International Journal of Pharmaceutics 416 (2011) 293–295

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Note

Reference material development for calibration and verification of image-based particle analyzers

Deepak K. Sharma, David King, Clark Merchant*

Brightwell Technologies Inc., 115 Terence Matthews Crescent, Ottawa, ON, Canada K2M 2B2

ARTICLE INFO

Article history:
Received 8 February 2011
Received in revised form 24 May 2011
Accepted 29 May 2011
Available online 23 June 2011

Keywords:
Sub-visible particles
Particle sizing
Particle counting
Protein aggregation
Micro-Flow Imaging
Image analysis

ABSTRACT

The need is well recognized for suitable reference populations for calibrating and verifying the size and concentration accuracy of particle analysis instruments for use in the measurement of suspended protein particles in biopharmaceuticals. Polystyrene bead standards are normally used as a reference material for calibrating and validating particle analyzers. However, these standards, unlike protein particles, are easily detected and do not challenge the sensitivity of optical instruments. Groups of instruments verified only with beads can still exhibit significant differences in measuring concentrations of more challenging protein particles. To minimize these and obtain consistent concentration measurements between instruments, reference populations must closely resemble protein aggregates in possessing high transparency and a refractive index close to typical protein matrix fluids. This paper describes work on evaluating a promising reference candidate and the use of this to harmonize the performance of Micro-Flow Imaging instruments. Results show that use of a suitable reference population can significantly increase measurement consistency when multiple instruments are used to characterize the same protein particle suspension.

The need to measure sub-visible protein particles in biopharmaceuticals has been stressed in a number of recent publications (Carpenter et al., 2009; Wuchner et al., 2010). Since such measurements may be used to guide decisions in formulation development, lot release, and for regulatory submission, achieving high sensitivity, accuracy and consistency are of prime importance. The USP ⟨788⟩ particle measurement method employs polystyrene (PS) standards as a reference population for calibrating light obscuration (LO) instruments. However LO, thus calibrated, have been demonstrated to count and size only a small fraction of the protein particulates present in many biopharmaceutical formulations (Wuchner et al., 2010; Sharma et al., 2010a). Micro-Flow Imaging (MFI) demonstrates higher sensitivity than LO in measuring protein aggregate populations (Sharma et al., 2010b). However, early work also found that instruments which displayed identical concentration measurements for populations of PS beads could still demonstrate significant differences when applied to more challenging protein populations (Huang et al., 2009; Sharma et al., 2007). This was attributed to the fact that PS beads, unlike protein particles, are easily detected and do not challenge the sensitivity of optical particle analyzers. In contrast, protein aggregates are transparent and have refractive indices close to their carrier fluids. In obscuration analyzers, the detector response is calibrated with polystyrene beads of known size. Protein aggregate particles of the same size interact less with the illumination, creating smaller signals at the detector and as a result are undersized or missed entirely. Imaging based instruments are less susceptible to particle properties in that, provided a particle interacts sufficiently and that the pixels within its image are identified by the system threshold, the particle will be measured correctly. Validation of sizing accuracy using PS beads is legitimate for a wide range of particle interactions. However, if the interaction is too weak, some or all pixels within a particle image will not receive adequate signals. These particle images may be undersized, fragmented, or missed entirely. The ability of an instrument configuration to minimize such effects is a measure of its sensitivity. For accurate measurement of transparent particles, this sensitivity should be as high as possible. For consistent measurement across a group of instruments, all should have the same sensitivity. While fine-tuning of the instrument can reduce variations, a suitable reference population against which instruments could be adjusted to a common sensitivity is required. Desirable properties of such a protein sensitivity reference material have been identified as (Ripple, 2010): irregular structure, aspect ratio ranging from fibrous to near-spherical, small refractive index between particles and matrix fluid, stable over long periods and with expected variations in use, viscosity of matrix liquid in range of water to typical protein formulations, and convenient to use. This paper describes work on developing a candidate reference material

* Corresponding author. Tel.: +1 613 591 7715; fax: +1 613 591 7716.
E-mail address: cmerchant@brightwelltech.com (C. Merchant).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.05.078

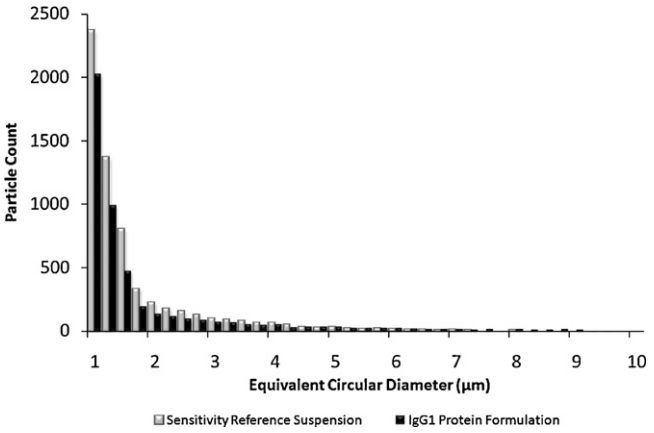

Fig. 1. Particle size distributions for SRS and the protein aggregate model.

(termed a sensitivity reference suspension, or SRS) and use of this material to calibrate the sensitivity of MFI instruments.

Ideally, a reference suspension would be an absolute standard where the concentration and size distribution measurements obtained with an instrument could be compared with absolute values known for the standard suspension. However, obtaining acceptable stability without compromising the need for high sensitivity makes creation of an absolute standard a difficult challenge. The initial objective has been to develop a reference suspension which is sufficiently challenging and stable to permit comparisons to be made between large groups of instruments. Initial work was aimed at identifying a protein particle population that could serve as an SRS. However, protein particles, such as precipitates which displayed reasonable stability, did not provide sufficient optical challenge. More challenging populations, such as aggregates, were not sufficiently stable.

Following an evaluation of a number of non-protein candidates, a promising SRS particle population was identified which consisted of a stabilized suspension of precipitated silica in an acidic solution (pH 2.0 ± 0.2).

The physical and optical properties of the silica particles and their relative behavior in MFI measurements were compared with a population of typical protein aggregates. Two protein populations were used during this study: a model protein drug formulation (IgG1) at a concentration of 1 mg/mL, and a proprietary customer-supplied protein formulation containing a near-transparent protein aggregate population. Protein particles were generated in the IgG1 mAb solution using a freeze–thaw method (Sharma et al., 2010a).

Table 1
Morphological characteristics of PS beads, SRS, and a model protein (IgG1).

Particle size	Brightfield intensity mean (0–1023 units)			Circularity (0–1)		
	PS beads	SRS	IgG1	PS beads	SRS	IgG1
1–10 µm	463.95	752.53	750.21	0.92	0.82	0.69
10–25 µm	316.58	748.16	707.92	0.93	0.46	0.58
>25 µm	210.33	630.39	668.31	0.94	0.56	0.46

The particle size distribution for the SRS and the model IgG1 protein population were found to be similar (Fig. 1). In contrast to the uniform circularity of the PS beads, the protein particles and SRS were observed to be highly heterogeneous in shape, ranging from small dense fibers to large ribbon-like aggregates (Fig. 2). The measured morphological parameters of the three particle types are presented in Table 1, illustrating similarity in intensity and shape values between SRS and IgG1 for particles of comparable size. This contrasts with the dark, highly circular images of the PS beads. Detailed examination of SRS particle images with respect to fragmentation and loss showed that these resembled a very challenging protein particle suspension. This suggests that differences observed in measurement made by different instruments with an SRS sample was likely to be strongly predicative of the subsequent differences which would be observed in protein particle measurement.

A group of fixed-focus MFI 4200 instruments were adjusted and verified for sizing and counting accuracy with PS beads alone. They were then used to measure total particle concentrations in the proprietary protein aggregate population in the range of 1–70 µm (Table 2). The PS bead size measurements between instruments are in good agreement, and are well within the instrument size accuracy specification. However, large inter-instrument variations with respect to the average of the three instruments were observed for the proprietary protein sample particle concentrations.

An adjustment procedure was developed to bring the total SRS concentration measurements made by the less sensitive instruments to within ±15% of the instrument measuring the highest concentration. Subsequent comparison of bead, protein particle and SRS measurements showed that the adjustment procedure significantly reduced variations for protein particle measurement

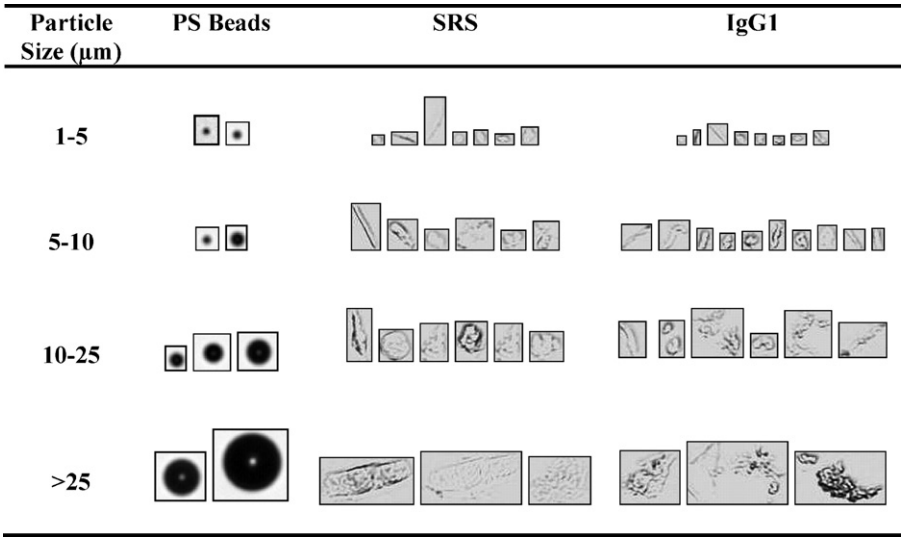

Fig. 2. Representative MFI images of PS beads, SRS and IgG1 particles of comparable size.

Table 2
MFI instruments showing good agreement in bead sizing, but large inter-instrument variations in protein concentration measurement.

MFI instruments	PS beads (10 μm)		PS beads (5 μm)		Concentration variation of protein aggregates[c]
	Size[a,b]	% Variation	Size[a,b]	% Variation	
1	10.11	−0.23%	5.01	0.13%	−29%
2	10.12	−0.13%	4.94	−1.26%	21%
3	10.17	0.36%	5.06	1.13%	8%

[a] PS bead size run-to-run standard deviation values less than ±0.01 ($n = 3$).
[b] Instrument specification ±5% of PS bead nominal mean size.
[c] Protein concentration run-to-run variations less than 3% ($n = 3$).

Table 3
Calibration of MFI instruments with SRS improves repeatability and counting of protein particles without affecting the sizing of conventional PS beads.

MFI instruments	PS beads (10 μm) Size[a]	PS beads (5 μm) Size[a]	Concentration variation of protein aggregates[b]	Concentration variation of SRS[b]
1	10.10	5.12	−1%	−2%
2	10.16	5.07	−6%	−9%
3	10.23	5.03	7%	11%

[a] PS bead size run-to-run standard deviation values less than ±0.01 ($n = 3$).
[b] SRS and protein concentration run-to-run variations less than 3% ($n = 3$).

without influencing PS bead sizing (Table 3), or concentration accuracy. The suitability of the SRS as a challenge population is also supported by the observation that inter-instrument variations, at least for the proprietary protein formulation's aggregate population, are lower than those for the SRS.

To be practically useful as a reference material, it is necessary that a batch of SRS provide consistent concentration measurements for a sufficient period of time and with respect to normal shipping and handling. The results have shown that the SRS formulation is stable (i.e. a variation of <±10%, for 200,000 particles/mL of ≥1 μm particles) for at least one month with prospects for further improvement. Round robin shipping and testing trials show that the formulation retains acceptable stability for inter-site comparisons and inter-instrument qualification/validation.

A reference particle population which emulates the optical properties of protein aggregates has been used to harmonize the sensitivity of MFI instruments during manufacturing. The procedure has been observed to increase the consistency of challenging protein particle concentration measurements when compared with instruments calibrated exclusively with PS beads. The same reference population has also been used to verify the sensitivity of MFI instruments in the field. The use of a technique for harmonizing instrument sensitivity, such as that employed with the SRS, is essential if consistent measurements are to be obtained between challenging protein samples using optical based particle analyzers.

References

Carpenter, J., Randolph, T.W., Jiskoot, W., Crommelin, D., Middaugh, C., Winter, G., Fan, Y., Kirshner, S., Verthelyi, D., Kozlowski, S., Clouse, K., Swann, P., Rosenberg, A., Cherney, B., 2009. Overlooking sub-visible particles in therapeutic protein products: gaps that may compromise product quality. J. Pharm. Sci. 98, 1201–1205.

Huang, C., Sharma, D., Oma, P., Krishnamurthy, R., 2009. Quantitation of protein particles in parenteral solutions using Micro-Flow Imaging. J. Pharm. Sci. 98, 3058–3071.

Ripple, D., 2010. NIST perspective on standards and GMP processes for subvisible particles in protein therapeutics. IBC Formulation Strategies for Protein Therapeutics, September 21, 2010, Rhode Island, Providence, USA.

Sharma, D., King, D., Moore, P., Oma, P., Thomas, D., 2007. Flow microscopy for particulate analysis in parenteral and pharmaceutical fluids. Eur. J. Parent. Pharm. Sci. 12, 97–101.

Sharma, D., King, D., Merchant, C., Oma, P., 2010a. Micro-Flow Imaging: flow microscopy applied to sub-visible particulate analysis in protein formulations. AAPS J., doi:10.1208/s12248-010-9205-1.

Sharma, D., Oma, P., Pollo, M.J., Sukumar, M., 2010b. Quantification and characterization of subvisible proteinaceous particles in opalescent mAb formulations using Micro-Flow Imaging. J. Pharm. Sci. 99, 2628–2642.

Wuchner, K., Buchler, J., Spycher, R., Dlamonte, P., Volken, D.B., 2010. Development of a micro-flow digital imaging assay to characterize protein particulates during storage of a high concentration IgG1 monoclonal antibody formulation. J. Pharm. Sci. 99, 3343–3361.