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Stability characterization and appearance of particulates in a lyophilized formulation of a model peptide hormone-human secretin☆

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ABSTRACT

Drug shortages and recalls are often caused due to particulate growth in parenteral products and can have serious clinical implications. Root cause analysis of such recalls and shortages may arise due to insufficient understanding of process, formulations issues and environmental effects than often reported filtration and inadequate personnel training. Therefore, the goal of this study was to use a model peptide hormone, secretin that is currently under drug shortage, and investigate the effect of excipients on the lyophilized secretin formulation and evaluate the effect of storage and excursion temperatures. Lyophilized formulation was assayed for secretin by reverse phase HPLC. Solid state characteristics of lyophilized formulation were determined by X-ray powder diffraction (XRPD), thermal and spectroscopic methods. Dynamic light scattering (DLS) was used to detect particulates in the formulation after reconstitution. To assess the environmental impact, the lyophilized samples were stored at −20 °C, 4 °C, 25 °C and 25 °C/60%RH and analyzed at time 0, 1, 4, and 8 weeks. HPLC analyses exhibited a decrease in secretin concentration by 8 week (20–27% fold decrease). Visual observation and DLS showed particulates and increased reconstitution time (*e.g.*, at 25 °C/60%RH, particle size of ~390 nm at day 0 to >2 μm as early as week 1; reconstitution time of ~20 s at day 0 to ~67 s at week 8). XRPD, thermal and spectroscopic methods demonstrated polymorphic transitions of mannitol and increased crystallinity in the lyophilized formulations with time. These studies potentially address the effect of product excursions outside the proposed label storage conditions which is −20 °C for secretin formulation and this is the first time it has been investigated. These observations indicate that both environmental factor and excipient may have an impact on the stability of secretin formulation and appearance of particles in the product.
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1. Introduction

In recent times there has been lot of measures undertaken to overcome recalls and drug shortages due to particulates in parenteral products (June et al., 2014; Wang et al., 2014). United States Pharmacopeia categorizes particulates in injectable as extrinsic, intrinsic and inherent particles (USP, 2012). Extrinsic particulates in parenterals usually arise from external factors like glass, dust, fibers and including those caused by personnel. Commonly observed intrinsic particulates to mention a few are generated from packaging material, delamination of glass vials, silicone oil, and rubber stoppers (Langille, 2013). Although sources and nature of extrinsic and intrinsic particulates have been widely investigated and published, more light should be shed on inherent particulates spawned due to formulation issues, protein aggregates and formation of drug crystals during stability (Das, 2012). Particulate matter in injectable can cause mild to severe clinical reactions in patients, for example, local inflammation at the site of injection, phlebitis, pulmonary embolism, pulmonary granulomas, infarction and death (Doessegger et al., 2012; Langille, 2013).

Lyophilization is the most preferred method to stabilize biopharmaceuticals like proteins and peptides (Carpenter et al., 2002; Pikal et al., 1991). Ideally, a lyophilized product should possess the following characteristics: long term stability; elegant cake appearance; short reconstitution time; protein should regain its original form and particle size distribution following reconstitution without any structural loss (Bedu-Addo, 2004; Kasper and Friess, 2011). While lyophilization prolongs the shelf life of the protein products, some proteins are inactivated during the lyophilization process or degraded during

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storage (Carpenter et al., 1997; Hageman, 1988). Some degraded protein/peptide would also lose their therapeutic effects and may cause local inflammation, mechanical disruption to tissues or trigger immune response to particulates in the formulation (Doessegger et al., 2012; Wang et al., 2014). The issues in the product quality lead to drug shortages and several drug product recalls by the FDA (FDA, 2014a,b,c). Too often, root cause analysis of particulates in parenteral products has been reported to be due to filtration problem during processing or operator training problems (Friedman, 2005; Langille, 2013) but particulates may arise due to poorly understood manufacturing process or inadequately optimized formulation parameters (FDA, 2012, 2014b; Siddiqui et al., 2014).

The choice of excipients within the formulation often dictates the characteristics of final lyophilized product. Most commonly used excipients are sugars, polyols, amino acids and polymers as stabilizers; salts for tonicity adjustment and surfactants for reducing adsorption and aggregation (Izutsu et al., 1994; Jorgensen et al., 2009). Mannitol, a polyol, is widely used as an excipient because of its ability to crystallize from frozen aqueous solution and high eutectic melting temperature of the mannitol/ice mixtures. Due to these physical properties, its addition to formulation produces a pharmaceutically elegant cake structure and allows usage of higher primary drying temperature thereby increasing the efficiency of freeze drying cycle-time (Kim et al., 1998). Mannitol exists in different polymorphic forms: amorphous, anhydrous (α, β, δ forms) and hydrate form depending on processing conditions and other excipients used in the formulation (Burger et al., 2000; Nunes et al., 2004). The amorphous or crystalline nature of mannitol during freeze drying and storage dictates the protective effect of mannitol on the protein drug (Costantino et al., 1998; Herman et al., 1994). Amorphous state of mannitol has been demonstrated to protect certain enzymes during freeze drying (Izutsu et al., 1994). The crystalline form, which undergoes polymorphic transition between mannitol hydrate and crystalline anhydrous form tends to impact stability of protein/peptide during lyophilization and storage (Cao et al., 2013).

Amongst a number of excipients studied, addition of salts effectively suppresses the crystallization of mannitol during freeze drying (Costantino et al., 1998). In some cases addition of salts to mannitol–protein mixture lowered the rate of aggregation (Costantino et al., 1998). The impacts of sodium chloride and sodium phosphate on physiochemical properties of mannitol during freezing, annealing, and drying have been well documented (Costantino et al., 1998; Hawe and Frieb, 2006).

In order, to better understand the effect of excipients on the formulation product, a model peptide hormone, secretin, was chosen that is currently under drug shortage (FDA, 2014a). Secretin, a peptide hormone released from the duodenum, is a 27 residue peptide (heptacosipeptide) with amidated carboxyl terminus (Bayliss and Starling, 1902). Its primary function is to stimulate bicarbonate secretion by the pancreas and inhibits gastrin-stimulated gastric acid secretion. It has been reported that aqueous solution of secretin is unstable and loses its activity even when stored at −20 °C (Beyerman et al., 1981; Suenaga et al., 1976). Although the biological function and instability of secretin has been well investigated (Mutt and Jorpes, 1971; Onoue et al., 2006; Vaudry et al., 2000), there has been no scientific publication to the best of our knowledge that focuses on strategies to formulate and evaluate human secretin as a diagnostic peptide except for a patent filed (Suenaga et al., 1976). FDA has approved only one secretin product so far. It is an orphan drug that aids in the diagnosis of pancreatic cancer and pancreatic exocrine dysfunction. Drug shortage of such similar products may arise due to particulate growth within the lyophilized formulations. Therefore, our goal is to investigate the effect of excipients on the lyophilized secretin formulation and evaluate the effect of storage and excursion temperatures on the product. This is the first time such study has been reported for secretin peptide formulation and will give valuable information on product quality.

2. Materials and methods

Synthetic human secretin peptide was purchased from Bachem Americas Inc., (Torrance, CA). Mannitol and Cysteine HCl anhydrous were purchased from Amresco (Solon, OH). Sodium chloride (NaCl) was obtained from Fisher Scientific (Pittsburg, PA). All chemicals and reagents used were of USP or analytical grade. Type I glass vials and an automatic crimp sealer were purchased from Wheaton Science Products (Millville, NJ). Lyophilization stoppers (Fluorotec 20 mm stoppers) and 20 mm flip top crimp seals were purchased from West Pharmaceutical Services (Lionville, PA).

2.1. Preparation of secretin formulation

The secretin formulation was prepared by dissolving each component in following order: NaCl 0.9% w/v, Mannitol 2% w/v, CysteineHCl 0.15% w/v and Secretin 0.002% w/v in previously chilled autoclaved water under gentle stirring. The choice of the formulation composition was based on the information in the package insert of the marketed secretin product. The final volume of the formulation was made up to 1.2 L and sterile filtered using 0.22 μm filters and immediately transferred to the autoclaved lyophilization vials. The placebo formulation was prepared as mentioned above without addition of secretin.

2.2. Lyophilization

The secretin and placebo formulations were filled into 10 mL type I glass vials prearranged on a stainless steel tray. The fill volume of each vial was 2.5 mL and stoppered partially with 20 mm lyophilization stoppers. Empty vials were arranged in the periphery of the stainless-steel tray to minimize radiation effects (Awotwe-Otoo et al., 2012; Tang et al., 2006). Product temperature during the lyophilization process was monitored by placing thermocouples in the front, center, back and edge vials. Lyophilization was performed using a Lyostar3™ lyophilizer (SP Scientific, Stone Ridge, NY) using manometric temperature measurement mode (Auto-MTM). This mode allows the users to run a pre-determined lyophilization cycle. The samples were frozen at an initial shelf temperature of −45 °C. Primary drying was conducted at a chamber pressure of 0.166 mbar with shelf temperature adjusted to −25 °C and held for ∼20 h. Following primary drying shelf temperature was increased to 25 °C at a ramp rate of 0.08 °C/min and secondary drying was performed for ∼13 h. At the end of cycle, vials were automatically stoppered. The stoppered vials were successively crimp-sealed and stored at their respective storage temperatures (−20 °C, 4 °C, 25 °C and 25 °C/60%RH). At predetermined time points, samples were withdrawn and analyzed for changes in protein integrity and excipients.

2.3. Sample reconstitution

The lyophilized samples were reconstituted by injecting 10 mL of 0.22 μm filtered water obtained in-house from the Milli-Q Gradient A-10 water purification system (Millipore corp., Bedford, MA) into the vial through the rubber stopper using a sterile syringe–needle to yield a final concentration of 4 μg/mL of secretin per vial. Reconstitution time was immediately determined as the time taken for complete dissolution of lyophilized powder following addition of 10 mL solvent. Upon complete reconstitution, it is expected to obtain a clear solution free of particulates. Visual