Commentary

Theme: Aggregation and Interactions of Therapeutic Proteins
Guest Editor: Craig Svensson

Commentary: Current Perspectives on the Aggregation of Protein Drugs

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Protein drugs have revolutionized the pharmaceutical industry, offering new treatments for serious diseases. Since the first recombinant protein drug, Eli Lilly's Humulin®, was approved 30 years ago (1), protein drugs have grown from esoteric specialty products to a major drug class. Of the 20 top selling drugs in the USA in the third quarter of 2012, nine are proteins. Despite these successes, the inherent instability of protein molecules remains an impediment to their development and to their safety and efficacy. One of the most serious types of instability is aggregation, the self-association of native protein through covalent and/or non-covalent interactions. Protein aggregates have been associated with increased or decreased drug potency and with an increased potential for immunogenic side effects, which can be life-threatening. In this themed issue of the AAPS Journal, we have assembled research and review articles that address the aggregation of therapeutic proteins. The issue was inspired by presentations at the 10th Annual Garnet E. Peck Symposium in Industrial Pharmacy, held at Purdue University in West Lafayette, Indiana, on October 11, 2012.

At a practical level, interest in the aggregation of therapeutic proteins is driven by the need for stable formulations and robust manufacturing conditions. At the symposium, Dr. David Volkin (Dept. of Pharmaceutical Chemistry, University of Kansas) presented a series of case studies based on his recent work that address these issues. The cases showed the effects of excipients on the aggregation of an albumin fusion protein (2) and IgG2

monoclonal antibodies (mAbs) (3), the role of infusion bags in the solubility and aggregation of IgG4 mAbs (4), methods to ensure comparability during mAb process development (5), and an empirical phase diagram approach to identifying formulations that inhibit aggregation (6). In their review article in this special issue, Dr. Volkin and his coauthors summarize these case studies, present an overview of protein aggregation mechanisms, and describe high throughput approaches to monitoring protein stability (7).

In developing drug products, the industry makes use of accelerated stability studies to estimate shelf life at the storage temperature based on degradation rates measured at higher temperatures. This extrapolation usually assumes that the temperature dependence of reaction rates follows Arrhenius behavior. However, protein aggregation often exhibits non-Arrhenius temperature dependence, even over relatively narrow temperature ranges. At the symposium, Dr. Chris Roberts (Dept. of Chemical and Biomolecular Engineering, University of Delaware) presented his group's recent work on the mechanisms of protein aggregation and origins of non-Arrhenius behavior (8,9). In this themed issue, he and Dr. Wei Wang (Pfizer BioTherapeutics) summarize these mechanistic insights and discuss the implications for accelerated stability testing (10).

Ensuring that protein drugs are aggregate-free requires robust, reproducible analytical methods. The ideal method would resolve aggregate and monomeric species, quantify aggregate size and concentration, and provide a low limit of detection, all while achieving high throughput at moderate cost. Current methods fall short of this ideal. For example, size exclusion chromatography (SEC) assays for soluble aggregates are expensive and low-throughput. Gel

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electrophoresis (e.g., SDS-PAGE) gives somewhat higher throughput but generally is not quantitative. To address these limitations, Dr. Mary Wirth (Dept. of Chemistry, Purdue University) and her colleagues are developing novel chromatographic materials based on silica colloidal crystals. These ordered arrays of silica particles provide plate heights in the low nanometer range, allowing for high resolution and rapid analysis times. For example, using a capillary packed with silica colloidal crystals and pressure-driven flow, the group has separated a monoclonal antibody and its aggregates in less than a minute with baseline resolution (11). Dr. Wirth and her coauthors summarize the group's recent results in this themed issue (12).

Together, these articles provide insight into our current understanding of protein aggregation. Gaps in fundamental understanding and applied technology remain, however, impeding our ability to monitor and inhibit aggregation in biologics. These gaps include limited understanding of the relative importance of partial unfolding, colloidal interactions, and chemical reaction in the protein aggregation process; a lack of understanding of the chemical and physical determinants of the immune response to protein drugs and their aggregated forms; poor agreement among available analytical methods for determining aggregate size and concentration, particularly in the subvisible range; and an incomplete understanding of the mechanistic effects of process stresses and formulation variables. We hope that the practical and theoretical perspectives assembled here will stimulate additional discussion and research in this important area.

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