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A CASE STUDY OF PREVENTING IGG4 DRUG PRODUCT AGGREGATION

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Abstract

Protein aggregation is one of the major challenges in developing and manufacturing drug products based on monoclonal antibodies. The focus of the present work is the investigation of protein aggregation mechanisms that may occur during its manufacturing. The comprehensive approach to study of the target molecule stability was applied to all production stages.

Unique capability of therapeutic proteins to specifically bind with a particular target *in vivo* makes them ideal candidates for the next generation drugs development. However, protein aggregation can reduce the effectiveness and safety of target molecules, while increasing the drug immunogenicity. Protein aggregation is one of the major challenges in developing and manufacturing drug products (DP) based on monoclonal antibodies [1]. The mechanisms of protein molecules aggregation can be divided into five main types: aggregation of native protein, aggregation of chemically modified product, aggregation of conformationally-altered monomer, nucleation-controlled aggregation and surface-induced aggregation [1, 2]. It is worth noting that these mechanisms are not mutually exclusive, and for the same product aggregation can occur in different ways.

There is a wrong assumption that the drug product stability depends only on the finished formulation development stage, namely the correct choice of buffering agents, pH value as well as added excipients. At the same time, destabilization of the target protein can be triggered at different steps of the production and, therefore, affect the drug product stability during its long-term storage [3]. Establishing the degradation profile of the target molecule is one of the most important approaches to identify possible problems with the active substance stability throughout its life cycle even in the early development stages.

The main purpose of this investigation was to examine mechanisms of protein aggregation that may occur during manufacturing process of the drug product. For this, the degradation profile of the target molecule IgG4 produced by JSC "GENERIUM" was established using different forced degradation tests, such as high temperature, mechanical stress, light exposure, extreme pH range, oxidation and freeze/thaw cycles. The critical quality attributes of the target protein after stress tests were investigated using different methods where the most relevant were exclusion chromatography (aggregates and fragments), ion exchange HPLC (acidic and basic forms), capillary electrophoresis in reducing and non-reducing, denaturing conditions (related substances). Conformational and colloidal stability of the DP was characterized by establishing T_m and T_{agg} using differential scanning fluorimetry (DSF) and static light scattering (SLS) methods. The average hydrodynamic radius of the target molecule was analyzed using dynamic light scattering (DLS).

The most revealing experiments for the DP based on IgG4 were such stress tests as continuous shaking, high temperature and low pH. In particular, the purity (size exclusion HPLC) of the DP was found to be decreased to 30 % after shaking. The average size of the DP after this stress testing was shifted up to 3–4 nm compared to the control sample and indicated the aggregation process. To solve this problem possible aggregation mechanisms were investigated. It was found that the aggregation of the target protein could be related to the oxidation caused by its treatment with low pH (3.0–3.5) during chromatographic purification. After excluding this stage from the downstream process the DP stability was significantly increased.

The obtained results revealed that the comprehensive approach to the study of the target molecule stability should be applied at all production stages.

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