Pharmaceutical aspects of subvisible particles in protein formulations

Weinbuch, D.

CHAPTER 4

LIGHT OBSCURATION MEASUREMENTS OF HIGHLY VISCOUS SOLUTIONS: SAMPLE PRESSURIZATION OVERCOMES UNDERESTIMATION OF SUBVISIBLE PARTICLE COUNTS

Daniel Weinbuch[1,2], Wim Jiskoot[2] and Andrea Hawe[1]

[1] Coriolis Pharma Research GmbH, Am Klopferspitz 19, 82152 Martinsried-Munich, Germany

[2] Division of Drug Delivery Technology, Cluster BioTherapeutics, Leiden Academic Centre for Drug Research (LACDR), Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands

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Abstract

Light obscuration (LO) is the current standard technique for subvisible particle analysis in the quality control of parenterally administered drugs, including therapeutic proteins. Some of those, however, exhibit high viscosities due to high protein concentrations, which can lead to false results by LO measurements. In this study, we show that elevated sample viscosities, from about 9 cP, lead to an underestimation of subvisible particle concentrations, which is easily overlooked when considering reported data alone. We evaluated a solution to this problem, which is the application of sample pressurization during analysis. The results show that this is an elegant way to restore the reliability of LO analysis of highly viscous products without the necessity of additional sample preparation.

Introduction

Regulatory authorities require all parentally administered drugs/products to be tested for subvisible particulate matter. Light obscuration (LO) is the primary method described by the current pharmacopeias (USP <788> and Ph.Eur. 2.9.19) for the quantification of subvisible particles in parenteral products (1,2). However, for biopharmaceutical products other methods, such as flow imaging microscopy or electric zone sensing, are expected by the authorities as well (3). In light obscuration, a syringe pump draws the sample through the system, where particulate contaminants or impurities block a certain amount of light from a laser beam. The resulting "shadow" is detected by an optical sensor and converted into an equivalent circular diameter. However, for highly viscous products, such as high-concentration protein formulations for subcutaneous administration (4), LO measurements are potentially compromised (5) and a more time-consuming microscopy method has to be used, which is not always applicable to amorphous protein particles (1). High protein concentrations can impede light obscuration measurements because of an increased refractive index of the solution. Herewith the RI difference between proteinaceous particles and solution becomes so small that the particles become "invisible", which has been investigated previously by our group (6). In this study we focused on the influence of high viscosity to show that elevated sample viscosities from about 9 cP lead to an underestimation of subvisible particle concentrations, which is easy to overlook when considering reported data alone. We evaluated a solution to this problem involving the application of sample pressurization during analysis. The results show that this is an elegant way to restore the reliability of LO analysis of highly viscous products, e.g., highly concentrated protein formulations, without necessitating additional sample preparation.

Materials & Methods

Materials

Glycerol (≥ 99%) was purchased from Sigma Aldrich (Steinheim, Germany) and pharmaceutical grade sucrose was provided by Südzucker (Mannheim, Germany). Polyclonal IgG (Hizentra®) was obtained from a local pharmacy. NIST traceable 2-µm polystyrene sizing standards were purchased from Thermo Scientific (Ulm, Germany).

Sample preparation

Glycerol and sucrose solutions were prepared in purified water in the stated concentrations. A highly concentrated protein solution at elevated viscosity (48 cP) was

obtained by upconcentration of polyclonal IgG (Hizentra®) from 200 mg/mL to about 250 mg/mL by using a centrifugal filter unit with a 10 kDa molecular weight cutoff (Millipore, Schwalbach, Germany). Subsequently, all solutions were filtered through a 0.22-µm syringe filter (Millipore, Schwalbach, Germany). Samples were measured with or without the addition of polystyrene sizing standards (2 µm) in a final dilution of 1:10,000.

Light obscuration (LO)

Particle concentrations in a size range between 1 and 200 µm were measured with a PAMAS SBSS (Partikelmess- und Analysesysteme GmbH, Rutesheim, Germany) equipped with an HCB-LD-25/25 sensor, a 1-mL syringe pump, and a pressurizable sample chamber (Figure 1). Four measurements of 0.3 mL with a pre-run volume of 0.2 mL and a fixed flow rate of 10 mL/min were performed following the current draft USP <787> method (5) with or without pressurization of the sample chamber at 4 bar above atmosphere. The mean particle concentration was calculated from the last 3 (out of 4) measurements. Unless stated differently, samples were measured in triplicate and mean and standard deviations were calculated.

Viscosity measurements

A Paar Physica MCR-100 rheometer (Anton Paar GmbH, Ostfildern-Scharnhausen, Germany) equipped with an MK22 cone was used to measure the dynamic viscosity of a 1-mL sample at 20°C every 5 s during a shear rate ramp from 50 to 500 s^{-1} over 17 min with a cone-to-plate gap of 50 µm. All solutions showed Newtonian behavior. Samples were measured in triplicate and mean and standard deviations were calculated.

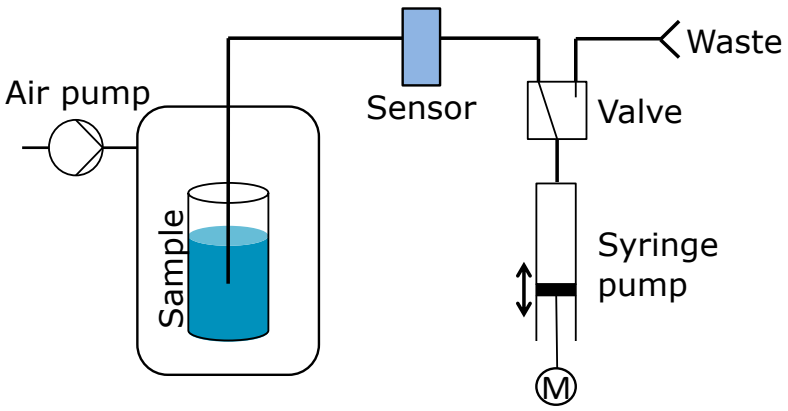

Figure 1: Schematic overview of the PAMAS SBSS light obscuration device

Results and discussion

Filtered solutions of sucrose and glycerol (0, 25, 50 and 75% w/v and v/v, respectively) were used to simulate high-viscosity samples. Analysis by light obscuration at 0 and 4 bar sample pressurization resulted in low background counts (< 100 particles/mL > 1 µm), showing that sample preparation and/or the light obscuration system itself introduce negligible quantities of foreign particulate matter (data not shown). Next, purified water, sucrose, and glycerol solutions were spiked with 2-µm polystyrene sizing standards, resulting in approximately $8x10^4$ particles per milliliter, as measured in purified water at ambient pressure conditions (Figure 2A). Results of the polystyrene sizing standards in purified water, measured under sample pressurization, showed similar particle counts. At increased concentrations of glycerol (> 50% v/v) or sucrose (> 75% w/v), however, particle concentrations apparently decreased when measured at ambient pressure conditions. A similar observation was made when a highly concentrated protein solution (polyclonal IgG at approx. 250 mg/mL) with a viscosity of 48 cP spiked with 2-µm polystyrene sizing standards was measured. Here the determined particle concentration, derived from a single LO measurement, decreased from $8.2x10^4$ particles per ml when measured at 4 bar to $4.3x10^4$ particles per ml when measured without sample pressurization (Figure 2B), showing the relevance of the problem to highly concentrated protein formulations.

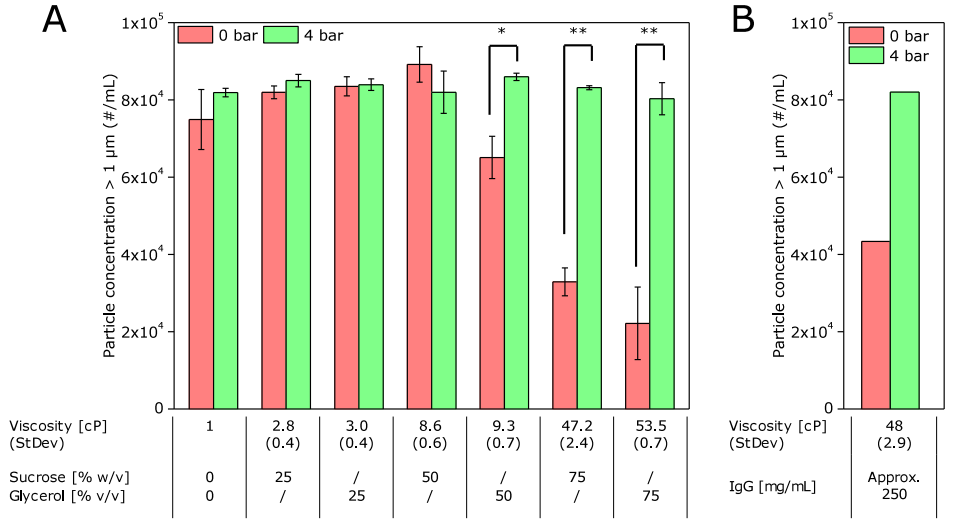

Figure 2: Measured particle concentrations by light obscuration, with and without sample pressurization, of A) water and different glycerol and sucrose solutions and B) high concentrated protein solution, all containing a fixed concentration of 2-µm polystyrene sizing standards. Error bars and values in brackets show standard deviations from triplicate particle concentration and viscosity measurements, respectively. *p<0.05, **p<0.005 (based on one-way ANOVA)

The reduction in particle counts can be explained by an intake of air into the syringe pump of the LO system (Figure 3). This air intake was also observed for the same highly concentrated glycerol and sucrose solutions without the addition of sizing standards, however, with no significant effect on the measured particle concentration. This indicates that the air bubbles did not pass the detector, because their high refractive index makes them easily detectable by the system (7). Thus, the air enters the light obscuration system between sensor and syringe pump cell through tubing connections and/or valves, as a result of an under-pressure created by the slow-moving high-viscosity solutions. This results in an overestimated measurement volume and, consequently, an underestimation of particle concentrations. The sample viscosity at which the effect started to occur in our tested system was approximately 9 cP (Figure 2A), a value that can easily be reached in concentrated protein formulations (8).

Figure 3: Fig. 3. Image of the PAMAS SBSS light obscuration syringe pump aspirating A) purified water and B) 75% (w/v) sucrose solution during sample measurement at ambient pressure conditions.

Since the air intake may depend on the state of the system tubing and valves, two other light obscuration systems (type PAMAS SVSS) —one equipped with a 1-mL syringe pump and the other with a 10-mL one— were tested as well. All of the tested systems showed a similar air intake at very comparable viscosity values (results not shown). This indicates that it is a general problem that is not related to one specific PAMAS system. Moreover,

depending on their maintenance state, individual LO systems might leak air at even lower viscosity values.

It is important to realize that the underestimation of particle concentration resulting from air intake may be overlooked when considering reported data alone. The syringe pump needs to be observed by the operator during method development and specifically tested for air intake when samples of increased viscosities are to be analyzed by LO. Alternatively, or in addition, one could follow the method described in this study and verify if counting or sizing standards spiked into water and into the formulation result in a similar increase in particle counts.

The application of light obscuration can, given the requirements of regulatory authorities and current pharmacopeias, only be circumvented by the application of microscopic techniques, which are more labor-intensive and less precise (9). The current draft USP <787>, which is tailored for the analysis of biopharmaceuticals, states that sample dilution with a low viscosity solvent (e.g., purified water) is a possible “last resort” solution. This, however, may have an influence on the composition, distribution or concentration of proteinaceous particles. Another more elegant way to measure high-viscosity solutions without sample dilution is the application of overpressure on the sample side. As shown in Figure 2, the application of 4 bar above atmospheric pressure can restore the reliability of light obscuration measurements for highly viscous solutions with viscosities of up to at least 50 cP.

Conclusions

We have demonstrated that particle concentrations in highly viscous samples, as measured by light obscuration, are potentially underestimated. This is due to an intake of air into the measurement system and consequently a reduced measurement volume. Importantly, this can easily be overlooked by the operator, since blank measurements are not affected, though they should be anticipated prior to the analysis of viscous samples. Sample pressurization is a simple and effective way to overcome this problem even for solutions with viscosities above 50 cP.

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