Particles in Therapeutic Protein Formulations, Part 1: Overview of Analytical Methods

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ABSTRACT: The presence of particles is a major issue during therapeutic protein formulation development. Both proteinaceous and nonproteinaceous particles need to be analyzed not only due to the requirements of the Pharmacopeias but also to monitor the stability of the protein formulation. Increasing concerns about the immunogenic potential together with new developments in particle analysis make a comparative description of established and novel analytical methods useful. Our review aims to provide a comprehensive overview on analytical methods for the detection and characterization of visible and subvisible particles in therapeutic protein formulations. We describe the underlying theory, benefits, shortcomings, and illustrative examples for quantification techniques, as well as characterization techniques for particle shape, morphology, structure, and identity. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:914–935, 2012

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INTRODUCTION

Approximately half of all new drugs approved by the United States Food and Drug Administration in the last few years are biopharmaceuticals,[1] mainly therapeutic proteins and especially monoclonal antibodies.[2] A major challenge during formulation development of these products is overcoming their limited stability. Among the various degradation mechanisms a protein can undergo[3,4] the formation of protein aggregates and particles is a particular concern.[5] Aggregates are generally defined as assemblies of protein monomers and can vary in many aspects such as size, reversibility, and structure. For instance, their size can range from dimers in the nanometer range to large aggregates of hundreds of microns, which are visible to the human eye. These larger aggregates are often also designated as particles.[6,7] However, not only proteinaceous particles but also nonproteinaceous particles, for example, originating from packaging material or excipients, can influence product quality and therefore need to be analyzed.[8,9]

Our review aims to give an overview on methods for both quantification and characterization of visible and subvisible particles in therapeutic protein formulations. On the basis of the current classification of protein aggregates and particles,[10] for this review article particles are defined as material with a size above 0.1 μm and are further classified into subvisible (0.1–100 μm) and visible particles (above 100 μm); submicron particles (0.1–1 μm) are a subgroup of subvisible particles.

Although particles above 10 μm have received attention in the development of therapeutic protein products already for a long time because of the requirements of the Pharmacopeias for parenteral

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products,[11–13] the detection and characterization of subvisible particles below 10 μm have only recently gained more importance. This is due to increasing concerns about the potential immunogenicity of subvisible particles—both proteinaceous particles,[14] nonproteinaceous particles,[15] and nonproteinaceous particles with adsorbed protein.[16,17] Moreover, new techniques for the analysis of subvisible particles have emerged in the last few years, enabling a more detailed characterization of these impurities or contaminants.[8,9]

Several reviews summarizing methods for the quantitative analysis of protein aggregates and particles[6,18,19] and one describing analytical methods for protein particles down to 2 μm[20] are available. Our review includes not only particle quantification techniques but also analytical characterization methods that provide information about particle characteristics such as shape, morphology, structure, and identity. Moreover, we discuss new developments in particle analysis. We provide a comprehensive overview of particle analysis for pharmaceutical protein products with the presented methods summarized in Table 1. The sections describing the individual analytical methods are sorted according to measurement principle and cover the underlying theory, advantages, shortcomings, and illustrative examples. Analytical techniques for nanometer protein aggregates with a very limited use for subvisible and visible particles such as size-exclusion chromatography (SEC) and analytical ultracentrifugation (AUC) are explained only briefly. For the pharmaceutical application of the described methods in development and production of therapeutic proteins, the reader is referred to Part II "Applications in the Pharmaceutical Industry" of the review article by Narhi et al.[21]

GOALS AND CHALLENGES ASSOCIATED WITH PARTICLE ANALYSIS

The tolerated amount of visible and subvisible particles in parenteral therapeutic protein products is restricted by regulations as described in the Pharmacopeias,[11–13,22] which make quantification of protein particles essential in development and production of therapeutic proteins. The size of visible particles is not specified in the Pharmacopeias, as the detection of particles by the human eye depends among others on personal eyesight, light conditions, and used test settings. However, the specifications for subvisible particles include particle sizes of at least 10 and at least 25 μm,[11,13] which make also size determination of the counted particles necessary.

Although the identification of particles present in parenteral protein products is not required by the Pharmacopeias, a distinction between nonproteinaceous and proteinaceous particles is relevant in case of protein therapeutics. Nonproteinaceous material, for example, particles shed from pumps or primary packaging material (including silicone oil droplets in prefilled syringes) or particles formed by degradation of excipients (e.g., polysorbate),[23] can trigger protein aggregation by heterogeneous nucleation and might be related to increased immunogenicity.[16,24,25] Root cause analysis to determine the source of the particles is an important part of any investigation and can result in minimizing the occurrence of nonproteinaceous particles. In addition, false positive "particles" such as air bubbles need to be distinguished from real particles for a correct evaluation of the particle load in the analytical characterization. However, only few techniques are able to discriminate between proteinaceous and nonproteinaceous particles, for example, Raman spectroscopy/microscopy,[26] infrared (IR) spectroscopy/microscopy,[27] and to a certain extent also methods involving fluorescent dyes[28] and flow imaging microscopy methods[29,30] (Table 1).

For proteinaceous particles, it can be helpful during formulation development to further discriminate the particles with respect to, for example, size, shape, or structure (Table 2). Depending on the (stress) conditions the protein had been exposed to, several types of aggregates and particles can be detected allowing conclusions about the susceptibility of the protein to distinct stress conditions and the identification of means to prevent this instability.[31]

Many analytical methods for (protein) particles are based on the interaction of particles with light (Fig. 1). Methods based on light scattering require a substantial difference in refractive index of the particles and the surrounding liquid. However, protein particles are often translucent with a supposed refractive index between 1.33 and 1.4.[29] This value is close to that of aqueous buffers and highly concentrated protein solutions, thereby hampering the detection by light-based systems.[29,32] However, to our knowledge the refractive index of protein particles has not been analyzed up to now. It likely depends on the type of particle, for example, degree of protein unfolding and packing, so the values described in the literature are only assumptions. Light-based systems for particle analysis rely on the calibration with standards, usually polystyrene beads of a clearly higher refractive index compared with protein particles. Therefore, the results obtained from these systems for protein particles need to be evaluated carefully and standards that resemble the proteinaceous nature of the particles more closely would be very helpful for data interpretation.[9] When comparing particle size results from several analytical techniques, algorithms for size determination need to be considered as particle size can be provided as various parameters (Table 2). A further challenge lies in the often dynamic, heterogeneous, and transient nature of particles, as size and