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Effect of cyclophosphamide on the solid form of mannitol during lyophilization

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ABSTRACT

Mannitol is a commonly used bulking agent in lyophilized formulations. It can crystallize into multiple solid forms during lyophilization thereby exhibiting phase heterogeneity and variability in product performance. In this manuscript, we studied the effect of cyclophosphamide (CPA), an anticancer drug, on the solid form of mannitol during lyophilization from aqueous solutions. Freeze-concentration studies were performed in the DSC while lyophilization was performed in a lab scale freeze dryer.

DSC experiments revealed two-stage crystallization of mannitol (1.5% w/v) during freeze-concentration, evident as two distinct exothermic events (at −18.2 °C and −30 °C) in the cooling curve. This was complemented by two eutectic melting endotherms in the subsequent heating curve. Addition of CPA (4.0% w/v) completely inhibited the exotherm at −18.2 °C, but enhanced the enthalpy of exotherm at −30 °C by five folds. Likewise, only one eutectic melting endotherm was observed in the subsequent heating curve. Lyophilization of the solution containing only mannitol, yielded a mixture of β- (major) and δ- (minor) polymorphs of mannitol. However, in the presence of CPA, only δ-polymorph was observed in the lyophilized sample.

This selective favoring of the metastable δ-polymorph over the stable β-polymorph, was explained by altered freezing kinetics of the solution in presence of CPA. The study provides mechanistic insights into solute crystallization behaviour during lyophilization of multi-component systems.

1. Introduction

Mannitol is the most commonly used bulking agent in lyophilized products (Baheti et al., 2010). This is attributed to its tendency to form a crystalline structure with high eutectic melting temperature (~ −1.5 °C) (Liao et al., 2007). Mannitol can exist in four crystalline forms including three anhydrous forms (Burger et al., 2000), designated as α-, β-, and δ-mannitol, and a hydrated form, mannitol hemihydrates (Nunes et al., 2004; Vanhoorne et al., 2016b; Yu et al., 1999). It can also remain in amorphous state when its crystallization is hindered (Izutsu et al., 1994; Telang et al., 2003). Different solid forms of mannitol can have variable effect on the stability and other quality attributes of lyophilized formulations (Grohganz et al., 2010; Izutsu et al., 1994; Kim et al., 1998; Mehta et al., 2013; Vanhoorne et al., 2016a; Xie et al., 2008; Yu et al., 1999). For example, anhydrous forms differ in their solubilities (Su et al., 2013; Seidell, 1919) and reconstitution properties (Kim et al., 1998), while hemihydrate and amorphous forms tend to dehydrate and devitrify, respectively, upon storage. This kind of conversion can increase the amount of free water in drug micro environment thereby affecting the product stability (Herman et al., 1994; Larsen et al., 2014; Yu et al., 1999; Dixon et al., 2009). Moreover, the rate of moisture absorption of δ-mannitol and β-mannitol was found to be different which may alter the characteristics of the freeze-dried samples if exposed to moisture (Yoshinari et al., 2002). In addition, mannitol containing formulations occasionally exhibit vial breakage, which has been attributed to the crystallization of mannitol during primary drying (Williams and Dean, 1991; Williams et al., 1986). Moreover, various processing parameters e.g. freezing rate, annealing and formulation variables e.g. concentration and formulation composition are known to affect solid form outcome of mannitol in freeze dried samples. Kim et al. has shown the influence of freezing rate and mannitol concentration on solid form outcome. Slow freezing of 10% w/v mannitol formed a mixture of α and β-mannitol while fast freezing of the same formed δ-mannitol. In a contrary, fast freezing of 5% w/v mannitol resulted in β-mannitol (Kim et al., 1998). Slower cooling rate and annealing at −20 °C, is reported to facilitate crystallization of β-mannitol during lyophilization (Haikala et al., 1997; Kett et al., 2003; Kim et al., 1998). Earlier Mehta et al. and Liao et al. have stated that annealing favours MHH

Abbreviations: API, active pharmaceutical ingredient; CPA, cyclophosphamide; CSD, Cambridge structural database; DSC, differential scanning calorimetry; FTIR, Fourier transform infrared spectroscopy; MDSC, modulated DSC; XRD, X-ray diffraction.

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in freeze dried samples (Mehta et al., 2013; Liao et al., 2007). As a result, understanding the effect of processing and formulation variables on the solid form of mannitol has been an active area of research (Cao et al., 2013; Grohganz et al., 2013; Jena et al., 2016; Larsen et al., 2014; Mehta et al., 2013; Oddone et al., 2016; Peters et al., 2016; Stärtzel et al., 2016).

Solid form of mannitol in lyophilized products can also be influenced by the presence of other excipients in the formulation (Grohganz et al., 2013; Hawe and Friess, 2006; Izutsu et al., 2007; Pyne et al., 2003; Telang et al., 2003; Yoshioka and Aso, 2007). For instance, sodium chloride (Telang et al., 2003), sucrose (Hawe and Friess, 2006), poly(vinylpyrrolidone) (Yoshioka et al., 1995), trisodium and tripotassium phosphates and citrates (Izutsu et al., 2007) inhibited mannitol crystallization in the frozen solutions and/or lyophilized products, while glycine (Pyne et al., 2003) facilitated its crystallization. Additionally, sodium chloride (Telang et al., 2003) and proteins (Grohganz et al., 2011; Grohganz et al., 2013; Pajander et al., 2015) altered the polymorphic form of mannitol in the lyophilized products.

Similarly, it is also possible that the presence of active pharmaceutical ingredients (APIs) in formulations can also affect the phase behaviour of mannitol during lyophilization. However, such studies are scanty and only one study reporting effect of acetylsalicylic acid on the crystallinity of mannitol has been published (Torrado and Torrado, 2002). Hence it would be interesting to study the effect of API on phase behaviour of mannitol during lyophilization.

The purpose of present study was to evaluate the effect of cyclophosphamide (CPA), an anticancer drug, on the solid form of mannitol during freeze-concentration and subsequent lyophilization. CPA was selected for this investigation as it is available in market as a lyophilized product containing mannitol as the only excipient (Baheti et al., 2010). Freeze-concentration studies were conducted in situ in a differential scanning calorimeter (DSC) while lyophilization was conducted in a lab scale freeze-dryer.

2. Experimental section

2.1. Materials

CPA monohydrate (>99.8% purity), D(−) mannitol and HPLC grade water were purchased from Alfa Aesar® Limited (England), Loba Chemie Pvt. Ltd. (India) and Fisher Scientific India Pvt. Ltd. (India), respectively. *Warning*: CPA is carcinogenic and toxic.

2.2. Differential scanning calorimetry (DSC)

DSC (model Q2000; TA Instruments, USA) equipped with a refrigerated cooling accessory, RCS90, was used. Dry nitrogen, at 50 mL/min, was used as the purge gas. About 15 mg of the solution was weighed in T_{zero} aluminum pan and hermetically sealed. Data was analyzed using Universal Analysis®, version 4.5A. The solutions were cooled to −80 °C at 1 °C/min, held for 15 min, and finally heated to 25 °C at 1 °C/min. For modulated DSC (MDSC) experiments, modulation amplitude of ±0.5 °C was applied over a modulation period of 30 s during the heating run. All the analyses were conducted in triplicate and mean onset temperatures have been reported throughout the text. Similar protocols have been utilized earlier for evaluation of phase behaviour in aqueous solutions (Munjal and Bansal, 2015a, 2015b).

Prior to analysis, the instrument was calibrated using T_{zero} calibration and cell constant calibration. T_{zero} calibration was conducted in heat-cool mode by running two experiments, one without samples, and the second using sapphire disks provided by the instrument manufacturer (without pans, weight approximately 100 mg). High purity standard of indium was used to calibrate the cell constant and temperature.

2.3. Lyophilization

Aqueous solutions containing mannitol alone (1.5% w/v) and mixture of mannitol (1.5% w/v) and CPA (4.0% w/v) were passed through 0.22 μ Nylon filters, filled into 5 mL glass vials (2 mL fill volume), and transferred to a bench top laboratory freeze-dryer (VirTis® Advantage ™, SP Scientific, Gardiner, New York). Vials were covered with gray butyl 2-pronged rubber stoppers (Fisher Scientific India Pvt. Ltd., India). Samples were lyophilized using four different lyophilization protocols. Samples were cooled to −50 °C at different cooling rates (i) 1 °C/min, (ii) 0.6 °C/min, (iii) 0.2 °C/min and (iv) annealed for 6 h at −20 °C after freezing at 1 °C/min. These samples were held isothermally for 2 h and then vacuum (60 Pa) was applied and primary drying was conducted at −10 °C for 12 h. Secondary drying was conducted at 10 °C for 5 h, with a vacuum of 60 Pa. Temperature ramp of 0.4 °C/min and 0.28 °C/min were employed during primary drying and secondary drying, respectively. In contrast to the conventional higher vacuum levels and temperature employed during secondary drying, milder secondary drying conditions (i.e., 5 h at 10 °C with a vacuum of 60 Pa) were utilized in the current work, to prevent dehydration of mannitol hemihydrate during this step. Previous studies have reported mannitol hemihydrates, crystallized during freezing, can subsequently transform to anhydrous mannitol under aggressive secondary drying conditions (Mehta et al., 2013; Nunes et al., 2004; Yu et al., 1999).

2.4. X-ray diffraction (XRD)

Powder X-ray diffraction (XRD) patterns were recorded at room temperature using Bruker's D8 Advance Diffractometer (Karlsruhe, West Germany) equipped with a 2θ compensating slit, using Cu Kα radiation (1.54 Å), at 40 kV and 40 mA passing through nickel filter with divergence slit (0.5°), anti-scattering slit (0.5°) and receiving slit (1 mm). The diffractometer was calibrated for accuracy of peak positions with corundum. XRD patterns were obtained by scanning in continuous mode over an angular range of 3–40° 2θ with a step size of 0.1° and a dwell time of 1 s. Diffractograms were analyzed using DIFFRAC^{plus} EVA (version 9.0) diffraction software. Characteristic peaks of the various polymorphic forms of mannitol were identified based upon existing literature (Campbell Roberts et al., 2002; Hulse et al., 2009; Mehta et al., 2013).

2.5. Fourier transformed infrared spectroscopy (FTIR)

The FTIR transmission spectra of β-polymorph (hereinafter referred to as β-mannitol) and δ-polymorph (hereinafter referred to as δ-mannitol) of mannitol, CPA and lyophilized products were recorded on a Perkin-Elmer spectrometer (Spectrum One, Perkin-Elmer, Buckinghamshire, U.K.) equipped with spectrum v3.02 software using attenuated total reflectance accessory. Each spectrum was scanned 32 times at a resolution of 2 cm^{-1}.

3. Results and discussion

3.1. Solid state characterization

The “as received” samples of mannitol and CPA were characterized using XRD and DSC. XRD pattern of commercial mannitol (Fig. S1 in supporting information) revealed characteristic peaks of β-mannitol at 2θ values of 10.5°, 14.6°, 16.8° and 23.4°. DSC heating curve (Fig. S2 in supporting information) also supported this information and showed a melting endotherm with onset temperature of 166.5 °C (melting point of β-mannitol) (Burger et al., 2000).

XRD pattern of CPA (Fig. S3 in supporting information) showed characteristic peaks of CPA monohydrate at 2θ values of 7.0°, 10.9°, 13.9°, 15.1°, 23.6°, 25.3° and 28.1° (Ketolainen et al., 1995; Kovalcik and Guillory, 1988). DSC heating curve (Fig. S4 in supporting information)