Characterization of Protein Rheology and Delivery Forces for Combination Products

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ABSTRACT: Characterization of a protein–device combination product over a wide range of operating parameters defined by end-user requirements is critical for developing a product presentation that is convenient for patient use. In addition to the device components, several product attributes, such as product rheology and product–container interactions, govern the functionality of a delivery system. This article presents results from a characterization study conducted for a high-concentration antibody product in a prefilled syringe. Analytical models are used to study the rheological behavior and to estimate delivery forces over a broad design space comprising temperature, concentration, and shear stress. Data suggest that high-viscosity products may exhibit significant shear thinning under the shear rates encountered under desired injection times. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:4472–4480, 2012

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INTRODUCTION

The need for higher dosage amounts coupled with patients' preference for fewer injections has resulted in the need for high-concentration parenterals. The commercialization of high-concentration antibody formulations poses several challenges related to formulation stability, analytics, and manufacturability.[1] In addition, the product presentation needs to mitigate any potential challenges related to the administration of viscous drug products. For many target therapeutic indications, the drug is expected to be self-administered by the patient via a delivery device such as an autoinjector. Such autoinjectors can be very effective in enhancing user convenience, driving a competitive advantage.[2] Market surveys and historical experience related to the therapeutic area in question can be used to develop end-user requirements and drive the final product presentation.[3] When developing high-concentration formulations, it is important to assess their injectability and functionality earlier during process development. This article presents theoretical and experimental framework on how such evaluations can be applied, with the goal of developing a robust formulation that is stable and optimal for delivery.

Three specific aspects of the protein–device combination product are explored in this study: (a) the characterization of product viscosity over a broad concentration and temperature range, (b) the characterization of product–syringe interactions and its impact on friction forces associated with delivery, and (c) the characterization of product rheological behavior under the high shear rates ($\sim$100,000 s^{-1}) associated with syringe injection. The effect of component variability on the delivery forces and injection time has been studied earlier and the results have been presented in a separate article.[4]

In addition to product rheology and product–syringe interactions, the relationship between the measured force and viscosity has been examined using theoretical predictions for laminar flow in a tube (the Hagen–Poiseuille equation).[5] Mechanistic models have been verified with experimental data, allowing for characterization over a wide design space beyond the operating set points. Such broad design space characterization is essential to understand the functionality of the device system, set appropriate

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performance specifications, and ensure that the final product remains within the specifications.

MATERIALS AND METHODS

Siliconized glass and plastic syringes from different vendors were used in the study. Brookfield standards of different viscosities (Brookfield 5, 10, and 50 cP) were used as Newtonian liquid standards for calibration purposes. The rheological behavior of several high-concentration antibody products (>100 mg/mL) was evaluated under different temperature and shear conditions.

Product viscosity measurements were performed using a double-gap rheometer (Malvern, Worcestershire, UK) under low-shear-rate (10–150 s^{-1}) conditions. The extrusion force measurements in syringes were performed using a mechanical testing system (Instron 5564; Instron, Norwood, Massachusetts), equipment designed to evaluate the mechanical properties of materials and components. The force measurements from the Instron 5564 were further utilized for viscosity characterization over a wide range of shear rates; the details of this methodology are discussed in the *Theory* section.

Mathematical models have been applied to model the rheological behavior over a wide range of temperatures and product concentrations. Analytical models discussed earlier in the context of estimating extrusions forces[4] have been applied to characterize viscosity over a range of shear stresses associated with syringe injection.

THEORY

The system under consideration is flow through a prefilled syringe as shown in Figure 1. The syringe consists of a barrel of radius r_b with an attached needle of length L_n and radius r_n. An external force is applied through the plunger rod to drive the fluid with an injection speed $\bar{v}$ (plunger rod speed in length over time dimensions). The break-loose force refers to the maximum force required to set the plunger rod into motion. The extrusion force is the force required to sustain the plunger rod in motion while maintaining the desired flow rate of product through the needle. This study characterizes the total extrusion force associated with delivery of a product through syringe injection.

Friction and Hydrodynamic Forces

The total extrusion force required to deliver an injection can be described as:

$$F_{\text{total}} = f_{\text{friction}} + F_{\text{hydrodynamic}}, \quad (1)$$

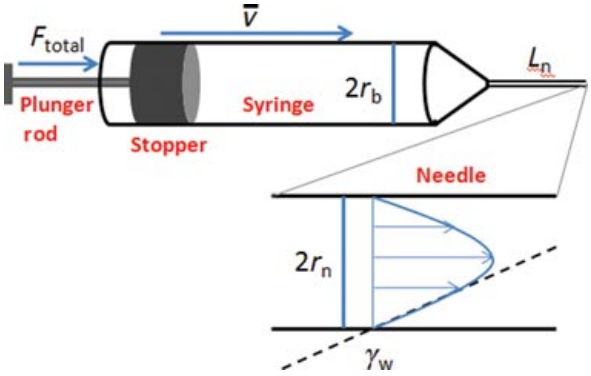

Figure 1. A schematic of the various components associated with the syringe delivery system.

where F_{total} is the total force needed for driving the plunger, f_{friction} is the friction force between the stopper and the syringe wall, and $F_{\text{hydrodynamic}}$ is the hydrodynamic force required to drive the fluid out of the needle. Hydrodynamic forces arising from the pressure drop associated with syringe barrel and entry losses into the needle are assumed to be negligible.

The f_{friction} arises from the interaction between the stopper and the glass syringe barrel. For siliconized syringes, a silicone oil layer serves as lubricant between the stopper and barrel surface. As discussed in a previous article,[4] the f_{friction} is dependent on the syringe and stopper geometry, the level of siliconization, and the injection speed. In addition, the friction can also be impacted by interactions between the filled product and glass barrel. As a result, in order to properly estimate the F_{total} using Eq. 1, it is important to estimate the f_{friction} for the specific product–syringe system under consideration. The f_{friction} for such a system (syringe barrel wetted with product) can be estimated by removing the needle from the syringe (making the hydrodynamic component negligible) and measuring the total extrusion force.

The $F_{\text{hydrodynamic}}$ results from the pressure drop required to drive the fluid out of the syringe. For Newtonian fluids, the relationship between the pressure drop ΔP required to drive the fluid at flow rate Q (units: volume/time) through a cylindrical tube is given by the Hagen–Poiseuille equation

$$\Delta P = \frac{8\mu L Q}{\pi r^4}, \quad (2)$$

where μ is the viscosity of fluid, r is the radius of the tube, and L is the length of the cylindrical tube.[5] The equation assumes laminar flow of an incompressible liquid through a channel of constant cross-section diameter of $2r$.

The hydrodynamic component of extrusion force has previously been estimated[4] as a function of solution properties, syringe dimensions, and injection

speed for both Newtonian and non-Newtonian fluids:

$$\text{Newtonian}: F_{\text{hydrodynamic}} = \left(\frac{8\pi\mu L_n r_b^4}{r_n^4}\right)\bar{v} \quad (3)$$

$$\text{Non}-\text{Newtonian}: F_{\text{hydrodynamic}} = \left(\frac{3n+1}{n}\right)^n \frac{2\pi K L_n r_b^{2n+2}}{r^{3n+1}}\bar{v}^n \quad (4)$$

where n is the power-law index ($n = 1$ representing the Newtonian case), K is defined as the flow consistency index, μ is the viscosity, r_b is the radius of the syringe barrel, r_n is the radius of the needle, L_n is the length of the needle, and $\bar{v}$ is the linear speed of the stopper.

Estimation of Product Viscosity from Injection Force Data

Equations 3 and 4 can be used to estimate the extrusion force associated with a syringe injection, given the knowledge of product rheological behavior.[6] Product viscosity measured at low shear rates (100–1000 s^{-1}) using common laboratory rheometers may not be completely representative of the rheological behavior under the high shear rates associated with commonly used injection times (100,000 s^{-1}).[7] The following sections present the theoretical framework used to characterize product viscosity under high shear rates. Standards of known viscosity have been used for calibration.

Newtonian Fluids

If the pressure drop across the syringe is known, then Eq. 3 can be used to calculate the rheological properties of a Newtonian fluid. The shear stress for the syringe system can be expressed as:

$$\tau_w = \frac{\Delta P r_n}{2L_n} = \left(\frac{F_{\text{total}} - f_{\text{friction}}}{\pi r_b^2}\right)\frac{r_n}{2L_n}, \quad (5)$$

where τ_w is the shear stress at the wall or barrel surface and ΔP is the pressure drop primarily governed by the flow through the syringe needle.[5] The shear rate is given by:

$$\gamma_w = \frac{4Q}{\pi r_n^3} = \frac{4\bar{v}r_b^2}{r_n^3}, \quad (6)$$

where γ_w is the shear rate at the wall.[8] Equations 5 and 6 can be combined to give the relation for the viscosity of the fluid as:

$$\mu = \frac{\tau_w}{\gamma_w} = (F_{\text{total}} - f_{\text{friction}})\left(\frac{r_n^4}{8\pi L_n r_b^4 \bar{v}}\right). \quad (7)$$

This equation allows the Instron 5564 to be used as a microcapillary rheometer to estimate viscosity for Newtonian fluids based on injection force and dimensions of syringe components.

Non-Newtonian Fluids

The shear stress for a non-Newtonian fluid can be calculated in an analogous fashion:

$$\tau_w = \frac{\Delta P r_n}{2L_n} = \left(\frac{F_{\text{total}} - f_{\text{friction}}}{\pi r_b^2}\right)\frac{r_n}{2L_n}. \quad (8)$$

The expression for the shear rate is:

$$\gamma_{app} = \frac{4Q}{\pi r_n^3}, \quad (9)$$

where γ_{app} is known as the apparent shear rate. The wall shear rate, γ_w, is related to the apparent shear rate as[8]:

$$\gamma_w = \left(\frac{3n+1}{4n}\right)\frac{4Q}{\pi r_n^3} = \left(\frac{3n+1}{4n}\right)\gamma_{app}. \quad (10)$$

The calculation of viscosity of non-Newtonian fluid involves estimation of two parameters: K and n. Equations 8–10 can be used to calculate the apparent viscosity as

$$\mu_{app} = \tau_w/\gamma_{app} = \left[K\left(\frac{3n+1}{4n}\right)^n\right](\gamma_{app})^{n-1}. \quad (11)$$

where μ_{app} is the apparent viscosity. The power-law index and K can then be calculated by fitting μ_{app} versus γ_{app}. The true viscosity and apparent viscosity are related as:

$$\mu_{true} = \left(\frac{4n}{3n+1}\right)\mu_{app}, \quad (12)$$

where μ_{true} is the true viscosity.

RESULTS AND DISCUSSION

Complete understanding of the injectability of a drug product requires knowledge of the product–syringe interactions (and their impact) as well as the product rheology. Small variations in the product concentration or operating conditions such as temperature could significantly impact the product viscosity and the resultant extrusion forces. The following sections

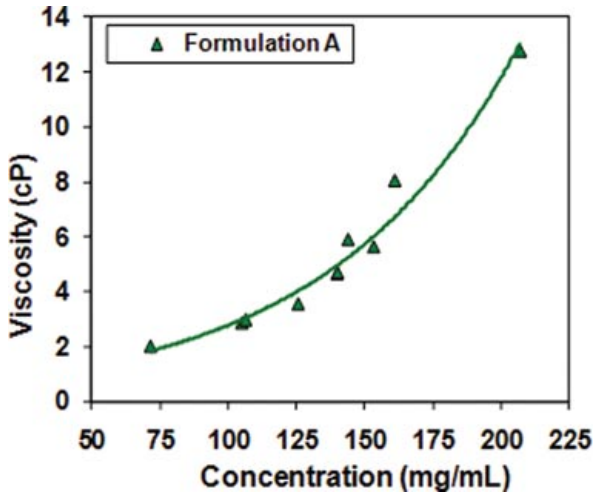

Figure 2. Concentration dependence of the viscosity for model antibody formulation. Note that the viscosity increases exponentially with protein concentration.

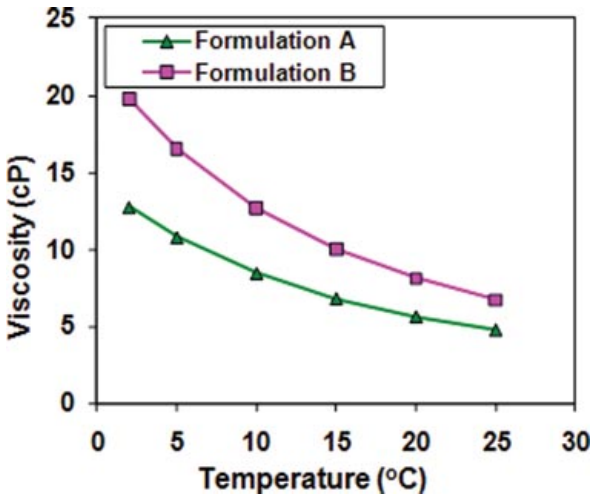

Figure 3. Temperature dependence of the viscosity of two different formulations of Product A. Note that the viscosity increases exponentially as temperature decreases.

present the results from characterization studies conducted to understand the friction forces and the product rheological behavior under high-shear conditions.

Product Viscosity Characterization Over A Temperature and Concentration Range

Viscosity is an important factor in the development of high-concentration therapeutics because of its strong dependence on protein concentration and temperature. This dependency is the result of macromolecular crowding (non-product specific) as well as product-specific intermolecular interactions.[1,7] It is common for biotechnology products to have an allowed tolerance of about 10% around the target protein concentration. Such tolerances are usually set based on the consistency of the manufacturing process as well as the variability associated with protein concentration measurement. Small variations (≤10%) in protein concentrations may not contribute to significant changes in product viscosity if the target concentration is low. However, for high-concentration products, such variations could result in a significant increase in product viscosity. Figure 2 shows that the solution viscosity of a model high-concentration antibody (Product A) increases exponentially as the protein concentration is increased beyond 100 mg/mL, highlighting the importance of accurately characterizing the viscosity over a wide concentration range.

Viscosity dependence on product temperature is equally critical, and as such has been studied extensively in the literature.[9–11] A robust device design should ensure functionality not only at a specific temperature, but also over a broad temperature range as defined by the end-user requirements. Figure 3 shows the exponential temperature dependence of the viscosity of two different formulations of Product A. Any injection time specifications should take into account the allowed operating temperature range and the associated impact on product viscosity.

A statistical analysis was performed to explore any cross-interaction between temperature and concentration of an antibody product. A leverage plot analysis using JMP statistical software (SAS Institute, Cary, North Carolina) showed that a cross-interaction term is indeed required to adequately capture the viscosity dependence on product concentration and temperature. The following equation was obtained after performing linear regression between $\ln(\mu)$, $1/T$, C, and a C/T interaction term:

$$\ln \mu = -12.103 + 0.0189C + \frac{3297}{T} + 26.7(C - 132.3)\left(\frac{1}{T} - 0.00352\right) \quad (13)$$

where C is the concentration of product in mg/mL, T is the temperature in Kelvin, and μ is the predicted viscosity in centipoise. This model can be used to represent product viscosity over a wide temperature and concentration space, as shown in Figure 4.

Product–Syringe Interaction and Its Impact on $f_{friction}$

As described earlier, the total delivery force is a sum of the $f_{friction}$ and the extrusion force needed to overcome the pressure drop across the syringe. Friction force in a syringe depends on the nature of the inner surface of barrel (e.g., lubrication with silicone oil) and the stopper properties. Friction force would be independent of the properties of filled product, provided the formulation does not interfere with the lubricating medium. However, in most practical cases,

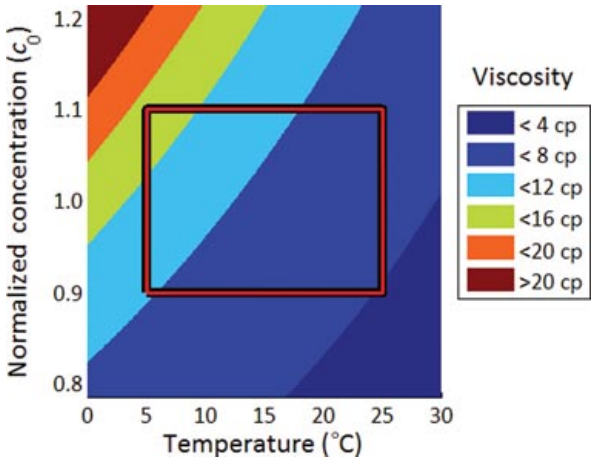

Figure 4. Product A viscosity as a function of temperature and concentration. The highlighted box indicates the range of interest for this product ($c_0 \pm 0.1c_0$ mg/mL, 5–25°C) based on intended usage.

the product formulation can interact with the lubricated glass barrel and modify the $f_{friction}$. Figure 5 provides a qualitative assessment of the impact of different formulations on the $f_{friction}$ for different syringe types (glass and plastic). The friction forces in glass syringes for water and buffer without Polysorbate-20 (PS-20, a commonly used surfactant) are similar to that of an empty syringe, indicating that these solutions do not interact significantly with the lubricating silicone oil layer on the glass barrel. The extrusion force profiles for the PS-20 free buffer samples showed reasonable consistency with a coefficient of variation (CV) of about 15%. However, buffer samples containing PS-20 showed significant variability in extrusion force profiles (CV of 40%). Measured friction forces in glass syringes for all buffer samples with PS-20 were higher than corresponding PS-20 free buffer samples, suggesting potential interactions between the surfactant formulation and the lubricated glass barrels. The behavior is different for plastic syringes, wherein the $f_{friction}$ for drug product without PS-20 is significantly higher than that for buffer and product with polysorbate.

Surfactants such as PS-20 can leach silicone oil and also serve as potential lubricant themselves, although silicone oil is a better lubricating agent than PS-20. Silicone oil leaching likely explains the observation that the presence of PS-20 increases the $f_{friction}$ in glass syringes. Conversely, plastic prefilled syringes do not have any silicone-oil-driven lubrication, and thus PS-20 may serve as a lubricating agent to reduce the $f_{friction}$. Protein present in the active formulation can also increase the surface activity of the solution, resulting in the leaching of silicone oil from glass syringes. Depending on product type, the protein molecules could also bind to the surface of the syringe barrel, particularly in poorly siliconized areas, and impact the necessary injection force. Complete understanding of protein–surface interactions is essential to estimate their impact on the friction forces over the duration of the shelf life of injection devices. Given the complexity and variability in these measurements, a quantitative assessment relying on a larger number of samples is needed to more accurately estimate the impact on friction forces.

Rheological Characterization of Products at High Shear Rates

As described earlier, the shear rates associated with a syringe delivery are typically much higher than shear rates employed by commonly used rheometers. For example, delivering a 1 mL volume through a 27 G needle over an injection time of 6 s would result in a shear rate of about 180,000 s^{-1}, whereas viscosity measurements using a double-gap rheometer are typically performed at shear rates under 1000 s^{-1}.

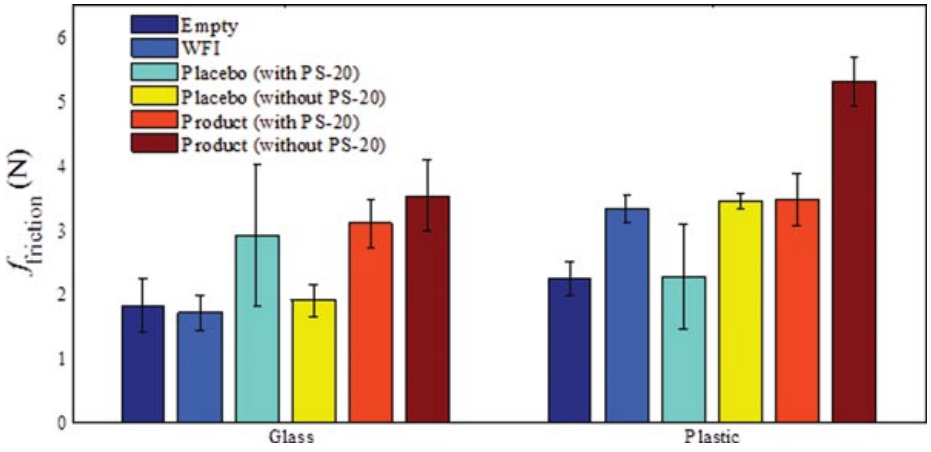

Figure 5. The effect of syringe material on the friction force for different injected liquids.

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