Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Engineering

Recent progresses of understanding the viscosity of concentrated protein solutions

Zhenhuan Zhang[1,2] and Yun Liu[1,2]

Viscosity control for concentrated protein solutions is very important for the manufacturability and drug delivery routes of many protein therapeutics of the pharmaceutical industry. Even though there are successful applications of colloidal theories to calculate or predict the viscosity of globular proteins with electrostatic repulsions, understanding concentrated protein solutions remains an open challenge for colloidal science. This is especially true when proteins have complicated interaction potentials and non-spherical shapes, such as monoclonal antibody proteins. This paper provides a brief review of the recent experimental and theoretical progress in understanding the viscosity of concentrated protein solutions with a focus on the experimental results.

Addresses
[1] Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA
[2] Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716, USA

Corresponding author: Liu, Yun (yunliu@nist.gov, yunliu@udel.edu)

Current Opinion in Chemical Engineering 2017, **16**:48–55

This review comes from a themed issue on **Nanotechnology**

Edited by **Norman Wagner** and **John Brady**

http://dx.doi.org/10.1016/j.coche.2017.04.001

Introduction

Protein therapeutics, such as rDNA-derived monoclonal antibody (mAb) drugs, have been tremendously successful in past decades to treat many diseases including some cancers. In fact, mAb based therapeutics have the global market value already over $40 billion. The large scale production of mAbs poses a big challenge for pharmaceutical scientists to create an appropriate formulation in order to meet all requirements of the target product profile such as drugs stability, compatibility with administration routes, and so on [1–3]. Even though most FDA approved mAbs have been administered intravenously at present, more convenient administration routes, such as oral, transdermal, pulmonary, and subcutaneous injection routes, becomes desirable due to the convenience for outpatient and home treatments [3]. Among them, subcutaneous injections become the preferred choice for some mAbs recently. But subcutaneous injection is limited to a small injection volume (<1.5 ml). Therefore it requires protein concentrations as high as 100 mg/ml or more [1,4,5]. The increased protein concentration increases the viscosity that can exceed the limit of 'syringeability' to subcutaneous routes as well as bringing manufacturing difficulties to industries. Thus, reducing viscosity while maintaining stability for a long shelf life becomes very important for pharmaceutical industries.

Many variables can affect the viscosity of protein solutions, such as surface charge, molecule shape, solvent viscosity, pH, ionic strength, temperature, and shear rate [5]. Thus, a clear physical picture for the high viscosity of highly concentrated protein solutions is necessary to provide guidance for protein formulation developments. Comprehensive rheological responses of protein solutions can be obtained through rheological measurements such as steady shear, small/large amplitude oscillatory shear, and creep/relaxation. Among them, the apparent shear viscosity is the most discussed property in literatures due to its practical importance on the drug delivery and manufacturability. It is also noted that simple model globular proteins have been studied with a hope to shed light to the understanding of more complex behavior of non-spherical protein systems [6,7,8•,9•,10]. In this paper, a brief review will be presented based on recently published literatures on the understanding of protein bulk viscosity and available measurement methods.

Controlling viscosity of concentrated protein solutions

The viscosity of concentrated protein solutions is determined by inter-protein structures in solutions, which depend on the protein–protein interactions (PPI). By controlling the experimental conditions, such as pH, and coions/counterions, PPIs can be altered [11•,12•,13]. Thus, there are three important steps that link the experimental control parameters to the protein solution viscosity: (1) the relationship between experimental control parameters and PPIs; (2) the relationship between PPIs and inter-protein structures, and finally (3) the relationship between inter-protein structures and solution viscosity. Protein and/or solution differences may affect different steps, which can alter the formulation strategy necessary to control the protein solution viscosity. Research efforts have been devoted to the understanding of one or multiple steps. By gaining enough understanding of each step, hopefully, a complete physical picture

can be formed in future to provide guidance for both industrial applications and academic researches. Because protein sizes are typically small, the ratio of convective to diffusive mass transport rate, or say Peclet number [14], of experiments on protein systems is usually so small that we will mainly focus on the discussion of the zero shear viscosity and only briefly discuss non-Newtonian behaviors.

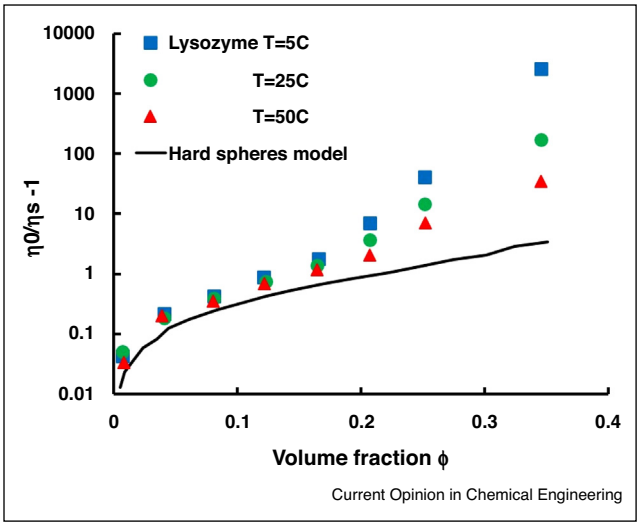
Viscosity of globular protein solutions

Proteins are, in general, very complex objects. To simplify the problem, globular proteins, such as bovine serum albumin(BSA) [9•,15,16•,17] and lysozyme [6,10], have been widely investigated as model systems. Many globular proteins can be approximated as spherical particles and further, the PPIs between them can be approximated as isotropic interactions. Therefore, well-developed colloidal theories for spherical particles have been applied to understand the viscosity of globular protein solutions in different buffer conditions [7,9•,16•]. It is noted that Sarangapani *et al.* recently questioned the suitability of using colloidal models to understand the solution viscosity of globular proteins [19] while many others believe that colloidal model theories are still valid for many cases [7,9•,16•,18].

For globular proteins with only electrostatic repulsions, colloidal theories based on spherical particles seem working reasonable well to calculate or fit experimental viscosity data. The PPI between BSA proteins can be modeled as a hard sphere core with an electrostatic repulsion. The viscosity of BSA proteins with and without salts have been measured up to about 100 mg/ml by Heinen *et al.* [9•]. Interestingly, the difference of the viscosity for BSA proteins with these two different buffer conditions is not very large. The theoretical viscosity calculated with the mode-coupling theory (MCT) seems to agree with the experimental values reasonably well [9•]. Sharma *et al.* have measured the viscosity of BSA proteins up to about 40% volume fraction in presence of a strong electrostatic repulsion. By considering an effective radius of BSA proteins due to the electrostatic repulsion, the viscosity data can be fitted well with colloidal theories [16•]. A recent study on α-crystallin solutions also indicate that the relative viscosity can be fitted well with Krieger–Dougherty equation up to about 50% volume fraction while for concentration higher than 50% volume faction, it needs to use the MCT to fit the data. Because excessive amount of salts is added to α-crystallin solutions to screen out the electrostatic repulsion, proteins in this experiment can be treated essentially as polydisperse hard sphere particles [7].

However, many proteins have also a strong short-range attraction. Without added salts to screen the electrostatic repulsion, the PPI has both a short-range attraction and long-range repulsion (SALR), where the range of the repulsion is, sometimes, comparable to the size of one protein molecule. Liu *et al.* demonstrated that the competition of the short-range attraction and long-range repulsion introduces the intermediate range order in a SALR system [10] that can affect the solution viscosity. There have been extensive studies in the past decade for SALR colloidal systems [20,21]. One widely studied example is lysozyme. The range of attraction between lysozymes in water is about a few angstroms. And its attraction strength can be controlled by temperature [8•,10,22]. The viscosity of highly concentrated lysozyme in D_2O was measured by a capillary rheometer and reported by Godfrin *et al.* [8•]. As shown in Figure 1, the theoretical calculation based on the MCT using the hard sphere interaction starts deviating from the experimental viscosity data when lysozyme's volume fractions are over ~0.15. And the viscosity at high protein concentrations dramatically increases when the temperature decreases from 50 °C to 5 °C. At the highest protein concentration, the viscosity shows an increase by a few orders of magnitudes. The increase of the attraction strength by decreasing the temperature introduces the intermediate range order in these lysozyme samples that drives the viscosity to a higher value. Interestingly, despite the very large viscosity change, lysozyme solutions remain to be Newtonian fluids at all tested concentrations [8•]. A shear thinning region can only be reached when the shear rate is up to $\sim 10^4\ s^{-1}$ [23]. Unlike the cases of BSA and α-crystallin proteins, new colloidal theories are still needed to be developed to successfully calculate/predict the viscosity of a SALR system at high concentrations.

Figure 1



Specific viscosity at the zero shear limit as a function of the lysozyme volume fraction relative to the viscosity of a hard sphere system. The figure was regenerated from the work by Godfrin *et al.* [8•].

Viscosity of monoclonal antibodies solutions

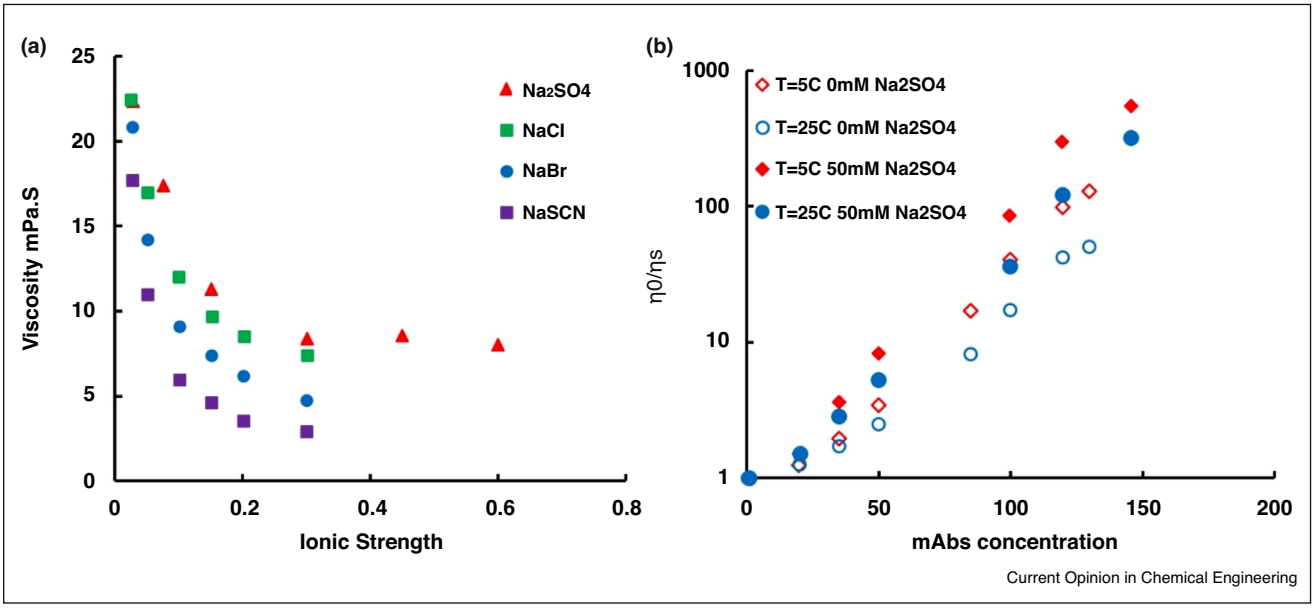
The viscosity control of mAb solutions is very important for both manufacturing and final administrations of such therapeutics. It has been reported that the viscosity limitation of syringeability is about 50 mPa·S for most cases [1,24]. However, the viscosity of some mAb solutions exceeds well above 50 mPa·S when the concentration is over 100 mg/ml, while many others stay below even 30 mPa·S at the similar concentration range [25•,26]. The pharmaceutical industry has showed a very strong interest in understanding the mechanisms in past decades. Therefore, the viscosity of mAb solutions has been widely studied as functions of concentration, temperature, pH, ionic strength, and different types of ions [1,5,11•,12•,13,27–30].

Controlling viscosity of mAbs solutions is much more challenging compared with cases of globular proteins due to the lack of understanding of the three relationships mentioned before. In fact, different mAb solutions, sometimes, show completely opposite trends of viscosity changes even though the buffer conditions, such as ionic strength and pH, are changed in the same way. Therefore, the clear understanding of viscosity control mechanisms can provide important guidance in developing successful mAb formulations.

A mAb protein is a 'Y' shaped molecule consisting of three domains: one fragment crystallizable (Fc) and two fragment antigen-binding (Fab) domains [1]. The anisotropic shape of mAb molecules makes it difficult to apply existing colloidal theories based on spherical particles to predict viscosity behaviors of concentrated mAbs solutions. However, experimental methods developed based on globular proteins can be still applied to study mAb solutions. And it is common to find that phenomenological equations traditionally applied to spherical colloidal systems are still used to understand experimental results of mAb solutions [5,31,32].

In the past decade, many works on mAb solutions have focused on investigating the structure-viscosity relationship. A series of papers have been published by Shire and co-workers investigating the reversible cluster formation in concentrated mAb solutions [13,27,33,34]. The viscosity of the investigated mAb solutions increases with increasing protein concentrations and can be fitted with the Mooney equation [27]. By studying 29 different mAbs, it is found that the electroviscous effect is not the governing factor for the viscosity of concentrated protein solutions [30]. The dramatic viscosity increase for some mAbs is attributed to the formation of reversible clusters driven by the electrostatic interaction. The viscosity of one mAb system is observed to decrease by over 80% by increasing the ionic strength as shown in Figure 2 (a) [27]. It's argued that added anions adsorbed on mAb surfaces disconnect this self-assembled structure and decrease the solution viscosity. Fab–Fab attractions instead of Fab–Fc interactions is found to be responsible for the formation of this structure. In order to directly probe the size of these self-assemblies, Yearley *et al.* use neutron spin echo to measure the short-time

Figure 2



(a) Solution viscosity of one mAb system decreases by adding different types of salts at the protein concentration of 125 mg/ml. The figure is regenerated from Kanai *et al.*'s work. [27] **(b)** Solution viscosity increases by adding Na_2SO4 at different mAb concentrations. The figure is regenerated from the work by Godfrin *et al.* [12•].

hydrodynamic radius of some mAbs, and conclude that the formation of small clusters with extended open structures causes the observed large viscosity in one of their mAbs [25•]. Adding salts disassembles these small clusters as indicated by smaller hydrodynamic radius, and decreases the solution viscosity. The driven force for this cluster formation is found due to the local eletrodipole effect [12•,26,35].

Interestingly, Godfrin *et al.* also show that the viscosity of a different mAb increases by adding salts, which shows different viscosity dependence on salt concentrations as shown in Figure 2(b) [12•]. Before adding salts (Na_2SO_4), this mAb can reversibly associate into a loosely connected transient cluster. Those clusters are composed of monomers as small moving units at short time limit. However, after adding 50 mM Na_2SO_4, this mAb forms elongated dimers first with a long life time. These dimers then associate hierarchically into large transient clusters at higher concentrations. The formation of the hierarchical clusters significantly increases solutions viscosity.

Despite the difference of the viscosity dependence on salt concentrations, the structure-viscosity relationship is actually the same for both mAbs reported by Godfrin and coworkers [12•,25•]. In both cases, the formation of clusters causes the increase of solutions viscosity. What is different is the first step of the three relationships for viscosity controls, that is, the relationship between experimental control parameters and PPIs is different for these two protein systems. For one case, adding salts weakens the short-ranged attraction [25•], while in another one, adding salts increases the attraction strength [12•].

Effects on solutions viscosity by irreversible clusters of mAbs have also been studied by Colby and coworkers [15,36]. Non-Newtonian rheology behaviors, such as yielding stress, have been detected in mAb solutions after long time incubation at 40 °C to introduce irreversible aggregates. However the mAb solution recovers Newtonian behaviors after these irreversible aggregations are filtered out.

Although reported control strategies of changing the solution viscosity seem different for different mAbs reported in literatures, the structural-viscosity relationship is actually consistent for most papers. The formation of clusters leads to the increased viscosity, which directly results from the different PPIs. Interestingly, a recent theory by Schmit *et al.* based on theoretical treatments of semidilute polymer solutions has shown some success in modeling the viscosity for some mAbs solutions [37]. More experimental tests for different theories and models are needed in future. Also we would like to point out that there is a report that the formation of large clusters in one mAb solution can result in the decrease of the solution viscosity [38].

Measurement methods of bulk viscosity of protein solutions

Conventional torsional shear rheometery systematically discussed by Macosko and Larson [39] and Bird *et al.* [40] has been widely used to measure solution viscosities. In general, the cone-plate geometry is preferred for highly concentrated samples since it generates a uniform flow profile during measurements. For low concentration samples, single/double gaps couette cell can be used in order to increase torque signal due to relative low viscosity of samples. However, these torsional shear rheometers usually cannot achieve a very high shear rate measurement.

When a torsional rheometer is used, speical attention has to be paid to eliminate or avoid interfacial effects due to the formation of protein 'films' at the air–liquid interface. Protein molecules tend to be adsorbed onto the air–liquid (or liquid–liquid) interface and form a relatively 'dense film' [16•]. Because the resulting torque measured by rheometers is the sum of both bulk and interfacial signals, the measured apparent viscosity has also the contribution from the interface viscosity which can be expressed as [41]

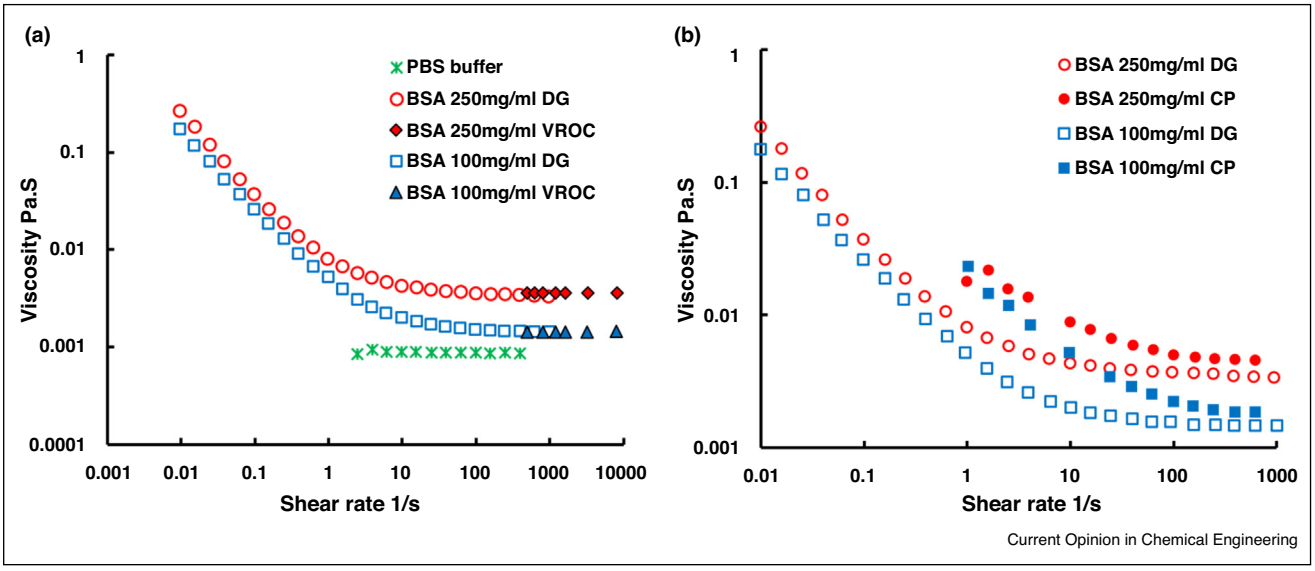
$$\eta_{measure} = \eta_{bulk} + {}^{\eta_{interface}}/_{k} \quad (1)$$

where k is the characteristic length of geometries and has been discussed in literature [15]. For protein solutions, strong interfacial films can produce a yielding behavior in low shear rate region as shown in Figure 3. Sharma *et al.* demonstrates that BSA solutions measured by a cone-plate geometry showing a 'clear' shear shining region while the bulk viscosity should show Newtonian behavior as indicated in Figure 3(a) [16•]. This measurement artifact has been proven to be due to the resistance generated by the 'dense protein film' formed at the interface and has been observed in many protein systems [15,42,43]. The measured viscosities by two geometries (cone-plate and double gaps couette cell) are different with each other in 'shear shining' domain due to different k values of geometries as shown in Figure 3(b).

To solve this problem, two methods can be used to obtain the bulk viscosity. First, surfactants, such as Tween 80 or SDS, can be added into tested samples [15,42,43]. Surfactant molecules can reduce interfacial viscosity by replacing protein molecules on the interface. Thus, the measured viscosity can be close to the bulk values. Secondly, interfacial viscosity can be independently measured by torsional rheometer with geometries such as DWR, bi-cone, and so on [44–46]. The bulk viscosity can then be calculated based on Eq. (1). However, it is sometimes difficult to have an exactly same measurement condition for both bulk and interfacial tests [42,43].

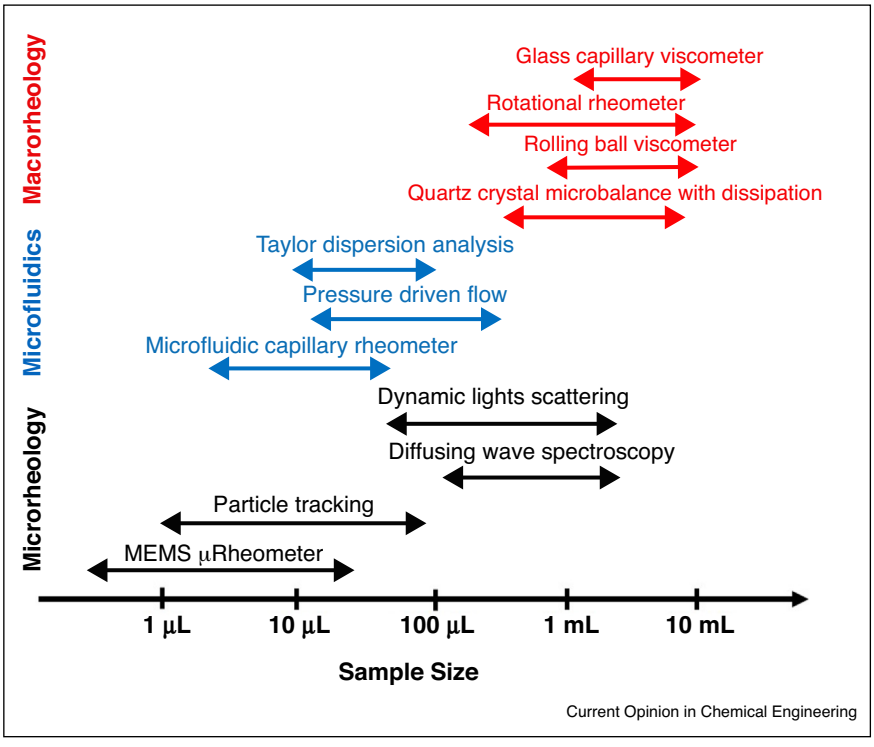
Other measurement methods, such as microfluidic capillary viscometers and microrheology technologies, which

Figure 3



(a) The apparent bulk viscosity of BSA protein solutions measured with a double gap geometry (DG) shows the characristics of a yield stress fluid while the true bulk viscosity measured by microfluid slit rheometer or viscometer-rheometer-on-chip (VROC) shows Newtonian behavior. This is due to the effect of the dense protein layer formed at the air-liquid interface. **(b)** Apparenent bulk viscosity of BSA proteins measured by double gap (DG) geometry and cone-plate (CP) geometry has different results due to the interfacial protein layer. The figure is regenerated from the work by Sharma *et al.* [16•].

Figure 4



Available viscosity measurement methods based on sample sizes. The figure is regenerated from the paper by Josephson *et al.* [50].

can eliminate the influence of an interface, become very useful to obtain the bulk viscosity of protein solutions.

Microfluidic capillary viscometer can measure the bulk viscosity up to a very high shear rate due to its micro-sized channel design. In some cases, it can reach the shear rate above 10^5 s^{-1} [47,48]. A comprehensive review of microfluids rheometery can be found in literature by Pipe and McKinley [49]. One apparent advantage of this technique is the small sample volume needed for a measurement compared to torsional rheometers, which is extremely desirable during early stage of mAb formulation developments due to limited sample resources for early screening studies.

Microrheology technologies, either active or passive methods, are also useful to measure the viscosity by tracking particle probes suspended in sample solutions, and are not affected by the dense protein layer at the interface. Passive particle tracking can be realized by observing particle movements driven by thermal fluctuations in solutions [50,51]. Active particle tracking by optical/magnetic tweezers can also be used in order to increase the threshold of material moduli accessible in measurements [51–53]. A short review about microrheology is published by Cicuta and Donald [54]. There are many other microrheology methods that has been studied such as light scattering [55], MEMS μRheometer [56]. Interested readers can learn those from cited literatures.

Overall, bulk viscosity measurements of protein solutions can be performed in various ways as shown in Figure 4 [50]. The choice of different methods depends on availability of sample volume and interested shear rate regions.

Summary

Much progresses have been made recently in understanding the viscosity of concentrated proteins solutions by focusing on the relationships between experimental control parameters, the PPIs, the inter-protein structures and protein viscosity. The behavior of some globular proteins with only electrostatic repulsions can be successfully explained by existing colloidal theories. However, when there is both a short-range attraction and long-range repulsion, the solution viscosity behavior becomes much more complex as demonstrated in lysozyme systems. New theoretical developments are needed to understand globular proteins with this type of complex potential. For mAb proteins, experimental results indicate that for most mAb proteins, the formation of clusters always increases the solution viscosity. This is also the case for globular proteins. However, there are very limited theories to calculate the viscosity behaviors of proteins with anisotropic shapes. Despite all the progresses, our understanding of the viscosity behavior of concentrated protein solutions is still at the infant stage. It remains an open challenge for colloidal science.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

1. Harris RJ, Shire SJ, Winter C: **Commercial manufacturing scale formulation and analytical characterization of therapeutic recombinant antibodies**. *Drug Dev. Res.* 2004, **61**:137-154.

2. Shire SJ: **1—Introduction**. *Monoclonal Antibodies*. Woodhead Publishing; 2015:1-15.

3. Mitragotri S, Burke PA, Langer R: **Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies**. *Nat. Rev. Drug Discov.* 2014, **13**:655-672.

4. Shire SJ: **6—Challenges in the subcutaneous (SC) administration of monoclonal antibodies (mAbs)**. *Monoclonal Antibodies*. Woodhead Publishing; 2015:131-138.

5. Yadav S, Shire SJ, Kalonia DS: **Factors affecting the viscosity in high concentration solutions of different monoclonal antibodies**. *J. Pharm. Sci.* 2010, **99**:4812-4829.

6. Cardinaux F, Zaccarelli E, Stradner A, Bucciarelli S, Farago B, Egelhaaf SU, Sciortino F, Schurtenberger P: **Cluster-driven dynamical arrest in concentrated lysozyme solutions**. *J. Phys. Chem. B* 2011, **115**:7227-7237.

7. Foffi G, Savin G, Bucciarelli S, Dorsaz N, Thurston GM, Stradner A, Schurtenberger P: **Hard sphere-like glass transition in eye lens α-crystallin solutions**. *Proc. Natl. Acad. Sci.* 2014, **111**:16748-16753.

8. • Godfrin PD, Hudson SD, Hong K, Porcar L, Falus P, Wagner NJ, Liu Y: **Short-time glassy dynamics in viscous protein solutions with competing interactions**. *Phys. Rev. Lett.* 2015, **115**:228302.
This paper focuses on all three relationships mentioned in this review article. It shows the interestingviscosity behavior of lysozyme systems where there is both a short-range attraction and long-range repulsion. By decreasing the temperature, the short-range attraction strength is increased resulting in a dramatic increase of solution viscosity. At the highest concentration, the viscosity increases by a couple of orders of magnitudes, and is caused by the formation of intermediate range order introduced by the competition of the two potential features. While all samples in this paper show Newtonian fluid behavior, the local dynamics of proteins in a few high concentration samples are actually similar to those of many colloidal systems in glass states.

9. • Heinen M, Zanini F, Roosen-Runge F, Fedunová D, Zhang F, Hennig M, Seydel T, Schweins R, Sztucki M, Antalík M, Schreiber F, Nagele G: **Viscosity and diffusion: crowding and salt effects in protein solutions**. *Soft Matter* 2012, **8**:1404-1419.
This paper shows the bulk viscosity of BSA protein solutions up to about 100 mg/ml. The results of two types of samples are compared with one having additional salts (150 mM NaCl) and another one having no added salts. Collective diffusion coefficients are measured with dynamic light scattering. And the protein-protein interaction potential is estimated by analyzing small angle X-ray scattering data. From the protein-protein interaction potential, the viscosity of their samples is calculated by estimating the contributions from high frequency viscosity and static shear viscosity. The details of the theories are reported.

10. Liu Y, Porcar L, Chen J, Chen W-R, Falus P, Faraone A, Fratini E, Hong K, Baglioni P: **Lysozyme protein solution with an intermediate range order structure**. *J. Phys. Chem. B* 2010, **115**:7238-7247.

11. • Dear BJ, Hung JJ, Truskett TM, Johnston KP: **Contrasting the influence of cationic amino acids on the viscosity and stability of a highly concentrated monoclonal antibody**. *Pharm. Res.* 2016:1-15.
This paper focuses on how different co-solutes affect the viscosity of concentrated mAb solutions. The co-solutes studied are Lysine, Histidine, Arginine, Imidazole, and camphorsulfonic acid.

12. • Godfrin PD, Zarraga IE, Zarzar J, Porcar L, Falus P, Wagner NJ, Liu Y: **Effect of hierarchical cluster formation on the viscosity of concentrated monoclonal antibody formulations studied by neutron scattering**. *J. Phys. Chem. B* 2016, **120**:278-291.

This paper focuses on the structure-viscosity relationship and illustrates that the formation of hierarchical clusters in mAb solutions greatly increases the solution viscosity. It shows that the moving units in solutions of one mAb are dimers when salts are added to the solutions. These dimers further associate into large transient networks. Neutron spin echo is very useful to probe strongly bounded small dimers and is not sensitive to the motions of large loosely bounded transient networks.

13. Liu J, Nguyen MD, Andya JD, Shire SJ: **Reversible self-association increases the viscosity of a concentrated monoclonal antibody in aqueous solution**. *J. Pharm. Sci.* 2006, **95**:234-235.

14. Chen DTN, Wen Q, Janmey P, Crocker J, Yodh A: **Rheology of soft materials**. *Ann. Rev. Condens. Matter Phys.* 2010, **1**:301-322.

15. Castellanos MM, Pathak JA, Colby RH: **Both protein adsorption and aggregation contribute to shear yielding and viscosity increase in protein solutions**. *Soft Matter* 2014, **10**:122-131.

16. • Sharma V, Jaishankar A, Wang Y-C, McKinley GH: **Rheology of globular proteins: apparent yield stress, high shear rate viscosity and interfacial viscoelasticity of bovine serum albumin solutions**. *Soft Matter* 2011, **7**:5150-5160.

This paper focuses on the measurement technique and elegantly demonstrates that the dense protein layer at the air-liquid interface can dramatically affect the measured apparent bulk viscosity using rotational rheometers. They have also measured the bulk viscosity of BSA proteins up to about 0.4 volume faction and used a colloidal theory to fit the data.

17. Yadav S, Shire SJ, Kalonia DS: **Viscosity analysis of high concentration bovine serum albumin aqueous solutions**. *Pharm. Res.* 2011, **28**:1973-1983.

18. Castellanos MM, Clark NJ, Watson MC, Krueger S, McAuley A, Curtis JE: **Role of molecular flexibility and colloidal descriptions of proteins in crowded environments from small-angle scattering**. *J. Phys. Chem. B* 2016, **120**:12511-12518.

19. Sarangapani Prasad S, Hudson Steven D, Jones Ronald L, Douglas Jack F, Pathak Jai A: **Critical examination of the colloidal particle model of globular proteins**. *Biophys. J.* 2016, **108**:724-737.

20. Godfrin PD, Valadez-Perez NE, Castaneda-Priego R, Wagner NJ, Liu Y: **Generalized phase behavior of cluster formation in colloidal dispersions with competing interactions**. *Soft Matter* 2014, **10**:5061-5071.

21. Zhuang Y, Charbonneau P: **Recent advances in the theory and simulation of model colloidal microphase formers**. *J. Phys. Chem. B* 2016, **120**:7775-7782.

22. Porcar L, Falus P, Chen WR, Faraone A, Fratini E, Hong KL, Baglioni P, Liu Y: **Formation of the dynamic clusters in concentrated lysozyme protein solutions**. *J. Phys. Chem. Lett.* 2010, **1**:126-129.

23. Dharmaraj VL, Godfrin PD, Liu Y, Hudson SD: **Rheology of clustering protein solutions**. *Biomicrofluidics* 2016, **10**:043509.

24. Daugherty AL, Mrsny RJ: **Formulation and delivery issues for monoclonal antibody therapeutics**. *Adv. Drug Deliv. Rev.* 2006, **58**:686-706.

25. • Yearley EJ, Godfrin PD, Perevozchikova T, Zhang H, Falus P, Porcar L, Nagao M, Curtis JE, Gawande P, Taing R: **Observation of small cluster formation in concentrated monoclonal antibody solutions and its implications to solution viscosity**. *Biophys. J.* 2014, **106**:1763-1770.

This paper focuses on the structure-viscosity relationship. Neutron spin echo is used to directly estimate the hydrodynamic radius of mAb proteins at high concentrations as neutron spin echo can measure the short-time self-diffusion coefficients even at very large protein concentrations. For this mAb system, mAb proteins behave like micelles. It exists as monomers at low concentrations and forms small clusters at large concentrations with the hydrodynamic radius independent of the concentration. The formed small mAb clusters have extended open structure that drives the viscosity unusually high..

26. Yearley EJ, Zarraga IE, Shire SJ, Scherer TM, Gokarn Y, Wagner NJ, Liu Y: **Small-angle neutron scattering characterization of monoclonal antibody conformations and interactions at high concentrations**. *Biophys. J.* 2013, **105**:720-731.

27. Kanai S, Liu J, Patapoff TW, Shire SJ: **Reversible self-association of a concentrated monoclonal antibody solution mediated by Fab–Fab interaction that impacts solution viscosity**. *J. Pharm. Sci.* 2008, **97**:4219-4227.

28. Shire SJ: **7—Strategies to deal with challenges of developing high-concentration subcutaneous (SC) formulations for monoclonal antibodies (mAbs)**. *Monoclonal Antibodies*. Woodhead Publishing; 2015:139-152.

29. Tomar DS, Kumar S, Singh SK, Goswami S, Li L: **Molecular basis of high viscosity in concentrated antibody solutions: strategies for high concentration drug product development**. *MAbs*. Taylor & Francis; 2016:216-228.

30. Connolly BD, Petry C, Yadav S, Demeule B, Ciaccio N, Moore JM, Shire SJ, Gokarn YR: **Weak interactions govern the viscosity of concentrated antibody solutions: high-throughput analysis using the diffusion interaction parameter**. *Biophys. J.* 2012, **103**:69-78.

31. Minton AP: **Hard quasispherical particle models for the viscosity of solutions of protein mixtures**. *J. Phys. Chem. B* 2012, **116**:9310-9315.

32. Shire SJ: **9—The molecular basis of high viscosity of monoclonal antibodies (mAbs) at high concentration**. *Monoclonal Antibodies*. Woodhead Publishing; 2015:163-192.

33. Scherer TM, Liu J, Shire SJ, Minton AP: **Intermolecular interactions of IgG1 monoclonal antibodies at high concentrations characterized by light scattering**. *J. Phys. Chem. B* 2010, **114**:12948-12957.

34. Yadav S, Sreedhara A, Kanai S, Liu J, Lien S, Lowman H, Kalonia DS, Shire SJ: **Establishing a link between amino acid sequences and self-associating and viscoelastic behavior of two closely related monoclonal antibodies**. *Pharm. Res.* 2011, **28**:1750-1764.

35. Chaudhri A, Zarraga IE, Kamerzell TJ, Brandt JP, Patapoff TW, Shire SJ, Voth GA: **Coarse-grained modeling of the self-association of therapeutic monoclonal antibodies**. *J. Phys. Chem. B* 2012, **116**:8045-8057.

36. Castellanos MM, Pathak JA, Leach W, Bishop SM, Colby RH: **Explaining the non-Newtonian character of aggregating monoclonal antibody solutions using small-angle neutron scattering**. *Biophys. J.* 2014, **107**:469-476.

37. Schmit JD, He F, Mishra S, Ketchem RR, Woods CE, Kerwin BA: **Entanglement model of antibody viscosity**. *J. Phys. Chem. B* 2014, **118**:5044-5049.

38. Johnston KP, Maynard JA, Truskett TM, Borwankar AU, Miller MA, Wilson BK, Dinin AK, Khan TA, Kaczorowski KJ: **Concentrated dispersions of equilibrium protein nanoclusters that reversibly dissociate into active monomers**. *ACS Nano* 2012, **6**:1357-1369.

39. Macosko CW, Larson RG: *Rheology: Principles, Measurements, and Applications*. New York, NY: VCH; 1994.

40. Bird RB, Armstrong RC, Hassager O, Curtiss CF: *Dynamics of Polymeric Liquids* . New York: Wiley; 1977.

41. Larson RG: *The Structure and Rheology of Complex Fluids*. Oxford University Press; 1998.

42. Zhang Z, Barman S, Christopher G: **The role of protein content on the steady and oscillatory shear rheology of model synovial fluids**. *Soft Matter* 2014, **10**:5965-5973.

43. Zhang Z, Barman S, Christopher GF: **Effect of interfacial viscoelasticity on the bulk linear viscoelastic moduli of globular protein solutions**. *Phys. Rev. E* 2014, **89**:052306.

44. Edwards DA, Brenner H, Wasan DT: **Chapter 7—measurement of interfacial shear viscosity**. *Interfacial Transport Processes and Rheology*. Butterworth-Heinemann; 1991:212-226.

45. Fuller GG, Vermant J: **Complex fluid–fluid interfaces: rheology and structure**. *Ann. Rev. Chem. Biomol. Eng.* 2012, **3**:519-543.

46. Vandebril S, Franck A, Fuller GG, Moldenaers P, Vermant J: **A double wall-ring geometry for interfacial shear rheometry**. *Rheol. Acta* 2010, **49**:131-144.

47. Kang K, Lee LJ, Koelling KW: **High shear microfluidics and its application in rheological measurement**. *Exp. Fluids* 2005, **38**:222-232.

48. Hudson SD, Sarangapani P, Pathak JA, Migler KB: **A microliter capillary rheometer for characterization of protein solutions**. *J. Pharm. Sci.* 2015, **104**:678-685.

49. Pipe CJ, McKinley GH: **Microfluidic rheometry**. *Mech. Res. Commun.* 2009, **36**:110-120.

50. Josephson LL, Furst EM, Galush WJ: **Particle tracking microrheology of protein solutions**. *J. Rheol.* 2016, **60**:531-540.

51. Mewis J, Wagner NJ: *Colloidal Suspension Rheology*. Cambridge University Press; 2012.

52. Brau R, Ferrer J, Lee H, Castro C, Tam B, Tarsa P, Matsudaira P, Boyce M, Kamm R, Lang M: **Passive and active microrheology with optical tweezers**. *J. Opt. A: Pure Appl. Opt.* 2007, **9**:S103.

53. Lin J, Valentine MT: **High-force NdFeB-based magnetic tweezers device optimized for microrheology experiments**. *Rev. Sci. Instrum.* 2012, **83**:053905.

54. Cicuta P, Donald AM: **Microrheology: a review of the method and applications**. *Soft Matter* 2007, **3**:1449-1455.

55. Instruments M: *An Introduction to DSL Microrheology*. Malvern Instruments; 2015.

56. Christopher GF, Yoo JM, Dagalakis N, Hudson SD, Migler KB: **Development of a MEMS based dynamic rheometer**. *Lab Chip* 2010, **10**:2749-2757.