# High Shear Rheology and Anisotropy in Concentrated Solutions of Monoclonal Antibodies

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*Received 5 February 2013; revised 26 April 2013; accepted 30 May 2013*

*Published online 19 July 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23647*

**ABSTRACT:** The high shear rheology of three concentrated solutions of immunoglobulin G1 monoclonal antibodies (mAb1, mAb2, and mAb3), differing only in their complementarity determining regions, was characterized using rotary and capillary rheometry. The more viscous solutions (mAb1 and mAb3) showed non-Newtonian behavior at high shear rates exhibiting both shear thinning and appreciable normal stress differences (NSDs) in the shear rate range $\gamma = 10$ to $10^4$ $s^{-1}$. The rheograms were retraced after $\gamma$ is increased and decreased, suggesting reversible self-associations under shear. In contrast, mAb2 solutions showed Newtonian behavior up to $\gamma = 6 \times 10^4$ $s^{-1}$. The critical shear stress $\tau_c$, corresponding to the onset of the reduction in the viscosity $\eta$, is a measure of mAb equilibrium cluster strength and increased rapidly with concentration for the high viscosity mAb solutions above 100 mg/mL. In addition, decreasing the temperature from 20°C to 5°C increased $\eta$ at low $\gamma$, but shear-thinning was enhanced and its onset occurred at a lower $\gamma_c$. Using an Arrhenius model $\eta = A \exp(E_a/kT)$, the activation energy for viscous flow $E_a$ was found to decrease for mAb1 solutions as $\gamma$ was increased from 10 to $10^4$ $s^{-1}$, suggesting mAb cluster disruption or rearrangement under shear. In contrast, for mAb2, this $E_a$ remained constant in the $\gamma$ range. Finally, mAb1 and mAb3 solutions showed appreciable NSDs, with their $N_1 > 0$ scaling linearly with $\gamma$ in the range $10^3$ to $10^4$ $s^{-1}$, whereas their $|N_2/N_1|$ was less than 0.25 in this region. These suggest anisotropy and deformation of their solution microstructure toward the extensional quadrant of the flow at high $\gamma$. In contrast, the NSDs for mAb2 were close to zero indicating that the solution microstructure under shear is practically isotropic. 
J Pharm Sci 102:2538–2549, 2013

**Keywords:** proteins; antibody; viscosity; injectables; formulation; colloid; relaxation time; concentrated solutions; anisotropy; cluster

## INTRODUCTION

The correlated motion of proteins in dense environments is critical to our understanding of physiological function as well as the behavior of biotherapeutic molecules in concentrated solutions. Recently, because of the growth of monoclonal antibody (mAb) therapy and the need to optimize formulations for delivery and stability, the viscosity behavior of high-concentration mAb solutions has become a subject of great interest and the topic of several publications.[1–8] mAbs tend to require several mg/kg body weight doses because of their relatively low potency. As more indications require home administration, the subcutaneous (SC) route of delivery has become a more convenient and attractive option. As SC administration typically has dose-volume constraints (e.g., <1.5 mL), injectable high-concentration mAb solutions have become an important strategy in addressing this challenge. Previous studies by Liu et al.[1] and Yadav et al.[2–4] on highly concentrated mAb

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Journal of Pharmaceutical Sciences, Vol. 102, 2538–2549 (2013)

solutions have revealed reversible self-associations that can increase mAb solution viscosity. Recent investigations by Kanai et al.[5] on the nature of these self-associations showed that Fab–Fab interactions between mAb1 molecules are likely responsible for the formation of these reversible mAb clusters. Equilibrium static light scattering studies by Scherer et al.[6] showed significantly higher scattering and apparent molecular weight for a high viscosity compared with a low-viscosity immunoglobulin G1 (IgG1) mAb sample, providing additional evidence of the relation between equilibrium self-associations and high viscosity. These reversible self-associations (or dynamic clusters) are thought to be because of the localized mAb–mAb attractions that arise from the heterogeneous distribution of charge on the surface of mAbs. This type of interaction cannot be clearly modeled by hard sphere or repulsive colloids. However, some insight could be gained from attractive colloidal systems where clusters can form and fill the space efficiently, resulting in hydrodynamic screening, high viscosities, and non-Newtonian rheology.[9–11]

For simple fluids like water at room temperature under typical process flows (about 0.1 up to $10^5$ $s^{-1}$), Newtonian or constant viscosity behavior is usually observed since Brownian collisions are sufficiently rapid compared to the flow time scale. These randomizing thermal collisions results in the solution microstructure having no angular dependence relative to the flow direction (i.e. it is isotropic) and also prevents long-range structures from forming. Due to the fast relaxation time of these fluids, shear flow is unable to change the rheological behavior from that at thermal equilibrium (e.g., viscosity is constant and normal stress differences are zero). In contrast, fluids such as polymer solutions and colloidal suspensions exhibit non-Newtonian behavior such as shear thinning and normal stress differences (NSDs), under typical process flow conditions.[12–15] This non-equilibrium behavior results from the competition between the flow and the arrangement of macromolecules or colloids in solution. Either the colloids or macromolecules are themselves large (and anisotropic) enough that shear causes them to orient or deform (e.g., high-molecular-weight polymers), or their correlated arrangement is modified by the flow. In the context of mAb solutions at high concentration, several studies have been conducted to understand the intermolecular forces that give rise to self-association or correlated arrangements.[1–5] However, the resulting non-Newtonian behavior caused by shear modification or disruption of these mAb arrangements has not been fully characterized.

In the present study, the rheological behavior of three IgG mAbs (mAb1, mAb2, and mAb3) as a function of concentration and temperature is investigated using rotational and capillary rheometry. In particular, the behavior of their viscosity $\eta$ and primary NSDs are thoroughly characterized as a function of shear rate. Both rotary and capillary rheometers were used to compare rheological measurements in the presence and absence of an air–liquid interface, with shear rates from 10 to $10^4$ $s^{-1}$ and $6 \times 10^4$ $s^{-1}$, respectively. The results are discussed in the context of what is known about mAb arrangements in solution. The importance of these results in processing and delivery of mAbs is also mentioned. Finally, a microstructural hypothesis for the anisotropy observed at high shear rate is presented.

## MATERIALS AND METHODS

The following materials and experimental techniques were utilized to investigate the rheological behavior of the three concentrated mAb solutions used in this study, namely mAb1, mAb2, and mAb3 molecules. These mAbs were full-length monoclonal IgG1 antibodies comprising κ-light chains constructed from identical human frameworks. The difference in amino acid composition among the mAbs resides in the complementarity determining region (CDR). The mAbs were expressed in Chinese hamster ovary cell lines and purified at Genentech (South San Francisco, California). The differences in the CDR sequence between mAb1 and mAb2, as well as some of their biophysical properties such as their net charge and their osmotic second virial coefficients $B_{22}$, have been previously published. The previously measured charges of mAb1 and mAb2 at pH 6.0 via membrane-confined electrophoresis were 6.3 and 11.9, respectively, in 15 mM ionic strength solutions.[4] In addition, the second osmotic virial coefficient $B_{22}$ of mAb1 was negative, suggesting attractive interactions, whereas that of mAb2 was positive, suggesting repulsive interactions. The proprietary mAb3 is an early version of a molecule in development that exhibited high viscosity but whose properties have not been fully characterized at the time of writing. Size-exclusion chromatography (SEC) was employed to characterize the size distribution of the mAbs upon dilution to 2 mg/mL. The percent monomer in mAb1, mAb2, and mAb3 were 96.8%, 99.5%, and 99.3%, respectively (see Supplemental Section).

Siloxane fluid viscosity standards rated at 20, 140, and 440 cP from Cannon Instrument Company (State College, PA) were used as controls in the rheology experiments, along with a 950 cP aqueous standard composed of 95% glycerin, 3.25% sucrose, and 1.75% water by weight.

### Sample Preparation

The monoclonal antibodies mAb1, mAb2, and mAb3 were buffer exchanged into 5 mM histidine buffer