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Research paper

Impact of freezing procedure and annealing on the physico-chemical properties and the formation of mannitol hydrate in mannitol–sucrose–NaCl formulations

Andrea Hawe *, Wolfgang Frieß

Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-University Munich, Munich, Germany

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Abstract

The goal was to investigate the impact of NaCl on the physico-chemical properties of mannitol–sucrose formulations during freezing and drying, with special focus on mannitol hydrate formation. Differential scanning calorimetry (DSC) and low-temperature X-ray powder diffraction (LTXRD) were used to study the frozen solutions. After lyophilization the products were analyzed with DSC, temperature-modulated DSC (TMDSC), X-ray powder diffraction (XRD) and Karl-Fischer titration. DSC showed an inhibition of mannitol crystallization by sucrose and NaCl during freezing. The glass transitions of the maximally freeze-concentrated solutions (Tg') were lowered by both mannitol and NaCl. By the application of an annealing step during lyophilization mannitol crystallinity could be increased. However, lyophilization with an annealing step promoted the formation of mannitol hydrate, which is known to undergo conversion into the anhydrous polymorphs of mannitol upon storage. LTXRD revealed that mannitol hydrate was formed at temperatures below −30 °C, but not at −27 °C. The tendency that mannitol hydrate is predominantly formed at lower temperature was confirmed by XRD of lyophilized products, produced at different annealing temperatures. For the development of lyophilization cycles the lowered Tg', as well as the tendency to mannitol hydrate formation predominantly at lower temperature needs to be considered.

Keywords: Freezing; Lyophilization; Annealing; NaCl; Mannitol hydrate

1. Introduction

Lyophilization is frequently used for the stabilization of biopharmaceuticals. To achieve stable products it is essential to add excipients that protect the active ingredient, e.g., a protein against degradation and damage during freezing and drying. One common way to achieve elegant lyophilized products is combining a crystalline bulking agent, e.g., mannitol or glycine with a second excipient that remains amorphous, e.g., sucrose, trehalose, human serum albumin [1–3]. Combinations of glycine with sucrose, respectively, mannitol with sucrose are often employed. Johnson et al., as well as Passot et al. used a combination of 4.0% mannitol and 1.0% sucrose to successfully stabilize different proteins [4,5]. Liao et al. have studied the impact of an incorporated protein on the physical state of mannitol in formulations with mannitol and sucrose [6]. During lyophilization mannitol can crystallize in the α-, β- or δ-modification or as mannitol hydrate depending on the applied freezing protocol, a potential annealing step and the process conditions during primary and secondary drying [7–9]. The presence of other excipients like buffer components, lyoprotectants or proteins can both inhibit and promote mannitol crystallization [10–12]. Especially salts, that are added as buffer components, isotonicity agents or stabilizers can have a major impact on the physico-chemical properties of mannitol and other excipients [13,14]. A slight increase in salt

* Corresponding author. Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-University Munich, Butenandtstr. 5-13, 81377 Munich, Germany. Tel.: +49 89 218077112.
E-mail address: Andrea.Hawe@cup.uni-muenchen.de (A. Hawe).

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concentration can lead to significant changes of the physico-chemical properties of the excipients during freezing and drying [15–18]. Besides the anhydrous modifications, mannitol can exist as a metastable crystalline hydrate after lyophilization [19]. The presence of mannitol hydrate can lead to stability problems during storage due to the release of hydrate water upon its conversion into the anhydrous crystal forms. Therefore, it is important to develop lyophilization cycles that result in products free of mannitol hydrate. Johnson et al. showed that mannitol hydrate content can be reduced by performing the secondary drying at temperatures above 40 °C [4]. Little is known about how an annealing procedure influences the formation of mannitol hydrate during lyophilization. Annealing is often applied for formulations with mannitol as bulking agent to maximize mannitol crystallization during the freezing step. On the other hand, several approaches are described to produce amorphous mannitol as lyoprotector, e.g., by adding NaCl, boric acid or sodium tetraborate [13,20,21]. Only in the amorphous state mannitol is able to adequately stabilize the active protein via molecular interactions [22,23]. However, amorphous mannitol tends to crystallize upon storage and thereby looses its ability to stabilize the protein [2]. Therefore, the employment of mannitol as crystalline bulking agent in combination with an amorphous lyoprotector is the more promising approach. Thereby, it is essential to ensure mannitol crystallization during lyophilization. Crystallization of excipients upon storage can reduce storage stability. This was described for *Humicola lanuginosa* Lipase formulated with sucrose by Kreilgaard et al., who attributed the reduced stability after crystallization to an increase in moisture content and a reduced glass transition (T_g) value of the remaining amorphous phase [24]. Different combinations of mannitol, sucrose and NaCl were used as model formulations for the studies. The impact of NaCl on the physico-chemical properties of the formulation during freezing, annealing and drying was studied. For the frozen state the glass transition of the maximally freeze-concentrated solution (Tg') of the formulations and the crystallization of mannitol were monitored. The lyophilized products were analyzed regarding mannitol modifications, as well as T_g and residual moisture. Special focus was set on the presence of mannitol hydrate in relation to the applied annealing steps.

2. Materials and methods

2.1. Materials

Mannitol was obtained from Caelo (Hilden, Germany), sucrose from Suedzucker (Mannheim, Germany) and NaCl from Sigma (Steinheim, Germany).

2.2. Low-temperature X-ray powder diffraction (LTXRD)

Crystallization was studied with LTXRD using Cu-Kα1 radiation ($\lambda = 154.06$ pm) on the powder diffractometer Stadi P from STOE (Darmstadt, Germany) with parafocussed transmission geometry. Germanium was used as primary monochromator and the scattered X-rays were detected with a linear PSD area detector. The sterile filtrated solutions were frozen in the rotating capillary (diameter 0.5 mm) in the cooling stage (Oxford Cryosystem) of the X-ray diffractometer. For the LTXRD experiment a temperature profile similar to the conditions during lyophilization was chosen. The samples were frozen to −50 °C with a cooling rate of 0.5 °C/min. At −50 °C the first measurement under isothermal conditions was performed. The temperature was subsequently increased to the different annealing temperatures with 1 °C/min and several measurements were performed at designated time intervals. The diffraction patterns were analyzed with the program WinXPOW from STOE (Darmstadt, Germany). Table 1 shows the XRD diffraction peaks used for the assignment of the different mannitol modifications.

Table 1
Assignment of X-ray diffraction peaks to the different mannitol modifications

Modification	Main peaks [° 2-Θ]	Intensity (%)	Peaks used for identification [° 2-Θ]	References
α-Mannitol	9.4	10	13.6	JCPDS-database
	13.6	20	17.2	
	17.2	45		
	18.7	100		
β-Mannitol	10.5	18	14.6	JCPDS-database
	14.6	65	16.8	
	16.8	85	23.4	
	18.8	100		
	23.4	90		
δ-Mannitol	9.7°	100	9.7	JCPDS-database
	20.4	50	No peak at 17.9	
Mannitol hydrate	9.6	80	9.6	[19]
	17.9	100	17.9	[19]