Characterization of Physical State of Mannitol after Freeze-Drying: Effect of Acetylsalicylic Acid as a Second Crystalline Cosolute

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Freeze-drying of mixed solutes is a preparative technique widely used in the pharmaceutical industry. The presence of an amorphous form or changes in the crystalline form can affect solid state stability. In this work, acetylsalicylic acid (AAS) was chosen as a model drug, and was mixed with mannitol, a commonly used bulking agent in formulation of tablets. Variations in the final freeze-dried crystalline forms were found after changing the ratios of the two co-solutes. Samples were analysed by powder X-ray diffractometry and differential scanning calorimetry. A major amorphous form and a minor crystalline δ-mannitol form were produced during the mannitol freeze-drying process. The crystal form of mannitol in the two-component system depended on the AAS : mannitol ratio. The AAS was mostly crystalline, regardless of the amount of mannitol present. A major δ-mannitol and a minor amorphous form were obtained when AAS was present in a high percentage (75% w/w). When AAS percentages of 50 and 25% (w/w) were present during the drying process, the mannitol was found in a highly crystalline form.

Key words acetylsalicylic acid; mannitol; frozen solution; crystallization

The physical characteristics of frozen solutions have been studied mainly with aqueous single solutes.[1] The complexities of solutions used for pharmaceutical formulations require information on the physical characteristics of multiple component solutions.[2]

When two different substances are mixed in a solution and then freeze-dried, some physical changes can affect the crystallographic characteristics of the final product.[2,3] Pikal *et al.* have reported the mutual inhibition of crystallization of mannitol and glycine during lyophilization.[3] However, there are not many articles which study the effect of a crystalline active substance on the physical state of mannitol forms.

Mannitol is an excipient broadly used in the freeze-drying process,[4—8] and its crystalline and amorphous forms have been extensively studied.[4,6—8] The freeze-drying process can produce a partially amorphous and partially crystalline material[6]; the crystallization of mannitol during this process can lead to different anhydrous polymorphs (α, β, and δ). The existence of crystalline mannitol hydrate obtained during the freeze-drying process has been confirmed by Yu *et al.*[7]

As a result of these changes to the crystal form of mannitol during freeze-drying there is a difference in the physical and chemical stability of the freeze-dried solid, which can induce adverse effects. Therefore, by studying the physical chemistry of freeze-dried mannitol containing formulations one can anticipate these changes and prevent adverse effects.

During the freeze-drying process, samples are subject to different cycles of freeze and vacuum drying. These processes can lead to modifications of the initial crystalline form of the drug.[4,5,8,9] The effects of freezing rate, temperature and mannitol concentration may also induce crystallographic changes producing a previously known mannitol form.[6,7] Yu *et al.* have shown that mixtures of δ (major) and β (minor) polymorphs were produced by freeze-drying a 4% w/v pure mannitol solution.[7] Nevertheless, other authors when employing a fast freezing process favored the formation of β form when 5% (w/v) mannitol solution was freeze-dried.[6] The difficulties which arise when attending to control these variables (as temperature and cooling rate) during the freeze-drying process are shown by Yu *et al.*,[7] who identify several factors that can contribute to the vial-to-vial variation in the amount and stability of the mannitol hydrate.

In the current study, acetylsalicylic acid (AAS) is used as model crystalline drug. Possible changes in crystallinity of AAS can be easily detected because its crystallographic characteristics are well known.[10,11]

Therefore, the objectives of this study are to (1) Identify and measure the different crystalline mannitol forms obtained when a second crystallizing solute is present. (2) Determine the effect of a similar to industrial scale freeze-drying process conditions on the physical state of freeze-dried mannitol present as a single component.

Experimental

Materials AAS USP 23 (Merck, Darmstadt, Germany). β Mannitol NF (ICI Americas Inc., U.S.A.). δ-mannitol was obtained using a procedure described by Kim *et al.*[6] All other ingredients were of reagent grade or better (Merck).

Methods. Powder Samples Physical Mixtures: Samples of AAS and β-mannitol (weight ratios 100 : 0, 75 : 25, 50 : 50, 25 : 75 and 0 : 100) were prepared and used as references for the different analytical methods.

Recrystallized Formulations: AAS and mannitol powder mixtures were prepared at various weight ratios (100 : 0, 75 : 25, 50 : 50, 25 : 75 and 0 : 100). All these formulations were dissolved in water as the solvent. The freeze-drying process was carried out using a Liolabor 7 (Telstat, Inc., Madrid, Spain). The freeze-drying vials were of 20 ml capacity with 24.7 mm i.d., different samples were prepared from a 5% w/v. The fill volume was 2.0 ml/vial. A fast freezing step at −40 °C was employed for the different samples and approximately 2 h were required to reach −40 °C. An extended primary drying was carried out at a shelf temperature of less than −20 °C for 40 h, followed by secondary drying at a shelf temperature of 25 °C for 6 h. The chamber pressure was set to 100 μm of Hg throughout the drying process.

Key Formulations Physical Mixtures: PM-100 : 0, PM-75 : 25, PM-50 : 50, PM-25 : 75 and PM-0 : 100.

Recrystallized Formulations: R-100 : 0, R-75 : 25, R-50 : 50, R-25 : 75 and R-0 : 100.

Differential Scanning Calorimetry Samples of 2—6 mg in covered aluminum pans were heated from −80 to 300 °C at the rate of 10 °C/min under nitrogen purge, with an empty, covered aluminum pan as the reference in a DSC Mettler TA 4000. Temperature was calibrated using indium as standard.

The differential scanning calorimetry (DSC) technique may be used to

confirm the existence of different crystalline forms of mannitol or to study the possible presence of amorphous phases of both substances.[2,12,13]

The crystallinities of AAS and mannitol in each freeze-dried formulation were obtained from the heat absorption at the melting temperatures and related to the corresponding fully crystalline raw materials. A mathematical program (PeakFit®) based on deconvolution curves was used to calculate the enthalpy of fusion.

Powder X-Ray Diffraction The structural characterization included conventional θ–2θ powder X-ray diffraction (Philips X'Pert-MPD) (CAI X-ray diffraction, School of Pharmacy, UCM) of all samples under study. Measurements were carried out with 2θ 5—40°, using a step size of 0.04° (2θ) and 1 s/step.

Two peaks at 7.86° and 15.87° for 2θ, corresponding to reflections 100 and 002, respectively, were used to detect changes in the AAS crystallinity after the freeze-drying process.

Several peaks were used to detect the crystalline forms of D-mannitol.

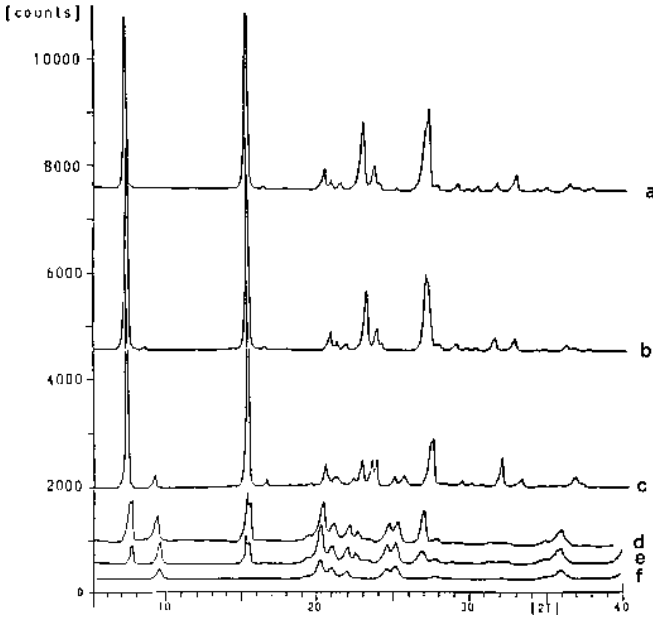

Fig. 1. Powder X-Ray Diffraction Patterns

a) $AAS_{Standard}$, b) R-100 : 0, c) R-75 : 25, d) R-50 : 50, e) R-25 : 75 and f) R-0 : 100.

Results and Discussion

Evaluation of the Crystalline Characteristics of Monocomponent Formulations (AAS or Mannitol) after Freeze Drying Samples of formulations containing pure AAS obtained by freeze-drying were evaluated and compared to a reference AAS by powder X-ray diffractometry (Fig. 1). The relative intensity values did not exhibit any change when compared with the diffraction patterns of the AAS crystalline forms according to the ASCIT tables (monoclinic crystal, PDF number 12-850).

Figure 2 shows the powder X-ray pattern of a standard β-mannitol and a freeze-dried mannitol sample as a single component (R-0 : 100) (α, β, and δ polymorphs patterns are shown for comparison). According to this figure the standard mannitol corresponds to the β form while the freeze-dried mannitol corresponds to the δ form. Variations in the intensity values can be attributed to the presence of a major amorphous form of mannitol in the freeze-dried sample. The absence of mannitol hydrate form was confirmed because its characteristic peaks at 16.5, 17.9, 25.7 and 27.0° (2θ) were not present in the assayed samples.[7] Most likely, this δ mannitol form is obtained when low concentrations of mannitol in solution (5% w/v) are employed. A similar result was obtained by Haikala *et al.*, when a 5% mannitol solution was freeze-dried.[4]

The DSC results of β and δ mannitol standard forms and a freeze-dried mannitol sample (formulation R-0 : 100) were compared, and the melting points were similar for all three samples (see Table 1). The enthalpy values of both mannitol forms were similar (ΔH–δ mannitol=270.6; ΔH–β mannitol=273.40 (J/g), therefore only one of these forms (β-mannitol) was added to the physical mixtures assayed through the deconvolution technique. However, there were great differences between the enthalpy values 25.70 J/g for mannitol freeze-dried formulation (R-0 : 100) and the two mannitol reference forms (ΔH=273.40, 270.60 J/g, see Table 1). These results confirmed that a major amorphous form and a minor δ form were obtained under our freeze-dried conditions. Similar mixtures of crystalline and amorphous manni-

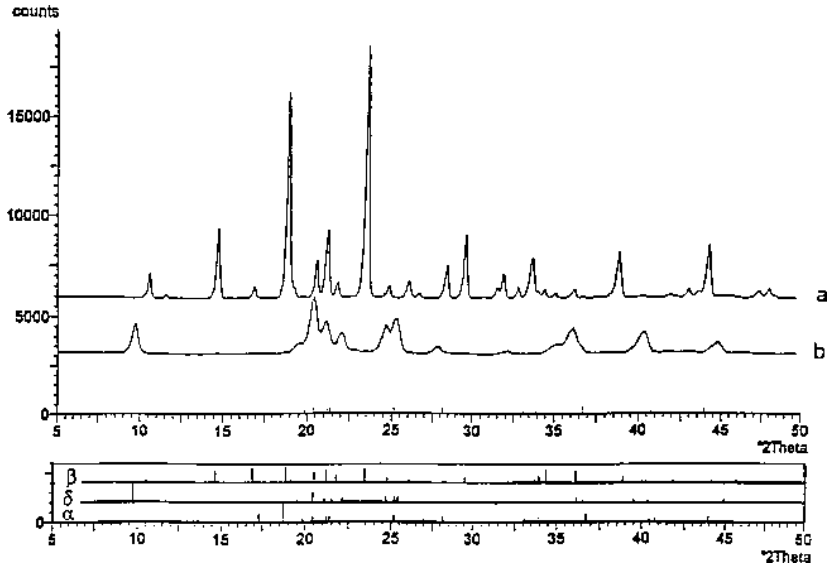

Table 1. Melting Points and Enthalpy of Fusion for AAS and Mannitol in Different Formulations

Product	Melting point (°C)		ΔH_{Total} (J/g)	ΔH_{AAS} (J/g)	$\Delta H_{Mannitol}$ (J/g)
	AAS	Mannitol			
$AAS_{Standard}$	142.1	—	198.00	198.00	—
R-100 : 0	139.3	—	167.50	167.50	—
R-75 : 25	139.4	142.6	203.03	193.93	230.32
R-50 : 50	137.8	150.9	221.61	179.52	263.70
R-25 : 75	135.2	154.4	235.49	168.18	257.94
β-$Mannitol_{Standard}$	—	167.4	273.4	—	273.4
δ-$Mannitol_{Standard}$	—	166.1	270.6	—	270.6
R-0 : 100	—	166.0	25.70	—	25.7

tol were obtained with different freeze-dried processes.[14] Even though the shelf temperature was controlled at −40 °C, positive control of the sample temperature is uncertain due to lateral heat transfer from the chamber walls.[7]

Evaluation of the Crystalline Characteristics When Mixtures of AAS and Mannitol Were Used in the Same Formulations The effect of AAS on the crystallinity of freeze-dried mannitol was studied by powder X-ray diffractometry and DSC.

The powder X-ray diffraction of formulations $AAS_{standard}$, R-100 : 0, R-75 : 25, R-50 : 50, R-25 : 75 and R-0 : 100 are shown in Fig. 1. The peak at 9.6 (2θ) suggests the presence of a δ-mannitol form in all the freeze-dried samples. Figure 3 shows the DSC scans: (a) shows the melting points of the standard mannitol and freeze-dried mannitol, and (b) shows that the melting points of AAS and mannitol were significantly close in freeze-dried samples. A deconvolution program was used to evaluate the DSC results in the recrystallized mixtures of AAS and mannitol. Similar techniques have been used previously by Lee *et al.*[15] and Lier *et al.*[16] to study mixtures of binary crystalline polymers and determine the DSC melt endotherm of individual crystalline substances. The measured endotherms were related to the percentage crystallinity of each component.

The enthalpy values of AAS and δ-mannitol (R-0 : 100) were evaluated by the physical mixtures PM-100 : 0, PM-75 : 25, PM-50 : 50, PM-25 : 75 and PM-0 : 100. From a statistical point of view, the best fit was obtained when the experimental data were fitted to a linear model: measured endotherm = a + b × crystallinity percent. AAS presented a good determination coefficient (r^2 = 0.9995) for values between 0—5 mg of AAS. R^2 statistics indicates that model as fitted explains 99.95% of the variability in measured endotherm. The intercept and slope values were 3.41 mJ and 191.0 J/g respectively. The slope value showed an AAS melt enthalpy was similar to that of the raw sample (ΔH = 198.00 J/g).

The mannitol determination coefficient was r^2 = 0.9988. This relationship confirmed the utility of the deconvolution technique in the determination of crystallinity percentage for a mannitol range of 1—5 mg. The intercept and slope values were −273.10 mJ and 284.71 J/g, respectively. The intercept (−273.1 mJ), confirmed the presence of an amount less than 1 mg. The slope (284.73 J/g) was close to the raw material melt enthalpy (ΔH = 273.40 J/g). These results are similar to those obtained by Haikala *et al.*[4]

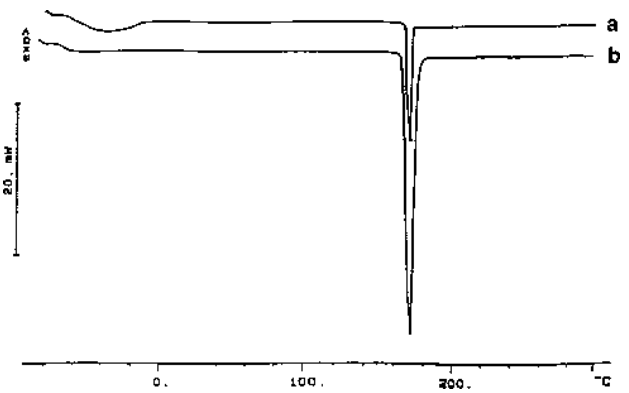

Fig. 3A. DSC Scans of a) Standard Mannitol and b) Freeze-Dried Mannitol (R-0 : 100)

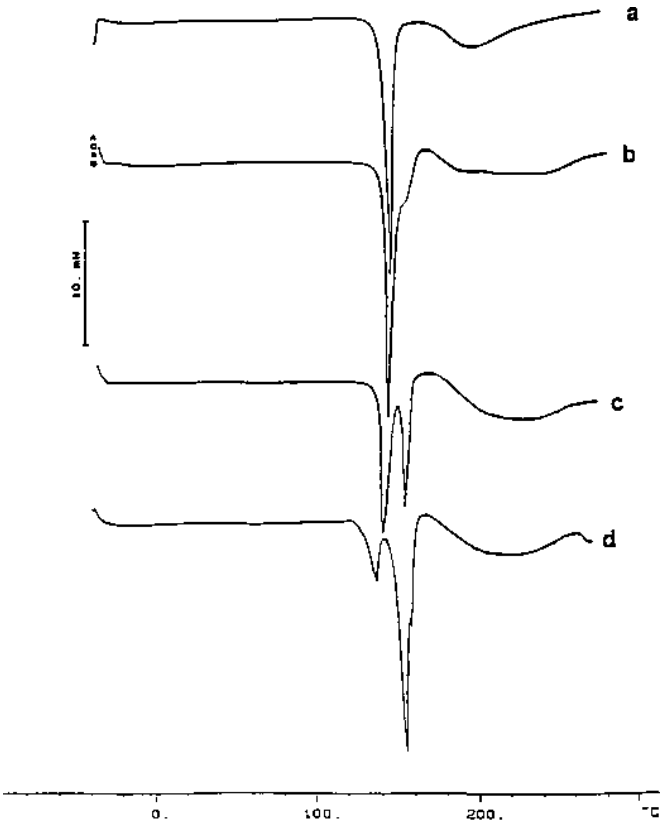

Fig. 3B. DSC Scans of a) Freeze-Dried AAS (R-100 : 0), b) R-75 : 25, c)

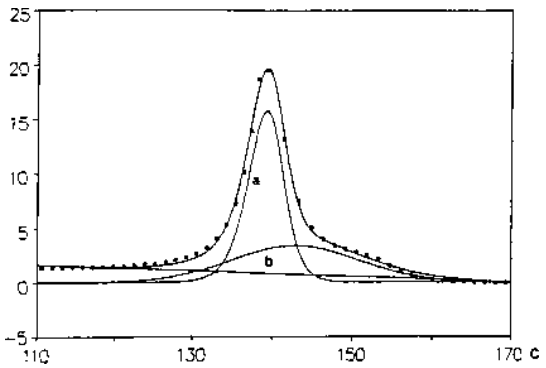

Fig. 4. DSC Scan and Deconvoluted Graphics of AAS : Mannitol 75 : 25 w/w Obtained by Freeze-Drying Process (R-75 : 25)

Curve (a) AAS and (b) mannitol.

143 °C) was due to the melting point and the second endotherm (181—183 °C) to the decomposition of this substance.

Figure 4 shows how this program resolves the DSC scan of formulation R-75 : 25. Both curves show the best fit (r^2=0.995) for two different mathematical models. The first curve (a) is sigmoidal and the melting point is 139.4 °C, close to that observed (139.3 °C) for R-100 : 0. Curve (b) corresponds to deconvoluted mannitol and shows a Gaussian distribution. The fit presents a lower δ-mannitol melting point (142.6 °C). This may be explained by a plasticizing effect of the amorphous mannitol form present in the cake. Herman *et al.*[17] considered that these changes can have an effect on distribution of water within the freeze-dried matrix. However, further work is needed in order to have a better understanding of the pharmaceutical significance of these changes within freeze-dried solids.

The AAS and mannitol enthalpies of the different formulations after the deconvolution process are shown in Table 1. There is an increase in the mannitol enthalpy of R-75 : 25 (230.32 J/g) when compared to R-0 : 100 (25.70 J/g). In sample (R-75 : 25), a major crystalline mannitol form and a minor amorphous mannitol form were shown to coexist. The presence of one phase in another can act as a focal point for spontaneous phase transitions such as crystallization.[18] Kim *et al.*[6] have described how a threshold concentration of 30% (w/w) of mannitol is required in mixtures with different non-crystallizing cosolutes to detect the crystalline phase. Under our experimental conditions, the presence of a second crystalline substance provides a heterogeneous nucleation site for the crystallization of δ-mannitol. The presence of AAS in ratios of 50 : 50 or 25 : 75 (AAS : mannitol) induce mannitol enthalpy values which are similar to the δ-mannitol reference form. Mannitol was found in a high crystalline form when 50 or 25% (w/w) of a second, crystallizing solute such as AAS was present in the cake.

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