Classification of Protein Aggregates

LINDA O. NARHI,[1] JEREMY SCHMIT,[2] KAROLINE BECHTOLD-PETERS,[3] DEEPAK SHARMA[4]

[1]Amgen Inc., Thousand Oaks, California

[2]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, California

[3]F. Hoffmann-La Roche Ltd., Basel, Switzerland

[4]Brightwell Technologies Inc., Ottawa, Ontario, Canada

Received 22 June 2011; revised 6 August 2011; accepted 22 September 2011

Published online 11 October 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22790

ABSTRACT: Comparison of protein aggregates/self-associated species between laboratories and across disciplines is complicated by the imprecise language presently used to describe them. In this commentary, we propose a standardized nomenclature and classification scheme that can be applied to describe all protein aggregates. Five categories are described under which a given aggregate may be independently classified: size, reversibility/dissociation, conformation, covalent modification, and morphology. Possible subclassifications within each category, several examples of applications of the nomenclature, and difficulties in making appropriate assignments will be discussed. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:493–498, 2012

Keywords: protein aggregation; particle size; stability; oligomers; amyloid; reversibility; dissociation; protein structure; modification; morphology

INTRODUCTION

The study of protein aggregation is rapidly evolving with much effort invested into the causes and pathways of aggregate formation. This work is revealing a growing array of aggregate states; however, there is much to be done for a coherent picture to arise. There are several excellent reviews available, which provide in-depth discussions of the mechanism of protein aggregation, techniques to analyze and characterize aggregates, and the potential biological effects of protein aggregates.[1–5] Work in this field is complicated by the imprecise terms used to describe the aggregates; one group's "subvisible particles" (SbVP) may be another group's "oligomer" and yet another researcher's "protofilament." This sloppy nomenclature presents an impediment to the comparison of results across labs and organizations, which must be overcome to achieve the interdisciplinary effort that will be required to solve the aggregation problem. This has been the topic of discussion at several recent meetings and in several professional organizations. At the Protein Aggregation and Immunogenicity meeting in Breckinridge, Colorado in July 2010, organized by the American Association of Pharmaceutical Scientists (AAPS) Focus Group "Protein Aggregation and Biological Consequences" and cosponsored by AAPS and the US Food and Drug Administration, there was a breakout session focusing on how to standardize the nomenclature used to describe protein aggregates (and particles). In this commentary (the result of that discussion), we attempt to remove this apparent language barrier by suggesting a standardized terminology to classify aggregates. This task is complicated by the fact that the aggregation community presently studies both amyloid formation and aggregates in pharmaceutical products, and it is thus unavoidable that some of the definitions will seem non-ideal to specialists in either of these areas. However, it is our hope that this initial awkwardness will be compensated for by the resulting clarity in communication.

One of the confounding semantic issues in this field is the use of the terms "aggregates" and "particles" themselves to refer to different species depending on

Abbreviations used: SbVP, subvisible particles; IUPAC, International Union of Pure and Applied Chemistry; AAPS, American Association of Pharmaceutical Scientists; SEC, size-exclusion chromatography.

Correspondence to: Linda O. Narhi (Telephone: + 805-447-3104; Fax: + 805-499-3654; E-mail: lnarhi@amgen.com)

"The first step towards wisdom is calling things by their right names" Chinese proverb

Journal of Pharmaceutical Sciences, Vol. 101, 493–498 (2012)

the area of research. In the purification and biochemistry fields, the term "aggregates" is often used when referring to dimer, tetramers, and other species that can be separated by size-exclusion chromatography (SEC). The term "particles" is sometimes used to refer to larger aggregates, often large enough to be detected with the naked eye. Because protein aggregation describes any protein self-association reaction, protein aggregates can range in size from nanometers to hundreds of micrometers, from dimers to amyloid and other complexes containing more than 1,000,000 subunits. In order to avoid the confusion inherent in the use of the same terms to refer to different specific species, a protein aggregate will be defined as any self-associated species, with monomer defined as the smallest naturally occurring and/or functional subunit. For example, for immunoglobulin G (IgG), the monomeric subunit would actually be the homodimer of a heavy chain/light chain pair.

The key to this classification scheme is the identification of five categories under which a given aggregate may be independently classified. These are size, reversibility/dissociation, conformation, chemical modifications (both covalent cross-links and modifications of individual amino acid residues), and morphology (Table 1; morphology is defined as the form and structure of an organism or its component parts. For protein aggregates, aspects of morphology include appearance of the aggregate, optical properties such as refractive index and transparency, any internal structure it may have, the number of monomeric units involved, and any foreign material it contains). All but the morphology category were also included in the nomenclature scheme recently proposed by Sharma and Kalonia.[6] In addition to the inclusion of morphology as a distinct descriptor of protein aggregates (including number of subunits in the aggregate when that information is available), the exact definitions of other categories are also slightly different between the two systems. The categories, subclassification within each category, and potential pitfalls in making assignments follow these guidelines will be discussed below along with several examples of the application of this nomenclature. Table 2 contains several terms that are commonly used in some fields and the corresponding term proposed using this nomenclature.

Table 1. Proposed Classification of Protein Aggregates

Category	Classification
Size	<100 nm, 100–1000 nm (submicrometer), 1–100 μm, >100 μm
Reversibility	Reversible, irreversible, dissociable, dissociable under physiological conditions, dissociable under defined (list) conditions
Secondary/tertiary structure	Native, partially unfolded, unfolded, inherently disordered, Amyloid
Covalent modification	Cross-linked, reducible cross-link, nonreducible cross-link, intramolecular modification, oxidation, deamidation, no modification
Morphology	Number of monomeric subunits, aspect ratio, surface roughness, internal morphology, optical properties, translucent, heterogeneous

Table 2. Definition of Some Commonly Used Protein Aggregate Descriptions and the Preferred Terminology Using this Nomenclature

Commonly Used Terms	Preferred Terminology
SEC high-molecular-weight species	Submicron aggregates
Oligomers	Nanometer (nm) aggregates
Subvisible particles	Micron aggregates (μm)
Visible particles	Aggregates greater than 100 μm

SIZE

Size is perhaps the most common characteristic used to classify aggregates; however, the use of subjective terms such as visible/subvisible and soluble/insoluble has hindered its usefulness. We suggest instead the use of quantitative categories such as >100 μm (previously visible particles), 1–100 μm (previously SbVP), 100–1000 nm (submicrometer, including aggregates that used to be defined as soluble), and nanometer (<100 nm, previously described as oligomers or soluble aggregates). An oligomer is defined as any aggregate that contains a few monomeric units, as defined by IUPAC; these would be generally submicron aggregates, depending on the size of the monomer. The actual size or ranges of sizes determined for the aggregate being discussed would be an even more precise descriptor and should be used when possible. Size must be accompanied with a description of the technique used to determine it, as this can have a strong effect on the apparent size observed. An oligomer would be defined as any aggregate that contains a few monomeric units, in keeping with the International Union of Pure and Applied Chemistry (IUPAC) definition; these would be generally in the submicron size range, depending on the size of the monomer.[7] Distribution of size into the four categories used here is somewhat arbitrary and is based on the ranges of the different techniques available for the measurements, as well as historical practice.

REVERSIBILITY/DISSOCIATION

A very important characteristic of protein aggregates is how the proteins are self-associated within these species. This can vary from association by a reversible thermodynamic equilibrium (The term "reversible"

is restricted to aggregates that exist in equilibrium with the native monomeric subunit under specific solution conditions, where the disassociation of protein aggregates may be observed on the experimental timescale simply by returning to the original solution condition. Reversible protein aggregation typically results from relatively weak noncovalent protein interactions and can be described thermodynamically.), which occurs between native molecules in solution, to irreversible aggregation (Irreversible aggregates are higher-molecular-weight species, which when formed cannot be dissociated short of the addition of denaturants or reducing agents.) as a result of stresses to which the solution has been exposed. The classification system presented here should be used to help differentiate between these different species. We suggest that the term "reversible" be restricted to aggregates that exist in equilibrium with the native monomeric subunit. For these species, disassociation of the aggregate may be achieved simply by diluting the solution. Frequently aggregation is induced by a perturbation in the solution conditions (pH, temperature, etc.) If the resulting aggregates are reversible (i.e., in thermodynamic equilibrium with the monomer), it follows that the aggregates may be disassociated by reverting to the original solution conditions, provided that the timescale of disassociation is not greater than the experimental timescale. Therefore, descriptions of reversibility should be accompanied with the timescale over which reversibility is observed (reversible on the minute timescale). When the aggregates are not reversible, or the timescale for disassociation is prohibitively long, it may still be possible to recover the monomer species through application of heat, buffers, or other conditions beyond a simple reversal of the conditions that led to aggregation. Such aggregates may be described as "dissociable", with the conditions under which dissociation occurs included in the description (e.g., thermally dissociable). A particularly important subcategory comprises those aggregates that dissociate when under physiological solution conditions. In summary, reversible association like any other reversible chemical reaction can be described by sets of equilibrium constants between different assembly states or aggregates present under those specific conditions. Dissociable aggregates are those which are not reversible, but where a monomeric solution may still be recovered by manipulation of the solution conditions (these aggregates require changes in the solution conditions to initiate disassociation, such as being placed into physiological conditions, changing the temperature or pressure, and so on). Note that the distinction between these categories depends in part on the experimental timescale by which reversibility is defined.

"Irreversible" aggregates are higher-molecular-weight species, which when formed cannot be dissociated short of the addition of denaturants or reducing agents. These species can often be chromatographically isolated and when reinjected into the column, will elute in the same position. Because of this, they can sometimes be purified and studied. Combinations of reversible, dissociable, and irreversible species are often present in aggregated protein samples.

CONFORMATION

The conformation of proteins in the aggregate can have implications for the underlying mechanism of formation, and also potential safety implications. The conformation of the aggregated protein can range from that of the original protein monomer, classified as native, to unfolded, and anything in between. The secondary and tertiary structure as well as the overall protein fold, stability, and surface hydrophobicity, are potential descriptors of the conformation of the aggregated protein. On the basis of these analyses, the proteins in the aggregate can be classified as "native," "partially unfolded," "misfolded," "inherently disordered," "unfolded," or "amyloid." The descriptor "native" refers to the conformation of nonaggregated, active protein, whether created through protein engineering or isolated from the biological organism in which the protein originates. The terms "partially unfolded," "misfolded," and "unfolded" must be defined for the specific system being studied and the analytical methods being used. "Partially unfolded" should be used to describe the aggregate in which changes in conformation can be detected beyond the variability of the method used, but which also retain some of the native structure. "Misfolded" should be used for proteins that have a fold different than that found in the native protein (e.g., increased beta sheet in a protein that is normally α-helical), and "unfolded" describes proteins with structure that is comparable, within the variability of the assay, to a protein that has been unfolded with fully denaturing conditions such as 6 M Guanidine hydrochloride. "Inherently disordered" should continue to be used to describe the conformation of synuclein, amyloid-β (Aβ), and other disordered proteins within the aggregates that do not show amyloid signature. "Amyloid" is defined by the approximately 4.7 Å and 1 nm signatures of cross-β diffraction pattern.[8]

CHEMICAL MODIFICATION

The individual protein molecules in an aggregate can be "chemically modified" either through cross-links between amino acids or modification of individual residues. Covalent cross-linking of the proteins can be an important factor in the formation of irreversible aggregates. This includes two types of disulfide cross-linking, which are reducible: intermolecular

cross-linking and intramolecular modification of the individual proteins. Other covalent nonreducible chemical cross-links such as thioether and dityrosine covalent bonds are also found in protein aggregates. Modification of individual amino acids such as oxidation of methionine or cysteines, deamidation, and so on are also found in a number of protein aggregates and can play roles of varying importance in aggregate formation. They also have the potential to provide clues to the mechanism of formation of aggregates found in protein therapeutics.

MORPHOLOGY

The morphology of the aggregate can provide important information that helps differentiate between the different types of protein aggregates, can provide information on the mechanism of protein aggregation, and can also help differentiate between protein aggregates and intrinsic and extrinsic foreign particles. Extrinsic particles are unexpected foreign materials that are introduced into the process due to insufficient cleaning of the production environment, product assembly protocols, and so on (e.g., insect parts or paint flakes). Intrinsic particles are those that can be traced to the normal manufacturing process, including delivery device (such as silicone oil), and can also be part of the original product, which was not removed by filtration prior to filling. Inherent particles are aggregates from the protein or formulation components, molecules that are a part of the particular drug product.[9]

"Aspect ratio" and "surface roughness," "how regular" or "amorphous" the structure appears, whether it is a "fiber" or a "sphere," and so on are all important characteristics, which can be used to differentiate between different protein aggregate species and between protein aggregates and other types of particles. Other important aggregate attributes are their "optical properties," including refractive index and transparency; these characteristics may affect detection by light-based methods while not having any impact on methods based on conductivity or other detection systems. These properties can also potentially be exploited to allow differentiation between protein aggregates, silicone oil droplets, air bubbles, and other nonproteinaceous particles.

"Aspect ratio" and "transparency" can be used to help differentiate between silicon oil droplets and protein particles. Many of the protein aggregates have similar density and refractive index properties, and, therefore, these characteristics often provide little impactful information for differentiating between types of protein aggregates. The density and refractive index of protein aggregates are much closer to the surrounding protein–buffer solution than any standards now available–a general weakness in obtaining appropriate controls for aggregate analysis. The arrangement of proteins in the particle, if known, should be included in the morphological category if a "regular array" of molecules are presented on the surface. The numbers of repetitive monomeric protein units in the aggregate, when known, should also be included as part of the description of the morphology. This is especially important for aggregates in the nanometer size range, as these are often dimers, trimers, and other oligomers. The nature of the multimer can often help determine control strategies, mechanism of formation, and so on.

Although many aggregates contain only protein, there are also aggregates that contain nonproteinaceous contaminants in addition to the therapeutic protein. These aggregates can be classified as "heterogeneous aggregates" and further described by including the identity of the other materials (glass particles, stainless steel particles, etc.). The morphology category can also be expanded to include internal characteristics, as these become experimentally accessible. These characteristics include the "solvent content" and "fractal dimension" of the aggregate as well as the "packing geometry" of proteins within the aggregate.

SPECIFIC DEFINITIONS

Moving beyond these classifications, we would like to discuss the definitions of some terms that are commonly used to describe aggregation, and terms whose current use leads to considerable ambiguity. For example, the term "oligomer," frequently used by protein biochemists, implies specific binding into a functional complex, whereas in the amyloid field, it is commonly applied to any nonfibrillar aggregate. The IUPAC defines an oligomer as "a molecule of intermediate relative molecular mass, the structure of which essentially comprises a small plurality of units derived, actually or conceptually, from molecules of lower relative molecular mass."[7] In the context of protein aggregation, this definition should be further refined to exclude fibril-like aggregates,[9] and restricted to aggregates in the submicron size range.

The term "protofilament" is another source of ambiguity. Following Bitan et al.,[10] we suggest the definition "elongated, fibril-like assemblies with curvi-linear morphology, a diameter of ~5 nm, and length not exceeding 100–200 nm."

APPLICATIONS/EXAMPLES

To help the reader understand how this nomenclature will be used, beyond the information above, we have provided a few examples.

Insulin is stable in both monomeric and hexameric forms. The monomer is the smallest active unit

and so, the hexamer would be defined as a reversible, native, unmodified submicron aggregate of organized, repeated units.

At high protein concentrations, it is very common to have oligomers or high-molecular-weight species formed, which are often identified as a prepeak, eluting before the main peak during SEC analysis. As reported by both Moore et al.[11] and Kanai et al.[12] for monoclonal antibodies, these species are usually reversible nanometer or submicron aggregates, have native conformation, and are not chemically modified.

Aggregates that have been traditionally designated as SbVP are often a very heterogeneous population of micron aggregates, with subpopulations of reversible, native, unmodified aggregates and irreversible, unfolded disulfide cross-linked aggregates, and everything in between, usually appearing translucent with irregular, amorphous structure.

The aggregates described by Mahler and coworkers,[13] which was formed following shaking and stirring of an IgG solution, would be described as a mixture of native and partially unfolded noncovalent aggregates ranging in size from submicrometer to $>100\,\mu m$ (determined by light obscuration). Aggregates created by stirring using a different process suggested by Joubert et al.[14] and Luo et al.[15] were consistent with this classification and further indicated that the aggregates were amorphous, translucent, contained oxidized methionines, and a mix of dissociable and irreversible species.

Aggregates created by freeze–thawing of interferons followed by pressure treatment[16] would be classified as micron aggregates (1–10 μm by Coulter counter) of native conformation, which are partially deamidated.

In contrast, aggregated species obtained by heating IgG at or above the melting transition are $>100\,\mu m$ (by light obscuration and micro-flow imaging), irreversible, unfolded, deamidated aggregates, which appear very dense or dark when visualized by flow imaging, many of which are filamentous.[14]

Protein aggregates intentionally formed by a process involving precipitation by water-miscible organic solvents in the presence of water-soluble, core-forming organic or inorganic excipients (protein-coated microcrystals) can be described as heterogeneous, partially amorphous, 1–100 μm (by HIAC), dissociable native aggregates. In this case, the core material is nonproteinaceous and the protein does not show changes in the secondary structure when investigated by solid-state circular dichroism, and dissolves readily upon placement into physiological buffers.[17]

There are several reports in the literature of foreign material such as glass and stainless steel acting as sites for protein adsorption and/or as nucleation sites for protein aggregation in the bulk. Tyagi et al.[18] described the shedding of nanometer and submicrometer particles of stainless steel from mechanical pumps, resulting in submicrometer to micrometer (Coulter counter) heterogeneous aggregates containing protein and metal, where the protein has a partially unfolded structure. Chi et al.[19] reported similar findings using glass particles and human platelet-activation factor acetylhydrolase. These aggregates would be classified as micrometer heterogeneous aggregates containing glass and protein with native or partially unfolded conformation.

One of the requirements in producing biotherapeutics is a solution that is practically free of particles; this refers to particles that are identified by visual inspection, including protein aggregates that are typically $>100\,\mu m$. Visible inspection is a probabilistic assay. The definition of what constitutes a visible particle depends on the light conditions, length of inspection, and acuity of the inspector's eyes, with a lower limit that can range from >20 to $>200\,\mu m$. Often these are foreign particles consisting of cellulose, hair, rubber, or other particles from the manufacturing process (extrinsic and intrinsic particles), but sometimes these are protein aggregates or heterogeneous aggregates, which contain protein. An example of these are the tungsten-induced protein particles reported by Liu et al.[20] Using this proposed nomenclature, these types of particles would be defined as dissociable, irregularly shaped heterogeneous aggregates greater than 100 μm as measured by light obscuration and visible inspection, which contain tungsten and protein with a native-like conformation.

Our classification scheme can also be used to describe amyloid aggregates. For example, consider the following aggregate states of Aβ. The Aβ-derived diffusible ligands of Lambert et al.[21] would be classified as 5 nm, reversible, intrinsically disordered, unmodified spherical aggregates. The "globulomer" state of Barghorn et al.[22] would be a nanometer, reversible, amyloid, unmodified, spherical aggregate. The βamy ball aggregate of Westlind-Danielsson and Arnerup[23] would be 20–200 μm, reversible/dissociable (depending on timescale), amyloid, unmodified spherical aggregates with gel-like internal morphology.

The above examples demonstrate how using this nomenclature can help differentiate between aggregates. This helps identify similarities and differences in the species obtained and used for subsequent testing, which in turn informs the interpretation and comparison of the results. Once a particular aggregate has been classified using this nomenclature, a shortened description such as "SbVP" can be used throughout a manuscript or presentation, with the abbreviated label understood to be as defined in that particular case.

The five categories proposed here should be useful in identifying key differences and similarities

between aggregated protein species formed across labs and proteins. As the tools to characterize protein aggregates evolve and more attributes are identified, the number of descriptors used can increase, providing even more specific details.

CONCLUSIONS

Protein aggregation is a complicated phenomenon, which is sensitive to solvent conditions, sample history, protein sequence, and so on. Our ability to understand aggregation will depend on the identification of patterns within this vast parameter space. It is our hope that these patterns will be more apparent with a more precise naming scheme. We propose using five categories: size, reversibility/dissociability, conformation, chemical modification, and morphology, to consistently describe protein aggregates. We have attempted to craft a nomenclature with the required precision as well as the flexibility to grow with a rapidly changing field.

ACKNOWLEDGMENTS

The authors would like to thank all members of the steering committee of the AAPS Focus Group on Protein Aggregation and Biological Consequences for many helpful discussions. We are especially grateful to Hanns-Christian Mahler, Ted Randolph, Devendra Kalonia, Jamie Moore, David Brems, and Wim Jiskoot for suggestions, discussions, and critically reading the manuscript.

REFERENCES

1. Carpenter JF, Randolph TW, Jiskoot W, Crommelin DJ, Middaugh CR, Winter G, Fan YX, Kirshner S, Verthelyi D, Kozlowski S, Clouse KA, Swann PG, Rosenberg A, Cherney B. 2009. Overlooking Subvisible Particles in Therapeutic Protein Products: Gaps That May Compromise Product Quality. J Pharm Sci 98:1201–1205.
2. Mahler HC, Friess W, Grauschopf U, Kiese S. Protein Aggregation: Pathways, Induction Factors and Analysis. 2009. J Pharm Sci 98:2909–2934.
3. Singh SK, Afonina N, Awwad M, Bechtold-Peters K, Blue JT, Chou D, Cromwell M, Krause HJ, Mahler HC, Meyer BK, Narhi L, Nesta DP, Spitznagel T. 2010. An Industry Perspective on the Monitoring of Subvisible Particles as a Quality Attribute for Protein Therapeutics. J Pharm Sci 99:3302–3321.
4. Narhi LO, Jiang Y, Cao S, Benedek K, Shnek D. 2009. A Critical Review of Analytical Methods for Subvisible and Visible Particles. Curr Pharm Biotech 10:373–381.
5. Wang W, Roberts CJ, Eds. 2010. Aggregation of therapeutic proteins. Hoboken, New Jersey: Wiley.
6. Sharma VK, Kalonia, DS. 2010. Experimental detection and characterization of protein aggregates. In Aggregation of therapeutic proteins; Wang W, Roberts CJ, Eds. Hoboken, New Jersey: Wiley.
7. Jenkins AD, Kratochvil P, Suter UW. 1996. Glossary of basic terms in polymer science. Pure Appl Chem 68:2287–2311 (IUPAC, available at http://www.iupac.org/reports/1996/6812jenkins/molecules.html. 08/04/2011).
8. Goda S, Takano K, Yamagata Y, Nagata R, Akutsu H, Maki S, Namba K, Yutani K. 2000. Protein Sci 9:369–375.
9. Vicéns M. 2009. Pharmaceutical Formulation & Quality. December/January 2009.
10. Bitan G, Fradinger EA, Spring SM, Teplow DB. 2005. Neurotoxic protein oligomers - what you see is not always what you get. Amyloid 12:88–95.
11. Moore JMR, Patapoff TW, Cromwell MEE. 1999. Kinetics and thermodynamics of dimer formation and dissociation for a recombinant humanized monoclonal antibody to vascular endothelial growth factor. Biochemistry 38:13960–13967.
12. Kanai S, Liu J, Patapoff TW, Shire SJ. 2008. Reversible self-association of a concentrated monoclonal antibody solution mediated by Fab-Fab interaction that impacts solution viscosity. J Pharm Sci 97:42419–4227.
13. Kiese S, Pappenberger A, Friess W, Mahler HC. 2008. Shaken, not stirred: Mechanical stress testing of an IgG1 antibody. J Pharm Sci 97:4347–4366.
14. Joubert M, Luo Q, Nashed-Samuel Y, Wypych J, Narhi LO. 2011. Classification and Characterization of Therapeutic Antibodies. J Biol Chem 286:25118–25133.
15. Luo Q, Joubert MK, Ketchum, R, Stevenson, R, Narhi, LO, Wypych, J. 2011. Chemical Modifications in Therapeutic Protein Aggregates Generated Under Different Stress Conditions. J Biol Chem 286:25134–25144.
16. Fradkin AH, Carpenter JF, Randolph TW. 2009. Immunogenicity of Aggregates of Recombinant Human Growth Hormone in Mouse Models. J Pharm Sci 98:3247–3264.
17. Bechtold-Peters K. 2010. Protein immobilization by crystallization and precipitation: An alternative to lyophilization. In Current trends in monoclonal antibody development and manufacturing; Shire S, Gombotz W, Bechtold-Peters K, Andva J, Eds. New York: Springer.
18. Tyagi A, Randolph TA, Dong A, Maloney KM, Hitscherich CJ, Carpenter JF. 2009. IgG Particle Formation during Filling Pump Operation: A Case Study of Heterogeneous Nucleation on Stainless Steel Nanoparticles. J Pharm Sci 98:94–104.
19. Chi EY, Weickmann J, Carpenter JF, Manning MC, Randolph TW. 2005. Heterogeneous nucleation-controlled particulate formation of recombinant human platelet-activating factor acetylhydrolase in pharmaceutical formulation. J Pharm Sci 94:256–274.
20. Liu W, Swift R, Torraca G, Nashed-Samuel Y, Wen ZQ, Jiang Y, Vance A, Mire-Sluis A, Freund E, Davis J, Narhi LO. 2010. Root Cause Analysis of Tungsten-Induced Protein Aggregation in Pre-filled Syringes PDA Journal of Pharmaceutical Science and Technology. PDA J Pharm Sci Technol 64:11–19.
21. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Kraft GA, Klein WL. 1998. Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci 95:6448–6453.
22. Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, Hillen H. 2005. Globular amyloid beta-peptide(1-42) oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. J Neurochem 95:834–847.
23. Westlind-Danielsson A, Arnerup G. 2001. Spontaneous in vitro formation of supramolecular beta-amyloid structures, "betaamy balls", by beta-amyloid 1-40 peptide Biochemistry 40:14736–14743.