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Communication

Effect of ethanol on spray-dried mannitol polymorphism as a function of particle size

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Abstract:

The aim of this study was to investigate the effect of ethanol on the polymorphic variation of spray dried mannitol as a function of particle size. A spray dried system with a mixed mannitol polymorphs was produced by using 10% ethanol solution. The obtained dry powders were then dispensed into different size fractions using a Next Generation Pharmaceutical Impactor (NGI). The morphology and polymorphic form of mannitol were analyzed using SEM and XRPD and Raman microscopy, respectively. Multivariate data analysis was applied to interpret the Raman spectra. X-ray powder diffraction (XRD) studies indicated that α- and β-mannitol were present in the 10% ethanol spray dried system. Further investigations with Raman studies revealed that more α-mannitol was present in smaller particles, suggesting that the presence of ethanol may affect the drying kinectics of particles inside the system, and by this means crystal arrangement of mannitol. Image analysis from the SEM showed a variation of size distribution between NGI stages. In conclusion, the polymorphic forms of spray-dried mannitol could change as a function of particle size. This finding provides guidance to improve dry powder formulations, especially for inhalation purposes, as produced by spray drying, since particle size is a critical component for therapeutic delivery.

Keywords: Spray-drying; Polymorphic form; Mannitol; X-ray powder diffraction; Raman microscopy; Chemometrics.

1. Introduction

Solid compounds with the same chemical composition can often exist in different solid state polymorphic forms. These differences may affect the quality, long term stability and performance of the drug products e.g. bioavailability.

Spray-drying is a commonly used manufacturing method in obtaining solid state. By adjusting spray dry conditions (i.e. temperature and humidity), particles with varying solid state composition can be produced. The difference in the drying rate of droplets with various sizes may contribute to the formation of polymorph mixtures (1). To date, research has been focused on the characterization of solid state in bulk spray dried powder. It is still unclear whether the polymorphic composition changes homogenously throughout the entire size range or preferentially occur at one particle size range. Understanding alteration in polymorphism as a function of particle size from spray-drying processes is highly desirable in order to improve the current knowledge in solid state processing. In this study mannitol was used as a model compound since it is known to have three polymorphic forms designated asα-, β- and δ-form and the recent observed hemi-hydrate form in conditions relevant for lyophilisation (2). The effect of ethanol on spray-dried mannitol polymorphism as a function of particle sizes was investigated.

2. Materials and methods

2.1 Materials

D-Mannitol (β and δ polymorph) and ethanol (≥ 99.9%, analytical grade) were obtained from VWR International Ltd, Poole, England. Mannitol powders were stored in airtight, light resistant containers at room temperature until use.

2.2 Preparation of α-mannitol

α-Mannitol was prepared by dissolving 50g of D-mannitol in 450g of 70% ethanol until a clear solution was obtained (3). The solution was slowly cooled down to room temperature, and further cooled to 4^0C in the fridge for approximate 2 hours. Needle-like crystals precipitating out were filtered and dried under a fume hood overnight.

2.3 Preparation of spray-dried particles

Mannitol powders were dissolved in purified water and 10% ethanol mixtures to prepare feed solutions for spray drying using a Büchi B-290 mini spray-dryer (Falwil, Switzerland) under operating conditions as listed in Table 1. The spray-dried powders were collected into glass scintillation vials, sealed with Parafilm and kept in a glass desiccators containing silica gel as drying agent at room temperature until analysis.

Table 1 Composition of feed solutions and spray-drying conditions.

Feed Solution	Feed Conc. (g/ml)	Feed rate (%)	Atomisation (L/min)	Aspirator (%)	Inlet (°C)	Outlet (°C)
Mannitol in purified water	0.16	2	7.3	100	180	160
Mannitol in 10% ethanol solution	0.114	2	7.3	100	180	100

2.4 Particle Size Separation

The spray-dried mannitol powders in 10% ethanol solution were dispensed into different particle size fractions (Stage 1 – Stage 8) using a Next Generation Pharmaceutical Impactor (NGI) fitted with a United State Pharmacopoeia (USP) throat. Particles with different sizes are separated with the large particles captured at higher stage (i.e. Stage 1) and small particles were captured at the lower stage (i.e. Stage 8). Approximate 60-80mg of powder was filled into a Size 3 gelatin capsule, loaded into a Turbospin® device and pierced. The device was then connected to the USP throat via a mouthpiece adapter, and dispersion was performed at air flow rate of 60L/min for 20 seconds. Prior to dispersion, the air pump was first calibrated with a mass flow meter (Model 2063; TSI Incorporated, MN, USA). Dispersion runs were carried out several times until sufficient powders could be collected at each NGI stage. Powders were then transferred into small scintillation vials, sealed with Parafilm and stored in desiccators until analysis.

2.5 Particle Size and Morphology

The morphology and particle sizes of spray-dried particles from Stage 1, 4 and 8 of the 10% ethanol system was visually examined using a JSM-5200 Scanning Electron Microscopy (SEM; JEOL Ltd, Tokyo, Japan). As a preparation for SEM, samples were transferred onto carbon sticky tape and mounted in metal stubs, followed by sputter coating with a thin layer of gold-palladium for 120 seconds with a E5200 Auto Sputter Coater (BIO-RAD, Polaron Equipment Ltd, Watford, England) under Argon gas purge (Air Liquide, Taastrup, Denmark). The specimens were then imaged at accelerating voltage of 10kV energy at different magnifications ranging from 3500X to 10000X. Particle size and distribution from Stage 1, 4 and 8 were measured using the conventional image analysis method. Martin's diameter of 100 randomly selected particles was determined in a fixed direction (zero degree, from left to right) from the field of view of the SEM images.

2.6 Thermogravimetric Analysis (TGA)

The bulk samples were analysed using a thermogravimetric analyzer (Model 2050; TA Instruments, DE, USA) under a nitrogen purge of 90cm^3/min. Samples (approx. 10-15mg) were loaded onto an open platinum pan and heated up from 20°C to 120°C at a scan rate of 10°C/min.

2.7 Identification of solid state composition

2.7.1 X-Ray Diffraction

All samples were analysed using PANalytical X'Pert PRO MPD system (PW3040/60, Philips, The Netherlands) using Cu Kα radiation with λ = 1.542Å (45kV and 40mA) and automatic divergence slit. Samples preparation was carried out on flat a aluminium sample holders and scanned from 5° to 45° 2θ with a step size of 0.02626° and scanning speed of 0.0673° per second. The mannitol reference codes are obtained from Cambridge Crystallographic Data Centre (CCDC) ConQuest v1.12. The data from the reference codes DMANTL 08, DMANTL 09 and DMANTL10 (4) were used to calculate the theoretical diffraction patterns of α-, β- and δ-mannitol, respectively, by Mercury CSD v2.3. The experimental XRD patterns were compared to the reference patterns for analysis of polymorphism.

2.7.2 Raman Spectroscopy

Raman Microprobe (Model RXNI-785; Kaiser Optical System INC, Ann Arbor, MI) equipped with MKII Holographic Filtered Probe attached to a NRXN Spectrograph engine was used. Small amounts of sample were placed under the 10X Microscope (Leica DM LP Microscope) and the focus was adjusted to focus the laser beam directly onto the samples. The sample was excited from a 785nm Invictus^{TM} NIR diode laser with a standard resolution of 5cm^{-1} and ~100mW laser power. All sample measurements are performed in 4 replicates with 5 seconds exposure time with 4 accumulations.

2.8 Spectral pre-treatment and Multivariate data analysis

Partial least square discriminant analysis (PLS-DA) is originally a classification method, and can be considered as a variant of PLS. Unlike ordinary PLS where the response matrix Y consists of continuous values, the response matrix in PLS-DA consists of discrete logical values, 0 and 1s, to indicate the different classes. In the current study, the calibration matrix X consists of four spectra of α- and β-mannitol each, making a 8 x 2 response matrix. The PLS-DA model is validated using random subset internal cross validation, performed by randomly splitting the calibration set into a calibration part consisting of 5 spectra, and a validation part consisting of 3 spectra. The classification error is estimated from the PLS-DA submodel, and this process is repeated 8 times. The final misclassification error reported is the average of the 8 estimations. Other parameters useful to describe the PLS-DA model are model specificity and sensitivity:

$$\text{specificity} = \frac{TN}{FP + TN}$$

$$\text{sensitivity} = \frac{TP}{TP + FN}$$

Where TN, TP, FN, FP are numbers of predicted true negative, true positive, false negative and false positive respectively. In the current study PLS-DA model is built using the PLS-Toolbox v.5.8 (Eigenvector, US). Data preprocessing are performed using in-house routine programmed in Matlab v.7.10 (Mathworks, US).

Raman spectra were preprocessed using Standard Normal Variate (SNV) scaling (5) on the spectral part from 300-1500 cm^{-1}. Variable selection is based on optimized misclassification rate from the reverse interval-PLS algorithm with window size 60 (6, 7). The final variables selected are 300-587 cm^{-1}, 607-1087 cm^{-1}, 1126-1356 cm^{-1} and 1375-1500 cm^{-1}.

3. Results and Discussion

As shown in the SEM images (Fig. 1), the smallest particle size fraction was recovered from the lower stage (i.e. Stage 8) as compared to the larger particles on the higher stage (i.e. Stage 1). A statistical analysis using one-way ANOVA confirmed the significant difference ($P<0.05$) of the mean size between stages. A residual moisture analysis of the spray dried powders revealed no detectable weight loss was found in the TGA.

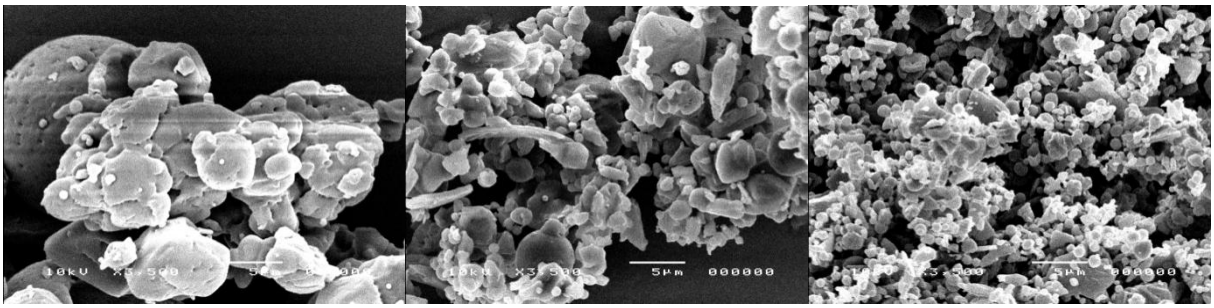

Fig. 1 SEM photographs of spray-dried mannitol powder from 10% ethanol solution on NGI Stage 1, 4 and 8 (from left to right). The bar in all images equals 5 micron.

Table 2 Particle size of the spray-dried powder.

Spray Dry System	Particle Size (S.D) (μm)		
	Stage 1	Stage 4	Stage 8
Mannitol in 10% ethanol solution	10.9 (3.4)	2.7 (1.7)	1.0 (0.4)

The XRD results in Fig. 2a indicate that a mixture of mannitol polymorphs (α- and β-forms) was produced from the present spray drying system, while spray-dried mannitol alone from a water solution

resulted in the most thermodynamically stable β form (Data not shown). Such observations are most likely due to the fact that ethanol increases the drying rate of the system, resulting in the formation of the less stable form (i.e. α-mannitol), according to Ostwald rule of stages (8).

Further examination of XRD patterns in Fig. 2a showed that the ratio of α-form to β-form of spray-dried mannitol on different NGI Stage varied with a certain trend. As shown in Fig. 2b, α-mannitol has characteristic peaks at 2θ position of 9.6, 13.8 and 17.3°, while an intensive peak at 14.7° and peaks at 10.6 and 16.8° can be attributed to β-mannitol. Comparing the intensity difference between peaks at position 13.8 (α) and 14.7° (β) in Fig. 2a, it shows the peak intensity at 13.8° (α) increased while the peak at 14.7° (β) decreased, from NGI Stage 1 to 8. Similar trends can be observed by comparing the differences at peak position 17.3 (α) and 16.8° (β). This indicated that as particles become smaller, the proportion of α-mannitol increased. This finding is supported by multivariate data analysis on the Raman spectra. As shown in Fig. 3, the content of β-mannitol decreased from NGI Stage 1 to 8. It suggests that the polymorphism of spray-dried mannitol from the 10% ethanol solution varies as a function of particle size.

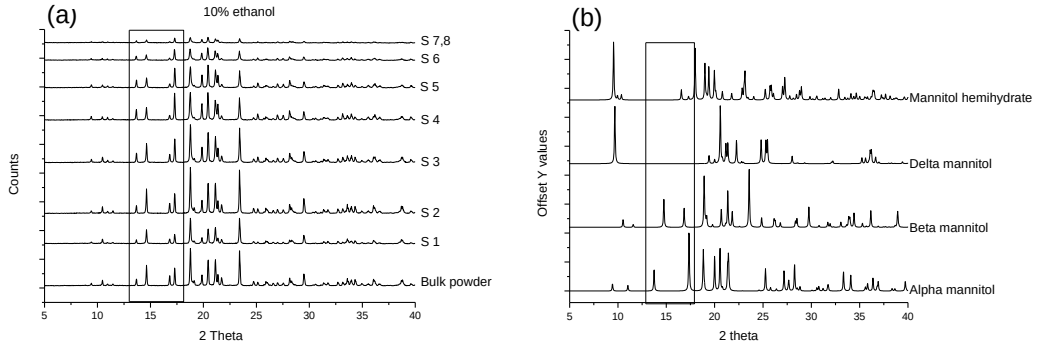

Fig. 2 XRPD patterns of spray-dried mannitol powder from 10% ethanol solution (a) and a calculated XRPD patterns of mannitol polymorphs (b).

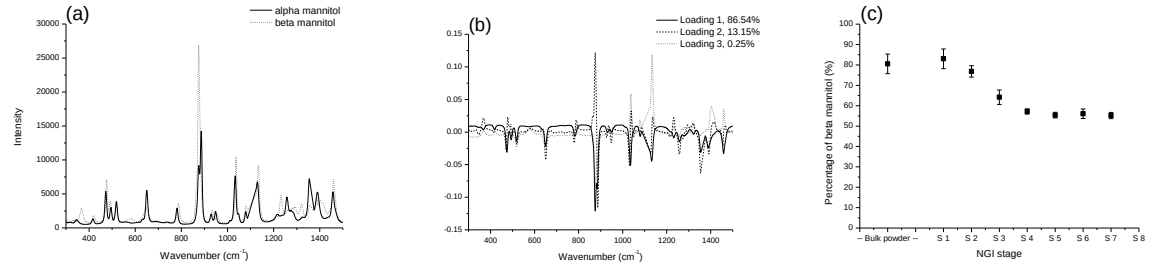

Fig. 3 Raw spectra of α and β-mannitol obtained using Raman spectroscopy (a). Loading plots from PLS-DA models built on Raman spectra (b). Percentage of β-mannitol content at various stages predicted from Raman spectroscopy (c).

The change in spray-dried mannitol polymorphism as a function of particle size can be explained by the fact that the droplets with various sizes have different drying rates. When heat energy was transferred to the droplet surface for solvent evaporation, the smaller droplets are likely to dry faster and will thus, experience a more rapid loss of solvent than the larger droplets. This faster drying leads to formation of meta-stable α-mannitol in the smaller particles, according to Ostwald rules of stages (8). On the other hand, the larger droplets will be able to retain more residue solvent at the time most of the residue solvent had been evaporated from the smaller droplets. This residual solvent in the larger droplets can act as a 'lubricant' to facilitate a stable crystal arrangement, which will explain the observed trend in the 10% ethanol system with more stable β-mannitol in the larger particles and vice versa.

4. Conclusions

Particle drying kinetics can determine the formation of particular polymorphic forms of mannitol in spray-drying process. Mannitol may not have sufficient time for crystal arrangements under fast drying associated with small droplets, thus producing a less stable polymorph. Big droplets dry slower than small droplets, which can retain more residue solvent, thus facilitating the crystallization to the stable polymorph. As scale-up production with commercial spray-dryers often produce dry powders with a broad particle size distribution, this finding can provide guidance to further improve the dry powder formulations, especially for inhalation, where particle size difference can affect the therapeutic efficacy.

Acknowledgments

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