Pharmaceutical aspects of subvisible particles in protein formulations

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CHAPTER 1

GENERAL INTRODUCTION

Immunogenicity of therapeutic proteins

Since the introduction of insulin as the first protein-based pharmaceutical product in the 1920s, the market for and the number of biopharmaceutical drugs has been rapidly growing. At present, about 100 different therapeutic proteins have been approved for clinical use by the US Food and Drug Administration (FDA) and they have acquired a key role in the treatment of various diseases such as several types of cancer, autoimmune and inflammatory diseases, and metabolic disorders (1). The first therapeutic proteins originated from non-human sources, such as equine antisera and insulin from bovine or porcine pancreas. Even though effective for therapy in humans, the large drawback of such proteins was their low purity and foreign structure to the human immune system, resulting in immune reactions in patients against the therapeutics (2). Extensive research has been performed in the past 30 years to improve safety and efficacy of biopharmaceuticals. With the development of improved molecular biology methods, recombinant expression techniques and better purification protocols, it has become possible to obtain highly pure recombinant human proteins. It was believed that those recombinant human proteins will not be recognized as foreign by the human immune system and will therefore not reveal the immunogenicity-related problems of former therapeutic proteins. However, clinical and post-market studies show that even these "human" products still induce immune responses in patients, suggesting that not just "foreignness" alone is responsible for the unwanted immunogenicity (3–5).

As we know now, unwanted immunogenicity of therapeutic proteins is a complex issue depending on patient-related factors (e.g., type of disease, genetic background), treatment-related factors (e.g., administration route, dosage regime), and product-related factors (e.g., product modifications, contaminants, and impurities) (6–8). An introduction of biological mechanisms potentially underlying unwanted immunogenicity can be found in Chapter 3. While it is still not entirely clear how each factor contributes to a drug product's potential for immunogenicity, it is generally recognized that the presence of aggregates is one of the main product-related risk factors for inducing immune reactions in patients (9–12).

Protein degradation and aggregation

Protein degradation can occur throughout the life cycle of a drug product, including manufacturing, storage, handling and administration to patients. The protein can thereby undergo various ways of degradation (13). Chemical modification for example include reactions such as deamidation, oxidation, isomerization, and peptide bond cleavage.

These can compromise the primary structure and thereby the conformational stability of a protein. Conformational stability can also be influenced by physical degradation including exposure to elevated temperatures, solid-liquid and liquid-air interfaces. In many instances, protein degradation results in protein aggregation.

Protein aggregation can follow a number of different mechanisms and pathways (Figure 1). These mechanisms are not mutually exclusive and can occur in parallel within the same product. The predominant mechanisms depends not only on the protein itself, but also on a variety of other factors, such as the formulation, the presence of impurities or contaminants, and the exposure to chemical or physical stress mentioned above (14–16). It is currently not fully understood how different aggregation mechanisms and the thereby resulting structural differences of aggregates influence their potential immunogenicity.

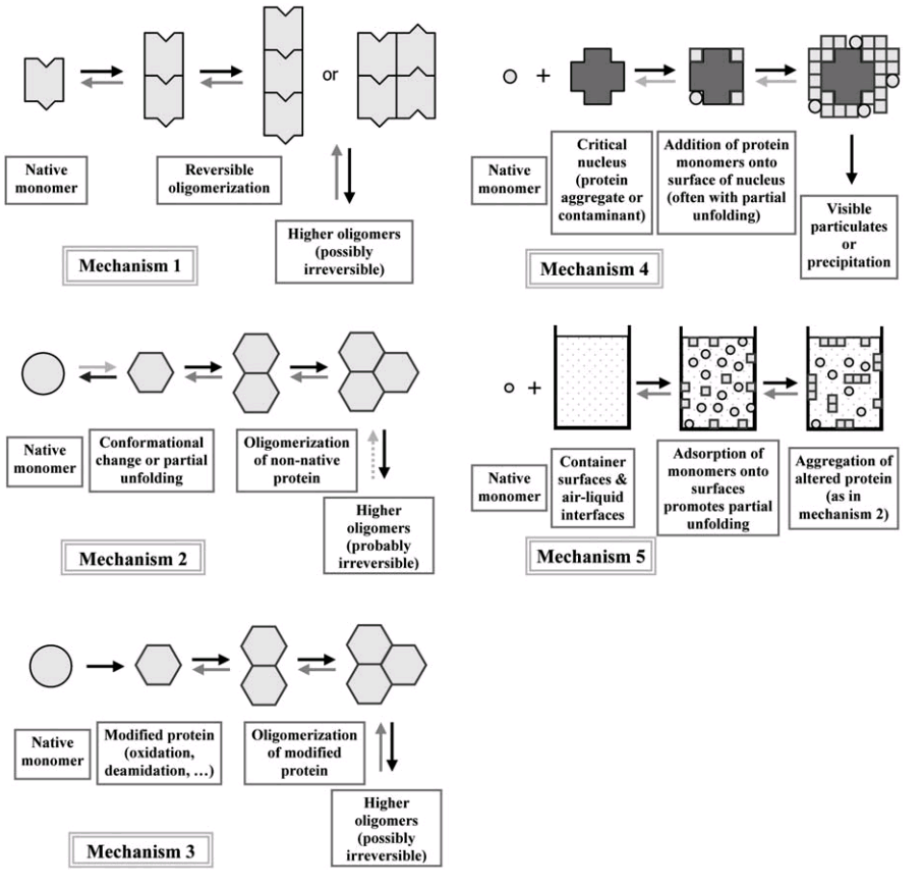

Figure 1: Schematic illustration of five common aggregation mechanisms (14).

Formulation development, an integral part of every biopharmaceutical drug product development program, aims to obtain a product that, amongst other things, maintains the stable and functional state of a therapeutic protein throughout the intended shelf life, while suppressing the potentially harmful degradation pathways. A detailed introduction into formulation development can be found in Chapter 2.

Analytical challenges

One major challenge during protein formulation development is the reliable characterization and quantification of potential degradation products, particularly aggregates and particles in the size range between around 0.1 to 10 µm (17,18). Importantly, proteinaceous particles in this size range are potentially the most immunogenic class of protein aggregates and are thus generally considered a critical quality attribute (19–22). While instrument manufacturers have worked on providing new analytical techniques to overcome an analytical gap in the subvisible size range identified in 2009 (19), there is a large demand of their critical scientific evaluation (23–26). Additionally, subvisible particles can be composed of non-proteinaceous material, such as particle sheds from pumps or primary packaging materials (including silicone oil droplets in prefilled syringes) or particles formed by degradation of excipients (e.g., polysorbate). While those are not necessarily harmful themselves, they can negatively impact protein stability and thereby compromise product quality (27–32). The presence of non-proteinaceous particles can also be indicative of problems during the production process (33). Unlike the compendial specification for particles ≥ 10 µm and ≥ 25 µm (34,35), there are currently no specifications for particle concentrations in the size range < 10 µm. It is therefore necessary for developers of innovator as well as biosimilar products to assess the nature and criticality of potentially present aggregates and particles case-by-case.

Aim and outline of this thesis

The aim of the work presented here was to evaluate and improve established and emerging analytical techniques for the characterization of aggregates and particles in the nm- and µm-size range, which are to be employed during research and development of biopharmaceutical drug products. These analytical techniques are then applied:

(i) to characterize particles in the nm- and µm-size range present in protein formulations and
(ii) to study the effect of nanoparticulate impurities from excipients on the stability of therapeutic proteins.

Chapter 2 is an introduction into the field of protein formulation development. It reviews literature on current protein formulation development strategies and summarizes current challenges formulation scientists are facing. **Chapter 3** is an introduction into the concept and underlying mechanisms of unwanted immunogenicity, as well as a review of various models currently employed to predict immunogenicity during the different stages of research and development of biopharmaceutical drug products. In **Chapter 4**, an improved version of the commonly applied subvisible particle counting technique light obscuration is investigated for its applicability to analyze formulations with high protein concentrations. The influence of sample viscosity on the results of different system setups is studied using highly concentrated drug products and model solutions with enhanced viscosity, which are spiked with polystyrene beads. **Chapter 5** is a comparative evaluation of Micro-Flow Imaging and Resonant Mass Measurement as emerging techniques for the differentiation of protein particles and silicone oil droplets in biopharmaceutical formulations. Artificially formed protein aggregates and silicone oil droplets in various concentrations and size ranges are analyzed individually and in different combinations by both systems. Furthermore, a novel mathematical filter, differentiating the particle types based on morphology, is developed and evaluated in comparison to currently used algorithms. In **Chapter 6**, four of the most relevant flow-imaging microscopy instruments are compared with the goal of identifying their differences, benefits and shortcoming. Artificially formed protein aggregates and silicone oil droplets as well as counting and sizing standards are used to test the instruments with respect to their accuracy and precision regarding size and concentration determination as well as their capability of differentiating particles of different morphology. In **Chapter 7**, an interference of sugar-containing formulations with light scattering based detection of nm-sized protein aggregates is investigated. The root cause of this interference is studied by using various different sugars, purification techniques and analytical instruments. In **Chapter 8**, nanoparticulate impurities found in pharmaceutical-grade sucrose are investigated and their effect on the stability of four therapeutic monoclonal antibodies currently on the market is studied in a time and concentration dependent fashion. In **Chapter 9**, the main findings are summarized and perspectives for further developments of analytical techniques and improvements of scientific knowledge in the field of subvisible particle analysis are briefly discussed.

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