A Microliter Capillary Rheometer for Characterization of Protein Solutions

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ABSTRACT: Rheometry is an important characterization tool for therapeutic protein solutions because it determines syringeability and relates indirectly to solution stability and thermodynamic interactions. Despite the maturity of rheometry, there remains a need for a rheometer that meets the following three needs of the biopharamaceutical industry: small volume; large dynamic range of shear rates; and no air–sample interface. Here, we report the development of a miniaturized capillary rheometer that meets these needs and is potentially scalable to a multiwell format. These measurements consume only a few microliters of sample and have an uncertainty of a few percent. We demonstrate its performance on monoclonal antibody solutions at different concentrations and temperatures. The instrument has a dynamic range of approximately three decades (in shear rate) and can measure Newtonian, shear thinning, and yielding behaviors, which are representative of the different solution behaviors typically encountered. We compare our microliter capillary rheometer with existing instruments to describe the range of parameter space covered by our device. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

The protein therapeutic market is growing rapidly in sales and number of drugs available.[1] In early-stage formulation development, a wide variety of different solution conditions, including various excipients, buffers, and pH, are screened for various properties including viscosity. High protein concentrations are part of the manufacturing process; sometimes therapeutic protein solutions are concentrated up to approximately 250 g/L. High protein concentrations are also sometimes required for the drug product, as there is a limit to the volume of fluid that can be administered to the patient, for some methods such as subcutaneous injection.

Viscosity is a critical property not only because it relates directly to syringeability, but also because it is a relevant parameter for purification, fill/finish, and drug delivery.[2,3] It is an indirect diagnostic of protein interactions and aggregation of the protein monomer.[4–9] Although there is not a clearly established relationship between viscosity and protein self-association and/or aggregation, considerable work is aimed at this goal.[10]

Despite the maturity of viscometry, there remains a need for a rheometer that meets the following three needs of the biopharamaceutical industry: small volume; large dynamic range of shear rates; and no air–sample interface. The small volume requirement is particularly important to the biopharmaceutical industry during the screening stage of product development. Traditional viscometric measurement methods include rotational rheometers such as the cone-and-plate, the cup-and-bob, or the double-gap cylinder geometries. The lower end of the volume range for these geometries is approximately 0.1, 3, and 4 mL, respectively, which places limits on the number of screening compounds that can be tested.

The requirement for a wide dynamic range in shear stems from the need for viscosity information in the high shear rate limit (e.g., shear rates greater than 10^5 s^{-1}) where syringeability is an issue and the concomitant need for low shear rate information (e.g., shear rates less than 100 s^{-1}), which relates to diffusive time scale of deformable structures.[11] The low shear rate range is particularly sensitive to the presence of irreversible aggregates.

Our third requirement of no air–fluid interface stems from work showing how the existence of such an interface in rotational rheometers sometimes complicates interpretation of results because of surface rheology and surface tension. The surface rheology complication[12] stems from the fact that the surface itself has an independent viscosity (units of Pa m s, as opposed to usual viscosity units of Pa s), which is convoluted with the viscosity measurement in situations that are common to protein therapeutics. The surface viscosity ranges significantly (from 10^{-9} to 10^{-5}) Pa m s for typical surfactants, and (from 10^{-5} to 10^{-2}) Pa m s for proteins (without added surfactant). If the surface rheology is measured separately with sufficient certainty, its effect can be accounted for.[13] Unfortunately, these precision-limiting surface effects are typically more significant in the smaller-volume geometries. The other complication[14] arises because surface tension serves as a major source of error in precision measurements at low torque because the nonaxisymmetric irregularities of the surface that frequently occur cause a net torque. Adding surfactant to the

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solution reduces and often essentially eliminates the effects of both surface rheology and surface tension. Surfactants are added to therapeutic protein solutions also to stabilize them against aggregation.[15]

Pressure-driven capillary viscometry[16] can meet the above three requirements; however, none to date have been designed and built to do so. The rheometer that we describe here measures the fluid viscosity over a wide range of shear rates (e.g., 1–10,000 s^{-1}) and temperatures (e.g., 0°C–80°C), yet it requires very small volumes (consuming ~3 μL at each temperature). In this report, we describe the approach and the resulting performance of this rheometer and then demonstrate its use to test protein solutions. We focus on the rheometer's capabilities and not on the rheological behavior of protein solutions, which can be explored with this instrument.

BACKGROUND

Here, we review the variety of viscometric techniques that are currently used to probe fluids under a wide range of conditions and discuss the relative merits with respect to the needs of the biopharmaceutical industry. Falling ball viscometry is sometimes used for protein solutions. The disadvantage is that the sample volume requirement is approximately 0.4 mL, and the shear rate range is typically limited. As the flow around the sphere is nonviscometric, the interpretation of this measurement is difficult when the fluid is non-Newtonian. In certain circumstances, when the diameter of the sphere is small compared with the cylinder containing the non-Newtonian fluid, and wall and end effects are eliminated, the zero-shear viscosity may be obtained by testing various spheres and extrapolating to zero stress, that is, to a sphere of infinitesimal size and neutral buoyancy.[17] This procedure will not work for all fluids, for example, not for yield-stress fluids. Moreover, commercial instruments are typically designed with Newtonian fluids in mind, when it is not necessary to use multiple or small spheres.

One class of small-volume viscometers is oscillators. A variety of geometries and approaches have been tried.[18] For many such cases, the so-called surface-loading limit[19] applies, because the evanescent shear wave penetrates a small distance compared with the fluid gap (i.e., unlike in a typical rheometer that operates in the gap-loading limit, in which the shear propagates entirely across the gap). For kilohertz (kHz) oscillators,[18,20] this penetration is roughly 10 μm or more, depending on viscosity. For megahertz (MHz) oscillators,[21,22] however, the penetration is typically a few hundred nanometer, so that bulk viscosity is measured only for simple fluids and monomeric protein solutions, and they are not suitable for complex clustering protein solutions. MHz oscillators have been applied to such systems and qualitative effects of clustering have been observed, but the measurement is ill defined and inaccurate when the shear penetration layer is not large enough to sample the structures within the fluid. Recently, a micro-electrical-mechanical (MEMs) microrheometer was reported, which is capable of low frequency behavior. As currently implemented, it contains an air–fluid interface that could act as a source of stress in protein solutions.[23]

Optical methods based on diffusive motions of proteins or tracer particles have been recently introduced. For example, dynamic light scattering[24,25] and particle tracking,[26,27] which both take advantage of the generalized Stokes–Einstein relation, GSER, need only very small volumes. The GSER is valid in some circumstances and not in others.[28] It fails whenever a probe particle changes the structure of the fluid to be measured.[29] This failure occurs in highly charged concentrated colloidal solutions,[30] of which protein solutions are a good example.[31] Another failure occurs when the probe particle is small compared with the size of structures in the fluid. This happens whenever protein solutions form large aggregates and thus become heterogeneous.[31] Guidelines involving a series of double checks to avoid the pitfalls of this method are elaborated by Amin et al.[32]

Lastly, we discuss capillary rheometers where many are commercially available and smaller-volume versions have appeared recently.[33,34] In a capillary rheometer, the flow is nonuniform (Poiseuille flow) but it is still viscometric. Even when the fluid is non-Newtonian, the shear stress and rate at the capillary wall is well defined provided the required Rabinowitsch correction (see Eq. (3) below) is made.[35] The basic principle underlying capillary viscometry is the Hagen–Poiseuille law describing nonturbulent flow through a tube:

$$R = \frac{\Delta P}{Q} = \frac{128\,\eta L}{\pi d^4} = K\eta \tag{1}$$

As such, the pressure drop (ΔP) and flow rate (Q) must be measured to determine the hydrodynamic resistance (R) and the fluid viscosity (η). K is a constant that depends only on the length (L) and diameter (d) of a capillary. R and K can be calculated similarly when the capillary or channel has rectangular cross-section.[36] The performance of any given system, in terms of volume required and the range of accessible viscosities and shear rates, depends on K, on the dimensions of the capillary (and any input tubing), and on the sensitivity and range of measurement of ΔP and Q (see Table 1). Several microfluidic rheometers have been developed.[33,37–45]

A recent capillary rheometer uses recirculation and thus enables dilution with the solvent or another solution mixture, in order to sample a whole range of concentration.[46] That

Table 1. Summary of Capability of Various Capillary and Microfluidic Rheometers Listed in Order of Approximate Sample Volume Consumed

Volume (μL)/Rate Sweep	η(mPa s)	$\dot{\gamma}_w(s^{-1})^a$	Reference
0.03	1 to 100	200 to 2000	39[b]
1	0.2 to 100	200 to 2000	37
1	1 to 600	10 to 600	38[b]
3	**0.4 to 2000**	**10 to 3000**	**This work**
10	0.9 to 100	10 to 1000	34
20	100 to 10^9	1 to 1000	42[b]
50	0.2 to 10^5	1000 to 10^5	33
100	0.2 to 10	100 to 2000	43
100	0.2 to 10	20 to 1000	45
750	0.2 to 1000	10 to 1500	46
1000	0.2 to 10	20 to 600	44
100,000	10 to 1000	1000 to 10^6	40

[a]The shear rate range accessible to a single sample with a Newtonian viscosity in the middle of the rheometer's range. Note that a wider range of shear rate is usually accessible if the sample is non-Newtonian.

[b]These rheometers are based on tracking the interface between immiscible fluids. The pressure at the sample interface must be accounted for, whenever the applied pressure is low (i.e., comparable to the sample interface pressure).

Values of the current work are in bold.

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