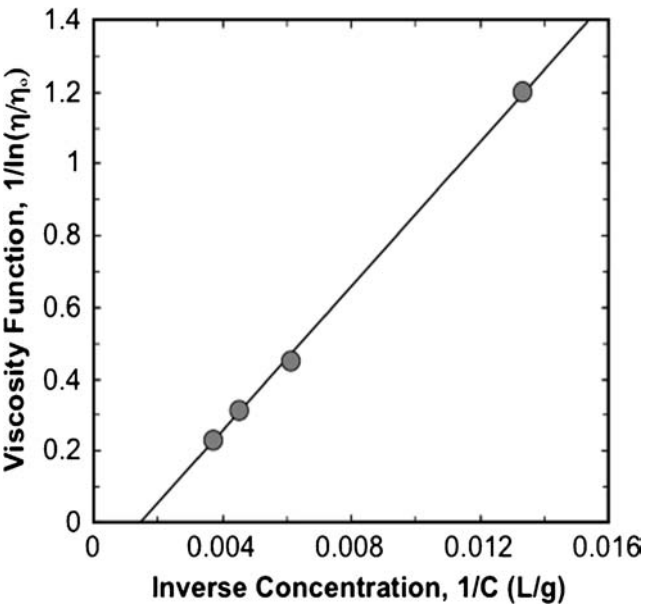

Fig. 4 Linearized plot of the viscosity data in a 5 mM acetate buffer with 20 mM added NaCl at pH 5. *Solid line* is linear regression fit using Eq. (4).

The viscosity data were all analyzed using Eq. (3) with the best fit values of the model parameters determined by non-linear regression using Mathematica Version 9.0. The high degree of coupling between b and C_{max} made it difficult to accurately determine both of these parameters from the available data, leading to very high error bars (95% confidence intervals). However, all of the fitted values of C_{max} were similar, with no clear dependence on solution pH or ionic strength. Thus, the data were re-fit using the average value of C_{max}= 800 g/L as determined from multiple data sets, with the best fit values of the parameter b then determined for each set of experimental conditions by non-linear regression. The solid and dashed curves in Fig. 2 are the resulting model calculations with b=0.0107, 0.0119, and 0.0126 L/g for pH 5, 6, and 7, respectively. The model fits are in very good agreement with the experimental data over the full range of antibody concentrations, providing further support for the use of Eq. (3) to analyze the viscosity of these highly concentrated monoclonal antibody solutions.

Virial Coefficients

The best fit values of the viscosity parameter b for the different buffer conditions are summarized in Table I along with results for the second and third virial coefficients determined for the same monoclonal antibody in the same buffers from osmotic pressure data obtained up to protein concentrations of 260 g/L (8). The viscosity parameter b increases with increasing pH and salt concentration, while the second virial coefficient decreases over the same range of parameters. This relationship between the viscosity and the second virial coefficient for monoclonal antibody solutions has been reported previously by Saito et al. (4) and Connolly et al. (10). However, the inverse correlation seen in Table I, and in the studies by Saito et al. and Connolly et al., is inconsistent with theoretical descriptions of colloidal systems which show that a net repulsive inter-particle potential produces a stress that causes a corresponding increase in the solution viscosity and the second virial coefficient through its effect on the potential of mean force.

The importance of reversible self-association on the behavior of highly concentrated solutions of monoclonal antibodies has been reported in a number of previous studies (2–4). These associations are due to local (short-range) attractive interactions that appear to dominate in very highly concentrated solutions despite the presence of long-range electrostatic repulsion caused by the overall net positive charge of the antibody. Binabaji et al. (8) discussed the importance of these local attractive interactions on the osmotic pressure, resulting in significant negative values of the third virial coefficients (B_3) as seen in Table I. These local interactions are likely electrostatic in origin, e.g., those associated with dipole-dipole attraction, as indicated by the significant decrease in the absolute value of B_3 with increasing ionic strength.

The results in Table I show a direct correlation between b and B_3, with both parameters increasing with increasing pH and salt concentration. In addition, the b value in the pH 5, 100 mM NaCl solution lies between the values in the pH 6 and pH 7 solutions (both with 20 mM NaCl), which is consistent with the very similar values of the third virial coefficient in these buffers.

The relationship between b and B_3 is examined more explicitly in Fig. 5 for experiments performed over a range of solution pH and NaCl concentration. The results are highly linear with r^2=0.97, suggesting that an increase in local attractive intermolecular electrostatic interactions (more negative values of the third virial coefficient) leads to a reduction in the viscosity of the antibody solution (smaller value of the viscosity parameter). The local attractive interactions can be thought of as reducing the effective diameter of an individual antibody (d_{eff} in Eq. 1) or as reducing the effective volume fraction of the suspension (by creating small "compact"

Table I Effect of Buffer Conditions on the Viscosity Coefficient (b), the Second and Third Osmotic Virial Coefficients (B_2 and B_3), and the Antibody Charge (Z) in a 5 mM Acetate Buffer at pH 5 with 20 mM Added NaCl

Buffer Condition	$b\left(\frac{m^3}{kg}\right)\times 10^2$	$B_2\left(\frac{m^3.mol}{kg^2}\right)\times 10^4$	$B_3\left(\frac{m^6.mol}{kg^3}\right)\times 10^7$	Z
pH 5				
10 mM	0.962	7.1 ± 1.3	−19 ± 7	12 ± 1
20 mM	1.08	4.4 ± 0.4	−11 ± 2	14 ± 1
100 mM	1.23	2.5 ± 0.4	−4 ± 2	24 ± 1
20 mM NaCl				
pH 5	1.08	4.4 ± 0.4	−11 ± 2	14 ± 1
pH 6	1.19	2.6 ± 0.3	−4 ± 2	6 ± 1
pH 7	1.26	1.7 ± 0.1	−3 ± 1	3 ± 1

* Error limits on B_2 and B_3 were determined using Mathematica (for the osmotic pressure data) or by propagation of error analysis (for Z values)

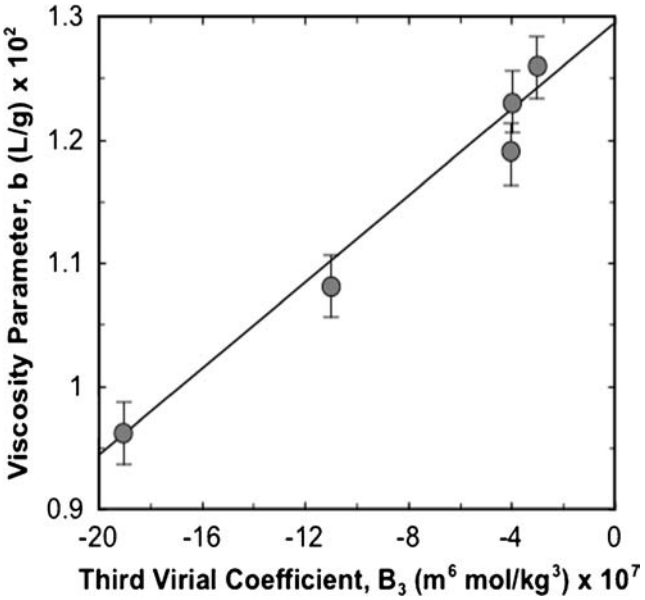

Fig. 5 Relationship between the viscosity coefficient (*b*) and the third virial coefficient (B_3) for monoclonal antibody solutions over a range of pH and salt concentration (data in Table I). *Error bars* were calculated from the 95% confidence interval on b determined using Mathematica.

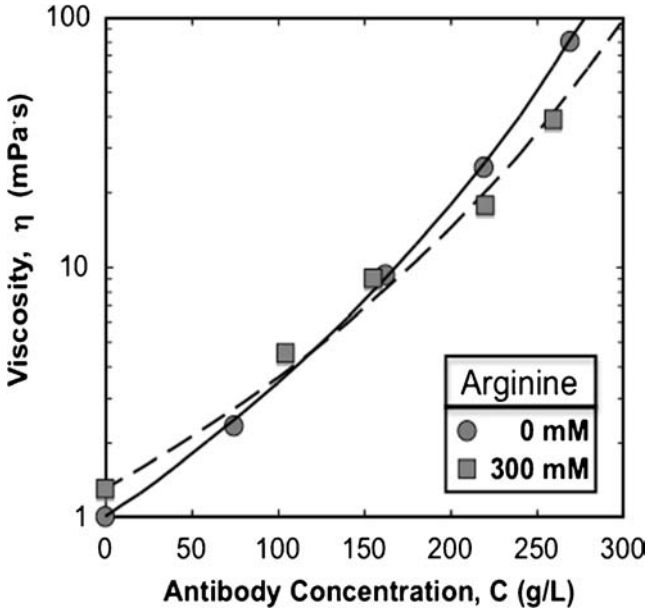

Fig. 6 Viscosity as a function of antibody concentration in a 5 mM acetate buffer at pH 5 with 20 mM NaCl both with and without added arginine. *Solid and dashed curves* are model calculations based on Eq. (3) with $C_{max}=800$ g/L with $b=0.0108$ L/g in the absence of arginine and $b=0.0090$ L/g in the presence of 300 mM arginine.

aggregates of several antibodies), in either case leading to a reduction in the solution viscosity.

Effect of Excipients

A number of different excipients are used to stabilize highly concentrated formulations of monoclonal antibodies and to help prevent protein aggregation. Viscosity data were also obtained in the presence of three commonly used excipients: sucrose, L-proline, and arginine-HCl. The effects of arginine-HCl on the viscosity of the antibody solution in a pH 5, 5 mM acetate buffer with 20 mM NaCl are shown in Fig. 6. At low protein concentrations, the viscosity increases slightly with increasing arginine concentration due to the known effect of arginine on the viscosity of water (in the absence of protein). In contrast, arginine caused a reduction in viscosity at high antibody concentrations (above about 150 g/L). This effect is quite pronounced at the highest protein concentration (≈270 g/L), with the viscosity decreasing from 80 mPa.s in the absence of arginine to 40 mPa.s in the presence of 300 mM arginine. This behavior is in good qualitative agreement with previous results by Bowen et al. (13) for a 246 g/L solution of an anti-CD4 monoclonal antibody that showed a reduction in viscosity from 330 mPa.s to 50 mPa.s upon the addition of 1000 mM arginine. Bowen et al. attributed this behavior to a reduction in self-association of the antibody due to the arginine, although no details were provided on the underlying mechanism.

The solid and dashed curves in Fig. 6 are the model fits using Eq. (3). The model is in very good agreement with the experimental results using $b=0.0108$ L/g in the absence of arginine and $b=0.0090$ L/g in the presence of 300 mM arginine. The addition of arginine also caused a small decrease in the value of the second virial coefficient from $B_2=4.4\times10^{-4}$ $m^3.mol/kg^2$ in the absence of arginine to $B_2=3.5\times10^{-4}$ $m^3.mol/kg^2$ in the 300 mM arginine solution (8). Arginine had a larger effect on the third virial coefficient, with B_3 going from -11×10^{-7} $m^6.mol/kg^3$ in the absence of arginine to -8.9×10^{-7} $m^6.mol/kg^3$ in the 300 mM arginine solution, corresponding to a reduction in the magnitude of the attractive multi-body interactions. This behavior is consistent with previous work by Arakawa et al. (14) who concluded that arginine reduces the extent of protein aggregation by suppressing short range protein-protein interactions through its association with the amino acid side chains and peptide bonds. Arginine also alters the structure of water, as seen in changes in the surface tension (14) as well as the solution viscosity (15).

The origin of the reduction in the viscosity coefficient *b* with added arginine is unclear; the data in Fig. 5 indicate that increasing the value of B_3 (i.e., reducing the magnitude of the negative value) would cause an increase in the value of *b*. Additional experiments were thus performed using the same buffer (5 mM acetate with 20 mM NaCl at pH 5) with 300 mM concentrations of the excipients L-proline and sucrose. In each case, the addition of the excipient caused an increase in the viscosity of water, which led to a small increase in the viscosity of the antibody solution at low protein concentrations. However, the excipients significantly reduced the increase in viscosity with increasing protein concentration, with

Table II Effect of Excipients (300 mM Concentration) on the Viscosity Coefficient (b) and the Second and Third Osmotic Virial Coefficients (B_2 and B_3) in a 5 mM Acetate Buffer at pH 5 with 20 mM Added NaCl

Excipient condition	η_0 (Pa.s)$\times 10^3$	$b\left(\frac{L}{g}\right)\times 10^2$	$B_2\left(\frac{m^3.mol}{kg^2}\right)\times 10^4$	$B_3\left(\frac{m^6.mol}{kg^3}\right)\times 10^7$
None	1.00	1.08	4.4 ± 0.4	−11 ± 2
Arginine-HCl	1.30	0.90	3.5 ± 0.1	−9 ± 2
L-Proline	1.39	0.84	4.1 ± 0.1	−10 ± 2
Sucrose	1.41	0.89	4.0 ± 0.4	−9 ± 2

* Error limits on B_2 and B_3 were determined using Mathematica based on the osmotic pressure data

the net result that the viscosity at high protein concentrations was lower than that for the solution without any excipient (similar to the results for arginine in Fig. 6).

The best fit values of the viscosity coefficient and the osmotic virial coefficients for the 300 mM solutions of the different excipients are summarized in Table II. All 3 excipients caused a reduction in the viscosity coefficient as well as a corresponding increase in the third virial coefficient; the effect of the excipient on the second virial coefficient was small.

DISCUSSION

The data obtained in this work provide an extensive study of the viscosity of a highly purified monoclonal antibody, focusing on the behavior at very high protein concentration and including a range of solution pH, salt concentration, and the presence of several commonly used excipients. The viscosity of the antibody solution was independent of the shear rate range over the range from 10 to 1000 s^{-1} even in highly concentrated solutions (up to 260 g/L). The viscosity at very high antibody concentrations (>250 g/L) was greater than 100 mPa.s at some buffer conditions. The viscosity increased with increasing pH (over the range from pH 5 to 7) and it also increased with increasing salt concentration (at pH 5).

The viscosity data were analyzed using the Mooney equation (11,12), providing a simple correlation for the concentration-dependence of the viscosity. The viscosity coefficient b was well-correlated with data for the third virial coefficient for the same antibody determined under identical buffer conditions based on osmotic pressure measurements (8). Note that Saito et al. (4) and Connolly et al. (10) both suggested a correlation between the viscosity and second osmotic virial coefficient based on data for a series of monoclonal antibodies (with the virial coefficient evaluated indirectly from sedimentation data). However, the inverse correlation seen in both of those studies is inconsistent with available models for the viscosity of colloidal dispersions. In addition, there is extensive previous work indicating that the viscosity of highly concentrated antibody solutions is governed primarily by short-range attractive interactions, which cannot be described by the positive values of the second virial coefficient. In contrast, the values for the third virial coefficient were negative over all experimental conditions, corresponding to short-range multi-body attractive interactions. The relationship between the viscosity behavior and the third virial coefficient has not been recognized previously, in large part due to the nearly complete absence of data for the third virial coefficient.

The results reported in this work may also help explain the very different behavior observed for monoclonal antibodies and other model proteins like bovine serum albumin (BSA). For example, Heinen et al. (16) found a reduction in the viscosity of concentrated BSA solutions with increasing salt concentration, exactly the opposite of the behavior seen in this work and elsewhere for a monoclonal antibody, even though the second osmotic virial coefficients are similar in magnitude for both proteins. However, the third virial coefficients for BSA are positive (17), in contrast to the negative values of the third virial coefficient for the monoclonal antibody examined in this work (8). It is not possible to extend this analysis to other proteins due to the lack of available data on the viscosity and third virial coefficients under comparable buffer conditions. Additional experiments with other antibodies will be needed to further explore this behavior given the unique properties of each protein.

The addition of the excipients, such as proline, arginine, and sucrose, caused an increase in the viscosity at low antibody concentrations where the viscosity is dominated by that of the solvent. The behavior is very different at high antibody concentrations where the excipients caused a significant reduction in the viscosity. For example, the addition of 300 mM arginine caused a two-fold reduction in the viscosity. This effect could not be explained by the change in the osmotic virial coefficients since the observed increase in B_3 was expected to cause an increase in viscosity due to the reduction in the magnitude of the short range attractive multi-body interactions. Additional experimental studies will be needed to clarify the origin of the complex behavior associated with these excipients.

CONCLUSION

This paper provides data on the viscosity of a highly purified monoclonal antibody, focusing on the behavior at very high

protein concentration. The antibody solutions showed Newtonian behavior at shear rates from 10–1000 s^{-1} even in highly concentrated solutions. The viscosity increased with increasing pH and increasing salt concentration, demonstrating the importance of electrostatic interactions. The viscosity data were analyzed using the Mooney equation. The viscosity coefficient (b) was well-correlated with data for the third virial coefficient determined from osmotic pressure measurements, suggesting that the viscosity is determined by relatively short range (primarily electrostatic) interactions in highly concentrated solutions.

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