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Issues Associated with Protein-Based Pharmaceuticals

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Extended Abstract

Abstract

Proteins are crucial molecules for various biological processes in our body. Production of pharmaceutical formulations of proteins is challenging because of the complexity of protein purification, synthesis and instability. This is why most of protein formulations are currently available in injection forms which overcome the poor bioavailability of proteins delivered by other route of administrations (e.g. oral and inhalation routes), provide greater control by the clinical administrator, and faster pharmaceutical development.

Introduction

Proteins are polymers of 20 L-amino acids which are connected by covalent amide bonds to form a polyamide backbone (protein primary structure) in addition to a range of side chains. Different arrangements of amino acid sequence result in the formation of protein's secondary structure. Proteins have advanced levels of structural organisation; tertiary (three-dimensional arrangement of the polypeptide chains in folded state) and quaternary (mixture of more than two polypeptide chains) structures. Hydration of the active site cleft of protein is significant for its activity. Removing water from protein's tertiary structure, especially in drying process, will highly result in biological inactive protein. However, overhydration of the protein is not preferred. It has been reported that 0.25 (g water)/(g protein) is considered as the maximum hydration level required to achieve a stabilise proteins; more than this level, water tend to act as a plasticiser, consequently, protein mobility will be noticed. In this review, issues associated with protein formulations will be addressed.

Stability of proteins

Production of biopharmaceutical formulations of proteins is challenging due to the inherent sensitivity of protein to various stress conditions during the protein formulations manufacturing process (e.g. process stresses and temperature) in addition to the high rate of protein degradation during long-term storage. Proteins instabilities are divided into two categories: chemical instabilities and physical instabilities. Chemical instabilities are disulphide exchange, deamidation, β-elimination, racemisation, oxidation and hydrolysis). While physical instabilities are precipitation, aggregation, denaturation and adsorption. The addition of additional additive is more common in protein formulations to help proteins to overcome processing stress such as heat.

High protein concentration formulations

Development of protein-based formulations require a high dose (in the order of mg/kg) of protein loading which is a problem for subcutaneous route (<1.5 ml is the permissible administration volume in this route that require >100 mg/ml of protein concentrations) and for solubility limited proteins (e.g. cytokines) that require the use of solubility enhancers. Such formulations also result in critical challenges in manufacturing, analytical, stability and delivery as proteins have high tendency to aggregate at high concentrations. Another problem associated with high protein concentration is the dramatic increase in the solution viscosity that leads to difficulties with pumping, filtering, filling and recovering the final product form vesicles, and can hinder the administration ability of the formulation in injection form.

Solvent used during processing techniques

The solubility and physical stability of proteins in a solvent used in processing technique could be a limiting step. For instance, lysozyme is soluble in aqueous media while it is insoluble in alcohol. Lysozyme was used in two different drying techniques; spray drying (use the power of heat to produce a dry product) and electrospraying (use charges to produce s dry product).

In electrospraying technique, the use of %100 water as a solvent is not possible as water will not evaporate by the effect of electric charges, in addition, the use of %100 of ethanol would result in protein denaturation. That is why a high proportion of the used solvent should be ethanol. For example, reported the use of %100 water solution in spray drying process of lysozyme while used (80/20 v/v) ethanol/water in order to improve lysozyme solubility in ethanol. The percentage (%) yield of the final product reported %53 and 25 for spray dried lysozyme with beta-cyclodextrin and electrosprayed lysozyme with beta-cyclodextrin, respectively.

Additives used in protein biopharmaceuticals

Due to protein sensitivity (that could lead to protein denaturation as a result of stresses associated with most pharmaceutical technologies) most of protein formulations now contain at least one type of additives to support protein stability during processing and storage. Choosing an additive that is compatible with protein formulation is essential. Additives will interact with proteins in the preparations thus it is important to select an additive that will not change the activity of the protein. Examples of most commonly used additives include: polymers (e.g. hyaluronic acid), sugars (e.g. sucrose), polyols (e.g. mannitol), surfactants (e.g. pluronic F-127), antioxidants (e.g. ascorbic acid), amino acids and certain salts (e.g. ammonium sulphate and chloride) which can prevent heat-induce aggregation of lysozyme.

Long-term storage of protein formulations

As mentioned above, stability of proteins in a challenging task to achieve especially at long-term storage. Proteins might show %100 biological activity after it has been prepared in a formulation form. However, this is not always the result after long-term storage. Number of factors affects protein stability in long-term storage: state of the formulation (solid form or liquid form), temperature and humidity. Dried protein formulations are usually more stable than liquid form formulations. This is due to the effect of water on the protein molecules as they become more flexible, accordingly, proteins conformation could be changed. Protein in solution form is more eligible for microbial contamination which would spoil the formulation. Moreover, in solution form, proteins are more susceptible for oxidation leading to aggregate formation within the solution due to protein collusion with other molecules in addition to the collusion with container surface.

Conclusion

Numerous challenges associated with the development of protein biopharmaceutical formulations arise from the complexity of protein purification, synthesis, chemical and physical instabilities. Maintaining the three-dimensional structure (tertiary) of proteins during processing and lone-term storage is vital for protein biological activity. By exposing proteins to various stress conditions during processing and storage might inactivate the protein either in reversibly or irreversibly ways. Accordingly, using additional additives is critical to prevent any chemical and physical instability of proteins in the formulation.