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Note

Pharmaceutical feasibility of sub-visible particle analysis in parenterals with reduced volume light obscuration methods

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ABSTRACT

The draft for a new United States Pharmacopoeia (USP) monograph ⟨787⟩ "Sub-visible Particulate Matter in Therapeutic Protein Injections" describes the analysis of sub-visible particles by light obscuration at much lower sample volumes as so far required by the European Pharmacopoeia (Ph. Eur.) and the USP for parenterals in general. Our aim was to show the feasibility of minimizing the sample expenditure required for light obscuration similar to the new USP settings for standards and pharmaceutically relevant samples (both proteins and small molecules), without compromising the data quality. The light obscuration method was downscaled from >20 ml volume as so far specified in Ph. Eur./USP to 1 ml total sample volume. Comparable results for the particle concentration in all tested size classes were obtained with both methods for polystyrene standards, stressed BSA solutions, recombinant human IgG1 formulations, and pantoprazol i.v. solution. An additional advantage of the low volume method is the possibility to detect vial-to-vial variations, which are leveled out when pooling several vials to achieve sufficient volume for the Ph. Eur./USP method. This is in particular important for biotech products where not only the general quality aspect, but also aggregate formation of the drug substance is monitored by light obscuration.

1. Introduction

Light obscuration has been the key technique for sub-visible particulate quantification in parenteral products, as it is required by most Pharmacopoeiae [1,2]. In cases where light obscuration is not possible, e.g. due to high turbidity or viscosity, microscopy is allowed as an alternative method [1,2]. If neither light obscuration nor microscopy are feasible due to too high viscosity it is allowed to dilute the samples for the analysis.

The currently valid pharmacopoeial methods were originally developed for quality control of parenterals in general and non-product related sub-visible particles, e.g. deriving from glass vials or stoppers were the main impurities to be detected. Both Pharmacopoeiae currently require large sample volumes to be measured (5×5 ml), which often translates into (i) extensive, error-prone sample pooling procedures, in many cases requiring 20 or more single doses, (ii) the inability to assess vial-to-vial variations and (iii) extraordinarily high costs especially for expensive protein therapeutics that are frequently marketed in low volume dosage forms like ready to use syringes, often with volumes of only 0.2 or 0.3 ml. Although it is technically possible to analyze sub-visible particles by light obscuration using clearly lower sample volumes as required by the Pharmacopoeiae [3,4] release testing, it still performed using high sample volumes.

Both Pharmacopoeiae give specifications for particulates larger than 10 and 25 μm, which originates from the fact that blood vessels might be blocked upon parenteral administration of such particulates. The significance of the specified limits for particles >10 μm and >25 μm (less than 6000 and 600 particles per container for low volume parenterals) for therapeutic protein formulations is heavily discussed in the literature [5,6]. Smaller sub-visible particulates in the size range from 1 to 10 μm, which are present in almost all commercial protein products [6], are so far not addressed by the Pharmacopoeiae.

In 2012, a draft version for a new United States Pharmacopoeia (USP) monograph ⟨787⟩ "Sub-visible Particulate Matter in Therapeutic Protein Injections" has been issued that describes measuring light obscuration at clearly lower sample volumes [7]. Instead of the so far required five aliquots of 5 ml, it is left to the user to measure five aliquots with a volume between 0.2 ml and 5 ml [7]. It can be expected that low volume methods will have a high regulatory impact, once this new monograph is made official.

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Our aim was to compare sub-visible particle levels measured by a low volume method to the results generated with the existing Ph. Eur./USP method for polystyrene standard beads and pharmaceutically relevant samples (heat-stressed BSA solutions, formulations of a monoclonal IgG, and a reconstituted pantoprazol i.v. solution). With our study, we support the draft of the novel UPS monograph ⟨787⟩ and show pharmaceutically relevant data for the new approach. Beyond this, we not only focused on data for particles $\geqslant$10 μm and $\geqslant$25 μm, but on a size range between 1 and 200 μm. Additionally, the suitability of low volume methods to assess vial-to-vial variations has been addressed.

2. Materials and methods

2.1. Materials

A mixture of polystyrene standards of 2 μm, 10 μm, and 25 μm (Duke Scientific, Palo Alto, USA) was prepared with a concentration of about 18,000 particles > 1 μm per ml, 2000 particles > 10 μm per ml, and about 50 particles > 25 μm per ml. Sufficient solution was prepared to perform all experiments from the same sample solution, and the solution was stored at 2–8 °C.

1.0 mg/ml bovine serum albumin (BSA) (Sigma–Aldrich, Steinheim, Germany) in 50 mM citrate (Sigma Aldrich, Steinheim Germany) pH 4.8 was thermally stressed for 30 min at 70 °C in order to create sub-visible particles. By dilution of the stressed BSA solution with buffer two particle concentrations per ml were prepared: BSA solution A with ~100,000 > 1 μm, ~170 > 10 μm, and ~2 > 25 μm; BSA solution B with ~10,000 > 1 μm, ~10 > 10 μm, and ~1 > 25 μm. The BSA solutions were filled into 2R glass type I vials (1.2 ml per vial) for the measurements.

Lyophilized formulations of a recombinant human IgG1 were reconstituted with 1 ml filtered (0.2 μm) water for injection per vial under gentle swirling. Lyophilized pantoprazol i.v. solution (Pantozol® i.v., Nycomed, Konstanz, Germany) was reconstituted with 10 ml filtered (0.2 μm) 0.9% NaCl solution (Serag Wiessner, Naila, Germany).

2.2. Light obscuration

The PAMAS SVSS-C Sensor HCB-LD-25/25 (Partikelmess-und Analysensysteme GmbH, Rutesheim, Germany) was used for light obscuration measurements. The linear concentration range is specified up to a total particle concentration of 120,000 particles per ml. This is clearly higher than for most instruments where ~20,000 particles per ml is the upper concentration limit [8]. The size range between 1 and 200 μm is covered. For measurements according to the Ph. Eur./USP, a pre-run volume of 1 ml was followed by 4 × 5 ml analyzed sample (in total 21 ml sample volume). A 10 ml syringe was used within the PAMAS SVSS-C Sensor HCB-LD-25/25, and the drawing speed was 10 ml/min.

For the low volume method, the system was equipped with a 1 ml syringe. During method development pre-run volume, measurement volume and number of subruns were tested. The final method uses a pre-run volume of 0.4 ml and 2 × 0.3 ml analyzed sample.

Sample preparation for the Ph. Eur./USP method required pooling of sufficient units, e.g. 20–25 units for the BSA solutions and the

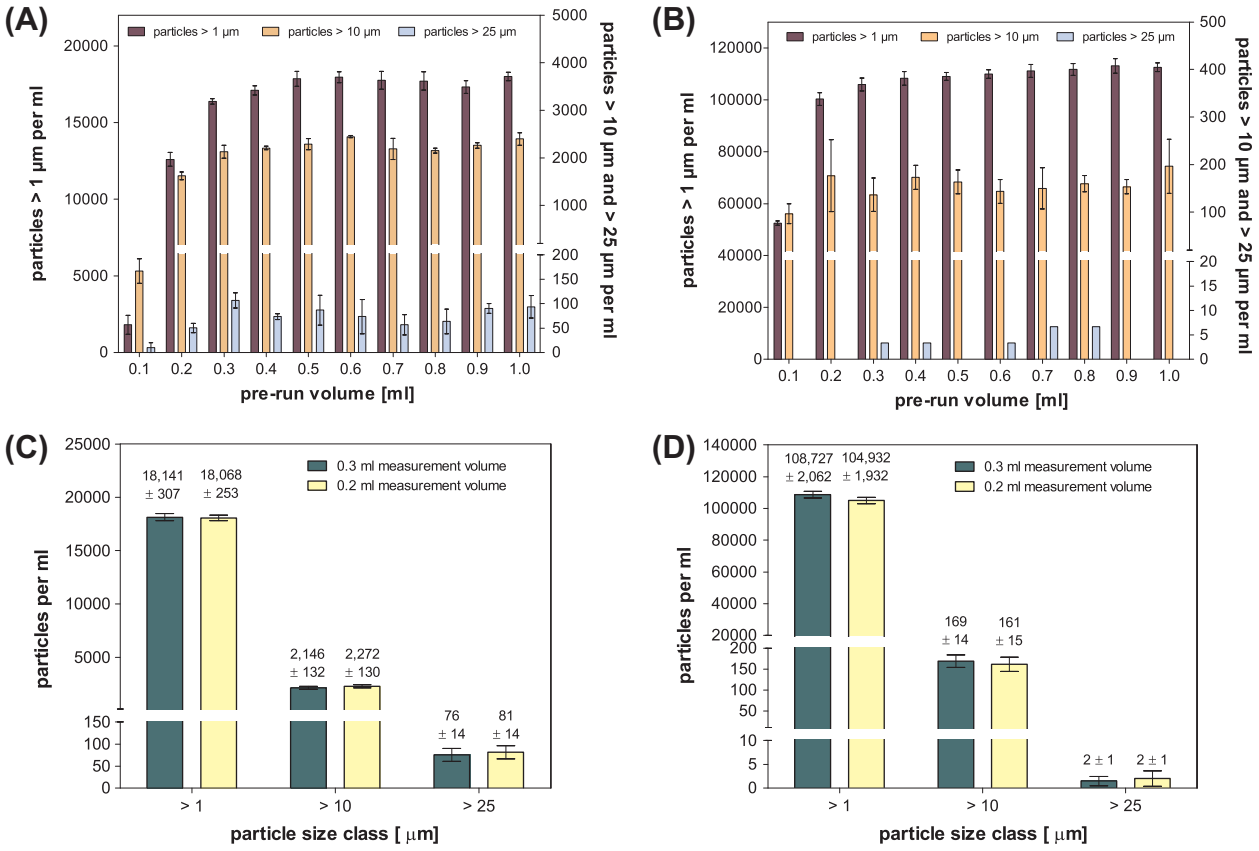

Fig. 1. Influence of pre-run volume on particles >1 μm, >10 μm and >25 μm for the polystyrene standard mixture (2 μm – 10 μm – 25 μm) (A) and BSA solution A (B). Results are given as mean and standard deviation of the $n = 3$ independent experiments. Reproducibility of 10 measurements at a measurement volume of 0.3 ml and 0.2 ml for the polystyrene standard mixture (2 μm – 10 μm – 25 μm) (C) and BSA solution A (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)