European Journal of Pharmaceutical Sciences 53 (2014) 95–108

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Effect of solution properties on the counting and sizing of subvisible particle standards as measured by light obscuration and digital imaging methods

Tobias Werk [a,*], David B. Volkin [b], Hanns-Christian Mahler [a]

[a] Pharmaceutical Development & Supplies, Pharma Technical Development Biologics EU, F. Hoffmann-La Roche Ltd., Basel, Switzerland
[b] Department of Pharmaceutical Chemistry, Macromolecule and Vaccine Stabilization Center, University of Kansas, Lawrence, Kansas 66047, USA

ARTICLE INFO

Article history:
Received 24 October 2013
Received in revised form 15 December 2013
Accepted 16 December 2013
Available online 23 December 2013

Keywords:
Light obscuration
Flow imaging
Viscosity
Turbidity
Coloration

ABSTRACT

Purpose: Protein formulations may contain subvisible particle (SbVP) impurities that can vary (e.g., in number, size, shape, density, refractive index and transparency) depending on the formulation composition, environmental stresses and the type of protein. Additionally formulation solutions may differ in their physical properties including turbidity, color, viscosity, density and refractive index. This study examined the impact of these formulation matrix parameters on the ability to size and count subvisible particles using a variety of analytical methods including two light obscuration (HIAC, Syringe) and two digital imaging instruments (MFI®, FlowCAM®). Several subvisible particle standards were tested, including polystyrene and glass beads as well as a new pseudo-protein particle standard, in order to also study of the effect of subvisible particles with different properties.
Results: The color and turbidity of solutions generally had a relatively small effect on SbVP sizing and counting. Solution viscosity and refractive index (RI), however, showed a more pronounced effect on the analytical results, especially with more translucent particles such as glass beads and the "pseudo protein standards", resulting in smaller sizes and lower counts of SbVPs, especially when measuring particles using light obscuration methods.
Conclusions: Each instrument showed certain advantages and disadvantages depending on the analytical parameter (i.e., accuracy, precision), type of subvisible particle, and solution properties. Based on these results, it is recommended to not only carefully consider physical solution parameters as part of analytical method assessment for counting and sizing SbVP in protein dosage forms, but also in terms of various typical QC validation parameters using actual protein formulations.

1. Introduction

Ongoing improvement of detection and quantification methods for subvisible particles (SbVP), that may be present in parenteral dosage forms of protein biotherapeutics, remains an important analytical challenge during formulation development (Das, 2012; Carpenter et al., 2009; Demeule et al., 2010; Krishnamurthy et al., 2008; Ripple and Dimitrova, 2012; Singh et al., 2010; Wuchner et al., 2010). The US and EU pharmacopeias require subvisible particle (SbVP) testing of parenteral drug products by either light obscuration (LO) or light microscopy with corresponding acceptance criteria (Ph.Eur 2.9.19, 2012; USP General Chapter <1788>, 2012; USP General Chapter <788, 2012). Subvisible particles (SbVP) are currently being discussed as relevant degradants in protein formulations due to the hypothesis and concern they may impact potency and/or immunogenicity. (Carpenter et al., 2009; Ripple and Dimitrova, 2012; Singh et al., 2010; Bee et al., 2012; Johnson and Jiskoot, 2012; Marszal and Fowler, 2012; Rosenberg et al., 2012) For small-volume parenteral products (with a fill volume of 100 mL or less), the compendia limits are: (a) using light obscuration: not more than (NMT) 6000 particles larger or equal than 10 μm and NMT 600 particles larger or equal than 25 μm, or (b) using the microscopic method: not more than (NMT) 3000 particles larger or equal than 10 μm and NMT 300 particles larger or equal than 25 μm. It is interesting to note that the Pharmacopeias acknowledge different numerical numbers for the different analytical methods for the quantification of subvisible particles. In general, these limits are not only applied to various parenteral drug products, regardless if small molecules or biologics, but are considered to be acceptable specifications for injectable drug products.

* Corresponding author. Address: F. Hoffmann-La Roche Ltd., Pharmaceutical Development & Supplies (PTDE-P), Bldg 64/443, Grenzacherstrasse 124, CH-4070 Basel, Switzerland. Tel.: +41 61 688 5417.
E-mail address: tobias.werk@roche.com (T. Werk).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2013.12.014

Small molecule parenterals may contain extrinsic process-related particulates, such as fibers, glass, as well as possible particles being generated from interaction of formulation components and primary packaging (e.g., barium sulfate). For biopharmaceutical drug products, all these components may also occur, however, additionally protein particles (as such or derived from interaction/induction of other extrinsic process related components) may be present. Thus, because of these product related particles, the total subvisible particle load in parenteral drug products containing biotechnology-derived APIs maybe be higher than for small molecule injectable dosage forms. Current analytical QC methods cannot specifically and reliably quantify proteinaceous, product-related particles from extrinsic, process-related particulates, though many new analytical techniques may be useful characterization purposes. In order to specifically describe subvisible particle counting in drug products containing biotechnology-derived APIs, specific USP monographs are currently in generation (USP <787> and USP <1787>), that will likely include specific considerations for sample handling and allow lower volume methods to minimize analytical costs.

As the name – light obscuration (LO) – implies, the degree of light blockage defines the particle count and size, but LO methods have been reported to have limitations for counting some types of translucent particles (such as protein particles) as well as for particles where the refractive index difference compared to the medium is small (Ripple et al., 2011; Zhao et al., 2012). Polystyrene particles are routinely used in calibration measurements of the LO method, however, protein particles have different shape and morphology as well as different optical properties compared to polystyrene particles (Ripple and Dimitrova, 2012; USP General Chapter <1788>, 2012; Ripple et al., 2011). These differences may result in difficulties in correctly detecting and accurately quantifying protein-based subvisible particles within liquid biopharmaceutical samples using the LO method (Huang et al., 2009; Narhi et al., 2009). The standard microscope compendia method for particle analysis also has several major drawbacks including the time required, both for sample preparation and for counting and measuring size properties of the particles. In addition, sample preparation may alter the results, e.g., protein particles may potentially dissolve during sample preparation or may be difficult to accurately detect on the filter surface.

Since LO has been reported to have some limitations with regards to counting and sizing protein subvisible particles, which are translucent and/or irregular shaped in nature and have a refractive index similar to that of the medium, there is increased interest in using newer analytical technologies for SbVP detection such as flow cells interfaced with digital imaging technologies (Huang et al., 2009; Narhi et al., 2009; Sharma et al., 2009, 2010a,b). These newer techniques may be viable add-ons to assess the number and size range of protein-based subvisible particles (Huang et al., 2009; Narhi et al., 2009; Sharma et al., 2009, 2010a,b; Oma, 2010; Strehl et al., 2012; Zölls et al., 2012). The digital imaging technique captures images of subvisible sized particles (~1–100 μm) as the sample is drawn through a flow-cell centered in a field of view, resulting in particle counts, particle sizing as well as additional particle shape information (Huang et al., 2009; Narhi et al., 2009; Sharma et al., 2009, 2010a,b; Oma, 2010; Strehl et al., 2012; Zölls et al., 2012). Despite of the similarities of FlowCAM™ and MFI™ digital imaging instruments, one must bear in mind their differences in particle size definition, optics and data analysis (Wilson and Manning, 2013). Both instruments typically also offer software filtering capabilities for detection of subvisible particles of varying physical properties allowing for counting, sizing and analyzing the morphology of translucent protein aggregates and particulates (Wuchner et al., 2010; Huang et al., 2009; Narhi et al., 2009; Sharma et al., 2009; Liu et al., 2011). As subvisible particles are heterogeneous in nature, and may vary widely in parameters such as morphology and size, refractive index and translucency, it is difficult to quantitatively describe the analytical limitations of Microflow digital imaging (MDI) and LO techniques for actual protein samples due to lack of appropriately characterized standards. The use of less frequently used or emerging analytical technologies for SbVP assessments, such as nano-tracking analysis, coulter counter, field flow fractionation, etc., were not in the scope of this study, given that LO and microflow digital imaging technologies are most advanced.

The ideal matrix for the analysis of subvisible particles would consist of a clear, water-like fluid with individual, freely dispersed particles of moderate buoyancy and high contrast (opacity, color, refractive index) such that each particle passing between the illuminator and sensor is effectively recognized (Sharma et al., 2010; Gregory, 1994, 1999; Lewis and Manz, 1991; Treweek and Morgan, 1977). In contrast, real-life protein formulation samples can differ from water in their solution optical properties such as turbidity, viscosity, density, color, and refractive index (Kanai et al., 2008; Liu et al., 2005). A protein formulation sample that does not have the clarity, color and viscosity approximately equivalent to water may provide erroneous data when analyzed by either LO counting method or MFI™ (Demeule et al., 2010; Chrai et al., 1987; Zolls et al., 2013). For example, due to their high molecular weight, protein molecules can represent a significant volume fraction in high concentration protein formulations which can contribute to solution viscosity. Moreover, solution non-ideality caused by protein–protein interaction in protein formulations may result in high viscosity and opalescence (Kanai et al., 2008; Liu et al., 2005). Opalescence and turbidity can be linked to phase separation, soluble aggregate formation, and/or generation of small particles, as is observed in emulsions (e.g., silicone and air bubbles) (Cromwell et al., 2006; Mahler et al., 2005). Coloration is typically observed in protein formulations and can be due to absorbing amino acid residues or their changes upon storage (e.g., Cysteine, Histidine, Phenylalanine, Tryptophan and Tyrosine) and/or reaction products with reducing sugars (e.g., Maillard reactions)(Pauson et al., 1980) and/or process impurities (e.g., vitamin B12).

We compared two LO methods and two digital imaging technologies (MFI™ and FlowCAM™) on counting and sizing of particles varying different formulation matrix parameters: color, opalescence, viscosity and a combination thereof. Our experiments were performed to better understand method performance with varying physical solution properties, as typically encountered in some (high concentration) protein formulations. Formulation matrix effects were studied using different types of subvisible particles including the use of different standards of known number and size (polystyrene beads, glass particles, and pseudo-protein standards). Similar to this study, Zölls et al. recently compared MFI™ and FlowCAM™, in terms of counting and sizing of polystyrene particles, and tested robustness of protein particle counting results by adding sucrose (Zolls et al., 2013). Several additional analytical issues (e.g., matrix effects on particle sizing, probing effect of mixed matrices, and comparison to light obscuration results) were not fully elucidated in their study, and thus are the focus of this work.

Though it would be preferable to use actual protein particles, protein particles induced by relevant means (e.g., shaking) were not stable and would change in size and number upon dilution, especially when using additives to mimic different solution parameters. The use of actual protein particles would thus significantly complicate analytical quantification results of this study. Further research is ongoing in our labs, comparing different subvisible particles methods with actual protein particles. In this work, it was shown that solution properties must be carefully accounted for to accurately determine subvisible particle size and number as