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Development of pellets for oral lysozyme delivery by using a quality by design approach

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ABSTRACT

The concept of Quality by design is of increasing importance in the pharmaceutical industry, especially in the development of biotechnologically produced active pharmaceutical ingredients (APIs). These proteins/peptide-like materials are usually more sensitive than small molecules to the environmental and process parameters.

The objectives of the presented study were to identify how the critical process parameters influence the critical quality attributes (CQAs) of a lysozyme-containing multiparticulate dosage form, and to determine the critical points of API activity preservation. The experiments were performed according to a 2^6 factorial design. The process parameters were recorded with Process Analytical Technology tools, based on in-line mixing speed, mixer torque and temperature measurements and at-line moisture content analysis. The hardness and geometry of the pellets, and the activity of the API were studied as CQA. Complex relationships were identified between the CQAs and process parameters, and the thermomechanical effects and the liquid distribution inside the mass during the texture-forming steps of the process were found to exert considerable effects on the mechanical and geometrical properties and also basically to determine the API activity.

1. Introduction

Biotechnologically produced APIs are of ever greater importance in the medication of patients, and the number of such APIs is currently increasing exponentially in the fields of anticancer therapy and the treatment of autoimmune or neurodegenerative diseases.

The formulation of these API macromolecules into stable and convenient dosage forms is a challenge for the pharmaceutical technologist because of their high sensitivity to environmental parameters or mechanical stress (Hamrang et al., 2013). Under these circumstances, the implementation of the Quality by design (QbD) approach as a quality assurance system is at present one of the hottest topics of the pharmaceutical and biotechnological industry (Rathore and Winkle, 2009; Rathore, 2009, 2014; Kontoravdi et al., 2013). The QbD concept replaces empirical pharmaceutical developments with a risk-based approach where the effects of the critical process parameters (CPPs) and their interactions on the critical quality attributes (CQA) should be evaluated from the aspects of production robustness and economy, treatment efficacy and patient safety. Furthermore, it aims at a more flexible production process and the reduction of failure costs through replacement of the currently used single-point validation-based processes with the application of design space-based production, which is a multidimensional combination of the acceptance regions of the studied CQAs within the factor space, and the modification of CPPs within this space is not regarded as change. The requirements of the relevant guidelines (ICH Q8, Q9, Q10 and Q11) contain

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recommendations concerning a full mathematical description and a detailed explanation of the above-mentioned effects of factors and factor interactions via the consecutive variation of CPPs based on the application of appropriate design of experiment (DoE) methods, which enables a reliable determination of the process design space (PDS).

At present, most of the formulations of biotechnology-derived APIs are parenterally administered liquid preparations, and the stabilization of the APIs in such an environment, together with patient compliance as concerns these products, are therefore critical issues (Awotwe-Otooa et al., 2012; Lindholm Larsen et al., 2014). Lyophilization is an effective way to improve the shelf-life of these products, but process optimization to avoid of the loss of activity during the re-dissolving process carries further challenges (Kasper et al., 2013). Protein delivery through inhalation or the nasal route could be alternatives (Forde et al., 2006; Maltesen et al., 2008), but the most convenient way from the aspect of patient compliance is still oral administration. The formulation of proteins or peptides into orally administered solid dosage forms could be very advantageous, but there are numerous concerns in connection with the processability of these drugs and their bioavailability in such dosage forms.

The most important issues during oral administration are the protection of the API from the low pH of the stomach, and from enzymatic degradation in the GI tract, and the ensurance of appropriate absorption (Morishita and Peppas, 2006). All of these issues may be solved through appropriate formulation, but the protection of the API from thermal and mechanical stress during the technological processes remains a problem.

Enzyme protection and absorption enhancement could be achieved through chemical modification (e.g. pegilation) of the molecules (Calceti et al., 2004), or the application of intelligent, phase-sensitive drug carrier systems (Wang and Wu, 1998; Singh and Singh, 2004; Toorisaka et al., 2005). However, the size of the microspheres still requires the functionalization of these particles, their production is complicated, often requiring the application of organic solvents, and the entrapment efficacy is questionable. The use of *in situ* gelling and biodegradable polymer systems (Kim and Peppas, 2003; Matricardi et al., 2006; Cchabra et al., 2007) allows similar conditions during administration, but results in a better entrapment efficacy than with microspheres. The storage stability and the corresponding shelf-life of these systems are the same as those of parenteral dosage forms.

The stability of macromolecules is better in the solid form, but crystallization is not always possible. Liquid materials may be processed into solid dosage forms through spraying onto the surface of a solid carrier. Kristó et al. (2008) observed that a protein solution can act similarly to solutions of other macromolecules, and can be used as a binder in a granulation process. Granulation or pelletization methods are also advantageous from stability aspects, as the impairment of the protective mechanisms in a dosage unit is less problematic in multiparticular systems than in single unit ones. Nevertheless, the proteins should be protected from the effects of mechanical and thermal stress during processing (Clausen and Bernkop-Schnürch, 2001; Kuni and Leuenberger, 2003). The role of conformational stabilizers in these formulations is of considerable importance. Trenktrog et al. (1996) developed a pellet formulation which combines the advantages of the formulations discussed above. Nevertheless, since pelletization is a considerably difficult process (Bölcskei et al., 2012), the implementation of the QbD attributes is of great importance in such developments (Garala et al., 2013), especially if the API is a biotechnologically produced macromolecule.

Despite its numerous advantages, the processing of macromolecular APIs into solid dosage forms is a poorly studied area of pharmaceutical technology. The aim of present work were to the identify how the CPPs influence CQAs of a lysozyme-containing multiparticulate dosage form, and to determine of the critical points of API activity preservation, and to provide deeper information about the mechanism of texture formation during an extrusion-spheronization process.

2. Materials and methods

2.1. Materials

Egg-white lysozyme (Lysoch 40000, Handary SA, Brussels, Belgium), an antibacterial preservative that is widely used in the food industry and can also be employed as a natural antibacterial agent against Gram+ bacteria in gastrointestinal infections, was utilized as model protein. The conformation of the enzyme was stabilized with mannitol (Hungaropharma Ltd., Budapest, Hungary) according to the findings of Singh and Singh (2004). Microcrystalline cellulose (Avicel pH 101, FMC BioPolymer, Philadelphia, USA) served as the plastic carrier of the formulation.

2.2. Methods

100 g of powder mixture containing lysozyme, mannitol and cellulose in a ratio of 1:4:5, respectively, was homogenized in a Turbula mixer (Willy A. Bachofen Maschienenfabrik, Basel, Switzerland) for 10 min.

The homogenized powder mixture was wetted and kneaded in a ProCepT 4M8 high-shear granulator (ProCepT nv., Zelzate, Belgium) with 60 ml of purified water, produced with a Simax Distilling Apparatus (Kavalierglass, Sázava, Czech Republic). The amount of water, the critical process parameters and the experimental settings were determined on the basis of preformulation data and pilot experiments. The impeller and chopper speeds, the rate of liquid addition, the impeller torque and the product temperature were recorded throughout the process via in-line measurements.

The wet mass was extruded with a Caleva mini screw extruder (Caleva Process Solutions Ltd., Sturminster Newton, UK) The extruder was water-cooled with the application of a laboratory-developed cooling jacket. The temperature was monitored on-line with a Maxwell MT 25-901 hand-held infrared thermometer (Maxwell-Digital, UK), every 30 s; the measured values were recorded manually. The moisture contents of the wetted mass and extruded samples were measured before and during the process with an at-line method, using a Mettler-Toledo HR73 (Mettler-Toledo Ltd., Budapest, Hungary) halogen moisture analyser. The moisture content of 1 g of material was determined at 105 °C on multiple occasions during and after the extrusion process. The extruded samples were stored in moisture-retentive containers so as to avoid evaporation before spheronization.

The spheronization was performed with a Caleva MBS spheronizer (Caleva Process Solutions Ltd., Sturminster Newton, UK); the speed of the spheronizer was measured on line the values being displayed digitally and recorded manually every 60 s. The length of the process was measured with a