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Formation of mannitol hemihydrate in freeze-dried protein formulations—A design of experiment approach

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ABSTRACT

Since the discovery of mannitol hemihydrate, this form of mannitol has been seen as potentially negative with regard to the stability of pharmaceutical formulations. The formation of mannitol hemihydrate is reported in several case studies; however, no systematic investigation has been performed so far. In this study, design of experiments was applied for response surface modelling of mannitol hemihydrate formation. The formulation parameters investigated in a composite face-centred design were the overall solid content, protein concentration, protein type and the ratio between mannitol and sucrose. Additionally, annealing as process parameter was included in a full factorial mixed design. For two proteins, models with a high goodness of fit (R^2: 0.82 and 0.93) and goodness of prediction (Q^2: 0.78 and 0.89) were achieved. Inclusion of the process parameter annealing resulted in models of similar quality. The successful application of design of experiments showed that the most prominent factors enhancing the formation of hemihydrate were a high protein concentration, low relative mannitol content and annealing at −20 °C.

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1. Introduction

Biopharmaceuticals have become an important class of drugs as advances in biotechnology have made it possible to synthetically and recombinantly prepare proteins for human use. As a consequence there has been an increase in the number of approved biopharmaceuticals. However, peptides and proteins have limited physical and chemical stability. Freeze-drying is often used to obtain a solid formulation with an acceptable shelf life (Chang and Pikal, 2009; Frokjaer and Otzen, 2005; Schwegman et al., 2005; Wang, 2000).

In order to develop a stable freeze-dried formulation it is important to understand the influence of formulations and process parameters (Carpenter et al., 2002; Jorgensen et al., 2009). Mannitol is a commonly used bulking agent as it readily crystallizes to form a stable matrix resulting in an elegant cake appearance (Pikal, 2002; Shalaev et al., 2008). Mannitol can exist in both an amorphous form and three known polymorphic forms of the anhydrate: α-, β-, and δ-mannitol. Furthermore a mannitol hydrate form has been reported to crystalize during freeze-drying (Yu et al., 1999). This mannitol hemihydrate (MHH) is generally undesired as it could release water into the finished product during storage possibly resulting in an unstable product (Nunes et al., 2004; Yu et al., 1999). The solid state of mannitol is strongly dependent on formulation and process variables and it is therefore important to gain knowledge about the formulation- and process parameters that favour the formation of MHH so that its formation can be limited or avoided (Cavatur and Suryanarayanan, 1998; Kim et al., 1998).

It is challenging to make a general statement on the effect of processing conditions such as cooling rate, as the solid state of mannitol is also dependent on the concentration of mannitol and the presence of other additives such as sodium chloride, cyclodextrins, polysorbate 80, and sucrose (Cao et al., 2006; Cavatur and Suryanarayanan, 1998; Cavatur et al., 2002; Haikala et al., 1997; Hawe and Friess, 2006; Kim et al., 1998; Liao et al., 2007; Martini et al., 1997; Mehta et al., 2013). The effect of annealing temperatures on the physical form of mannitol has been investigated. In absence of protein, raising the annealing temperature from −18 to −8 °C did not change the outcome of the physical form of mannitol as a mixture of δ-mannitol and MHH was found in both cases. In the presence of protein the high annealing temperature resulted in crystallization of δ-mannitol while inhibiting the formation of MHH (Liao et al., 2007). Annealing at low temperatures generally seems to facilitate the formation of MHH (De Beer et al., 2011; Hawe and Friess, 2006; Liao et al., 2007; Pyne et al., 2002; Sundaramurthi and Suryanarayanan, 2010). A recent study showed that the temperature at which crystallization occurs influences the solid state: MHH formed if crystallization occurred below −20 °C, while anhydrous forms crystallized at temperatures above −10 °C (Mehta et al., 2013).

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Whether primary drying has an effect on the solid state of mannitol is unclear as the cooling rate often is found to dictate the morphology of mannitol (Cannon and Trappler, 2000; Cavatur and Suryanarayanan, 1998; Liao et al., 2007). Primary drying above T_g' was reported to result in crystallization of MHH (Cavatur and Suryanarayanan, 1998). However, another study with primary drying above T_g' concluded that primary drying did not have an effect on MHH, as MHH was still present after drying at both −5 and −20 °C (Liao et al., 2007). Cannon and Trappler (2000) also showed that the physical state of mannitol did not depend on the primary drying, but that the crystallization was dictated by the cooling rate (Cannon and Trappler, 2000). The effect of secondary drying temperature is also discussed in the literature, as some authors state that secondary drying temperatures above +50 °C might be necessary to desolvate the MHH and others state that the hemihydrate easily loses its water already at ambient temperature (Cao et al., 2006; Cavatur and Suryanarayanan, 1998; Liao et al., 2007; Nunes et al., 2004; Yu et al., 1999). The lack of investigations including multiple formulation parameters thus makes it challenging to draw general conclusions on the effect of processing conditions, and underline the need for a more systematic approach.

Gaining knowledge about a pharmaceutical product in a systematic manner according to the quality by design (QbD) guideline can be done in various ways. X-ray powder diffraction (XRPD) is generally the standard method for the identification of polymorphs, but can only be applied in an off-line setting. Raman spectroscopy on the other hand is suitable for in-line process monitoring of freeze-drying and was applied for the differentiation of mannitol polymorphs (De Beer et al., 2007; Kauppinen et al., 2013). The identification of mannitol polymorphs by NIR could be confirmed both with Raman and XRPD (Grohganz et al., 2011). Apart from the use of in-line techniques, the application of design of experiments (DoE) is another part of the QbD guideline. DoE can be used to gain knowledge about combinations and interactions of input variables (e.g. formulation- and process parameters) and to establish a design space. DoE is used both in the development of new products and for optimization of existing processes as the aim of DoE is to optimize quality and performance of the product. Ideally DoE can lead to both reduced production costs and improved product quality. Once a design space has been approved by the regulatory authorities it is possible to work within the design space without it being considered as a change in the process. This adds more flexibility to the process, however good process understanding is important in order to stay within the design space (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009; Yu, 2008). Different needs require the use of different experimental designs. A full factorial design investigates all variables and interaction effects on the response(s) and can be used in screening and robustness testing. For optimization, a response surface methodology (RSM) design, that also takes non-linear behaviour of the main factors into account, can be used. Such RSM designs are for example the Box–Behnken design, the central composite circumscribed (CCC) or the central composite face-centred (CCF) design (Eriksson et al., 2008, 2001; Lundstedt et al., 1998).

The objective of this study was to investigate the factors influencing the formation of MHH during freeze-drying. To facilitate fast production and analysis of the high number of samples needed, freeze-drying was carried out in well plates (Aucamp et al., 2005; Chieng et al., 2013) and analysis performed using a well-plate reader XRPD setup. A CCF-design was used to investigate how varying protein concentration, solid content and sucrose fraction influence the content of MHH in the final product. The additional effect of varying the process parameter annealing was investigated using a full factorial mixed design.

2. Materials and methods

2.1. Materials

Mannitol and sucrose were both Ph.Eur. grade (Unikem, Copenhagen, Denmark). Bovine serum albumin (BSA, minimum 98% pure) and lysozyme (LYS) from chicken egg white (70,000 units/mg) were purchased from Sigma–Aldrich (St. Louis, MO). The freeze-drying was carried out in custom-made brass well plates. Each plate had 8 columns and 12 rows. The wells were 3 mm deep, 7 mm in diameter and were filled with 100 μL of sample solution each. Spray mount glue from 3M (St. Paul, MN) was used to seal the plates with transparent multi-layered composite film from R&G Faserverbundwerkstoffe (Waldenbuch, Germany). For a comparison with freeze-drying in vials, 3 ml glass vials were used with a fill volume of 0.4 ml and 1 ml, resulting in a fill height of around 3 mm (corresponding to the wells) and 7 mm, respectively (see Section 2.2.1).

2.2. Methods

2.2.1. Freeze-drying

The freeze-drying was carried out in an Epsilon 2-4 LSC laboratory scale freeze-dryer (Martin Christ GmbH, Osterode am Harz, Germany). The influence of formulation parameters was first investigated using a standard freeze-drying cycle: The cycle consisted of freezing to −50 °C with a freezing-rate of 1 °C/min and then maintaining the temperature for 20 min, before annealing at −20 °C for 3 h followed by primary drying at −20 °C for 8 h and secondary drying at +20 °C for 1 h. For the investigation of process parameters (full factorial mixed design) two additional cycles were applied: One cycle had an annealing step at −30 °C for 3 h (low temperature annealing) and one cycle was done without annealing at all. For all three cycles the pressure was kept at 0.2 mbar (hPa) during primary and secondary drying. Freeze-dried products were stored at room temperature and 11% relative humidity. For a comparison of selected samples from well plates with samples in vials, it should be noted that a direct transfer of process parameters from well plate to vial is not possible due to e.g. the interconnectivity of the wells and the material type influencing the heat transfer. Two freeze-drying cycles were investigated. In the first cycle, the fill height was kept constant at approximately 3 mm and the same cycle was used as for well-plates. For practically more relevant samples (volume 1 ml, resulting height 7 mm), the cycle was prolonged accordingly, i.e. the primary and secondary drying durations were doubled to 16 and 2 h, respectively.

2.2.2. Modulated differential scanning calorimetry

Modulated DSC (MDSC) was applied for the determination of the T_g' of selected corner points. Measurements were conducted using a TA Discovery DSC (TA-Instruments-Waters LLC, New Castle, DE, USA) under a nitrogen gas flow of 50 ml/min. Temperature and enthalpy calibration was performed using indium as a standard. 15 μl of sample solution was pipetted in an aluminium Tzero pan, crimped with an aluminium Tzero hermetic lid and then analyzed. Samples were equilibrated at −50 °C and kept isothermal for 15 min. MDSC thermograms were obtained with a scanning rate of 1.5 °C/min from −50 °C to 25 °C applying a modulation amplitude of 1 °C and period of 40 s. The midpoint T_g' was determined as the mean of two independent measurements. The analysing software was Trios (TA-Instruments-Waters LLC, New Castle, DE, USA).

2.2.3. X-ray powder diffraction (XRPD)

XRPD measurements were done using an X-ray powder diffractometer from PANalytical X'Pert PRO with a PIXcel detector (PANalytical B.V., Almelo, The Netherlands). Diffractograms

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