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Crystallisation of amorphous mannitol is retarded using boric acid

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Abstract

An approach to inhibit the crystallisation of amorphous mannitol was investigated. Boric acid was selected as a model additive for a fundamental study of its ability to retard crystallisation and to facilitate characterisation of the properties of the amorphous solid. At concentrations above 5% (w/w) of boric acid, the DSC scans indicated that a totally amorphous solid could be prepared by cooling the melted pre-mixture under ambient conditions. An increase in the glass transition temperature (T_g) was observed with a corresponding increase in boric acid content, and their relationship was well fitted by the Gordon–Taylor equation. This result suggested that mannitol and boric acid mixed homogeneously.

The crystallisation profiles of the resultant amorphous compositions were best described by the Avrami–Eroféev equation ($n = 1/3$), which indicated that random nucleation and three-dimensional crystal growth was the best-fitting mechanism of this crystallisation. The activation energy of crystallisation decreased with increasing boric acid content, indicating that the temperature dependency for crystallisation decreased with increasing boric acid content. Furthermore, the rate of crystallisation at 30 °C for mannitol alone was 7000 times higher than that of mannitol containing 7.5% (w/w) of boric acid.

Keywords: Mannitol; Boric acid; Amorphous state; Crystallisation; Anti-nucleant; Crystallisation inhibitor

1. Introduction

Amorphous systems have a number of drug delivery applications. One example is in the formulation of therapeutic proteins. Proteins generally have stability problems, which are more complex than those encountered with small molecules because their higher order structure is important for efficacy. Conformation change and aggregation are common problems. Careful formulation of protein products is required to enable them to survive processing stresses and storage without loss of potency or increase in the level of denaturation. Freeze-drying or spray-drying processes are often required for an adequate shelf life of protein pharmaceuticals (Lai and Topp, 1999; Wang, 1999). Generally, the dried solid is more stable than the corresponding aqueous solution, however, some proteins are inactivated during the drying process due to changing hydrogen bond networks, which can be formulation dependent. In addition, degradation and loss of activity during storage are sensitive to both physical and chemical formulation change and, in particular, to the levels of residual water in the dried solid (Pikal et al., 1991; Costantino et al., 1998a; Naini et al., 1998).

Many sugars, polyols and amino acids are used as cryoprotectants to prevent inactivation during drying or storage. These excipients preferentially interact

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with the surface of proteins, and stabilise them thermodynamically against heat- and cold-denaturation. Molecular interactions, such as hydrogen bonding, between proteins and excipients are reported to be necessary for stabilisation (Arakawa and Timasheff, 1982; Arakawa et al., 1991; Carpenter and Crowe, 1988, 1989; Costantino et al., 1998b). Crystallisation of an excipient from the amorphous state during drying or storage is an important contributory factor towards the stability of protein pharmaceuticals. Crystallisation is believed to remove solutes from the protein phase and result in the loss of a molecular interaction with protein. Therefore, maintenance of the amorphous or partially amorphous state of cryoprotectants is important for protein stabilisation. Mannitol is commonly used as a bulking agent in freeze-dried formulations. However, it is also well known that mannitol has a strong tendency to crystallise. The vial breakage phenomenon is a striking illustration of this tendency. Izutsu et al. (1993, 1994) and Costantino et al. (1998c) reported that crystallisation has been observed in freeze-dried cakes and that the stabilising effect decreased with an increase in the crystallinity of mannitol.

Amorphous systems can effect particle functionality Kawashima et al. (1992) performed studies on powdered phospholipid nanospheres, which are spray-dried products of phospholipid aqueous dispersions with various sugars. They concluded that the ability to render the sugar phase amorphous was important to maintain the re-dispersibility and initial particle size of the dispersion.

Hot melt-cooled mannitol was employed by Kanig (1964) to improve mannitol compressibility and compactibility. This improvement was mainly attributed to a temporary reduction of crystallinity. However, these advantages gradually diminished with time due to an increase in the crystallinity of mannitol.

For adequate pharmaceutical performance of mannitol as a protective agent, it is important to maintain an amorphous or partially amorphous state. Solid dispersions of drugs in polymers have been widely used to obtain and maintain the amorphous state of materials. This approach relies partly upon the significant increase of the glass transition temperature (T_g) of the solids. However, since large amounts of polymer are required to increase the glass transition temperature significantly, the solid solution approach was not deemed suitable for our purpose.

It is well known that sorbitol is readily obtained as an amorphous phase during the spray-drying or freeze-drying processes (Kibbe, 2000). Sorbitol is an isomer of mannitol, which differs only in the planar orientation of hydroxyl group on the second carbon atom. It was suggested that the difference in the ease of forming an amorphous state between these isomers could be attributed to their different hydrogen bonds networks (Young and Jeffrey, 1971; Quinquenet et al., 1988). In this study, we test the hypothesis that it is possible to retard mannitol crystallisation from the amorphous state if the hydrogen bond network can be altered through the addition of a small amount of additive.

Capillary isotachophoresis with conductometric detection has been used for separating polyols such as mannitol, sorbitol, dulcitol and xlitol (Pospísilová et al., 1997). This separation method distinguished between the ability of the various polyols to form complexes with boric acid. The hydroxyl groups of the polyols react rapidly with borate to give an anionic complex in aqueous solution. Boric acid (Kim et al., 1968) or heteropolyanions (Trovão et al., 1998) such as $H_3PW_{12}O_{40}{\cdot}nH_2O$ and $H_4SiW_{12}O_{40}{\cdot}nH_2O$ have been used for the selective-precipitation of a meta-stable form of mannitol from solution. These reports suggested that such additives alter the hydrogen bond network. Based on these solution-based findings, our hypothesis was that the additives may function to inhibit crystallisation in the solid state. Boric acid was selected as a model oxo-acid. Whilst acknowledging that boric acid is not an excipient of choice for all routes of administration, it serves as a useful model of an inhibitor, and we now present a detailed and kinetic analysis of its ability to retard mannitol crystallisation in the solid state.

2. Materials and methods

2.1. Material

Mannitol and boric acid were obtained as the commercial product provided from Merck (Darmstadt, Germany) and Sigma (St Louis, MO), respectively, without any further purification.

2.2. Preparation of samples

Kim et al. (1998) reported that amorphous mannitol could not be obtained using the freeze-drying technique and that their only method to successfully obtain mannitol in an amorphous state is to use the quench-cool method. Therefore, this same method was selected to obtain mannitol in an amorphous state without resorting to the use of additives. For this purpose mannitol was heated up to 170 °C in a DSC oven and the melted mannitol sample was cooled rapidly using liquid nitrogen. The sample was then stored at −30 °C until required for analysis. To investigate the ability of boric acid to maintain mannitol in an amorphous state, physical mixtures of mannitol and boric acid were melted in an oil heater and then cooled to ambient conditions. During such processing it is likely that boric acid is subjected to an intramolecular dehydration at a temperature above 100 °C to produce metaboric acid. This dehydration of boric acid was observed during the preparation of the samples in our studies. All procedures were carried out under a dry nitrogen gas purge to avoid exposing the sample to moisture and oxygen.

2.3. Differential scanning calorimetry (DSC)

DSC scans of the samples were obtained on a Perkin-Elmer DSC7 differential scanning calorimeter connected to a Perkin-Elmer 7700 computer via the TAC7 microprocessor controller. Pyris® software was used to calculate extrapolated onset temperature, peak temperature and enthalpy values for each thermal event. The temperature axis was calibrated with pure indium, with a melting point of 156.60 °C and was checked using a zinc standard, with a melting point of 419.47 °C. Using pin-holed sample pans, sample mass (5–10 mg) and heating rate (5–60 °C/min) were varied according to the purpose of the experiments. The thermal profiles for crystallisation were obtained under isothermal conditions using DSC apparatus. The crystallinity was calculated using the following equation:

$$\text{crystallinity at time } (t) = \frac{\Delta H_t}{\Delta H_{\max}}$$

where ΔH_t and $\Delta H_{\max}$ are the value of the relaxed enthalpy for crystallisation until time (t), and until reaching the base line again, respectively. For non-isothermal study, the scan rate sensitivity of the crystallisation peak was evaluated.

2.4. X-ray diffraction patterns (XRD)

The powder X-ray diffraction patterns of the samples were obtained using a Siemens, Model D5000 diffractometer fitted with a scintillation counter and Cu Kα radiation source (wavelength = 0.15418 nm). The divergence and detector slits were of 0.3 and 0.18° aperture, respectively. Data were collected between 3 and 40° of 2θ in a step mode using a step size of 0.02° of 2θ and collecting time of 1 s/step. A controlled temperature cell was used to detect diffraction pattern changes on heating.

2.5. Hot-stage microscopy (HSM)

Samples were heated on a Stanton Redcroft hot-stage unit at a controlled rate of 5 °C/min by using a universal temperature programmer (Stanton Redcroft, London, UK). During the scanning, a video camera, connected to the microscope, was used to record thermal events.

3. Results and discussion

3.1. Thermal characterisation

A representative DSC scan of quench-cooled mannitol prepared in the absence of additives is shown in Fig. 1. The scan is typical of a sample in the amorphous state, in that it displays a glass transition (with an onset at 7.4 °C), a small exothermic peak (with a peak at 23 °C), followed by a relatively large exothermic peak (with a peak at 56 °C). Kim et al. (1998), using powder X-ray diffraction measurements, confirmed these events to be crystallisation of a meta-stable and stable form of mannitol, respectively. The DSC scan of the mannitol sample without additives after melting and subsequent cooling to ambient conditions is displayed in Fig. 2(a). It displays a very small endothermic and subsequent exothermal peak between 140 and 155 °C and is followed by a large endothermic peak at 167 °C. Jones and Lee (1970) investigated the polymorphism of mannitol using the fusion method. They suggested

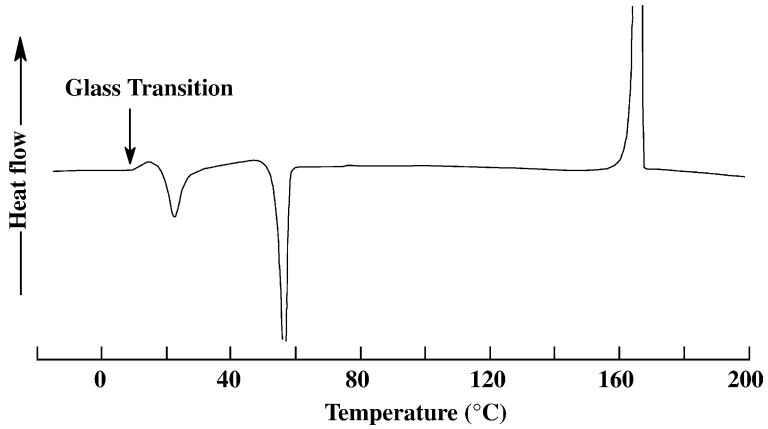

Fig. 1. DSC scan of quench cooled mannitol without any additives (scan rate 5 °C/min).

that a mixture of meta-stable and stable forms existed after the melting and cooling process. These minor thermal events could also be attributed to a polymorphic transition (Yoshinari et al., 2002) from the α to the β form according to Walter-Lévy's classification (1968). The DSC trace obtained for mannitol containing 1% (w/w) boric acid is shown in Fig. 2(b). Relatively larger peak areas at around 140–155 °C were observed than that in the case of mannitol without boric acid, which indicate that the relative amount of meta-stable form in the solid is increased. Because boric acid has been used as selective precipitating agent to promote crystallisation of the meta-stable form from aqueous system (Kim et al., 1968), it seems reasonable to assume that boric acid displays a similar effect for the melt-cooled system studied here. The effects of increasing boric acid content on the DSC traces are shown in Fig. 3. Marked differences between the DSC curves are readily apparent depending on boric acid content. When boric acid content was

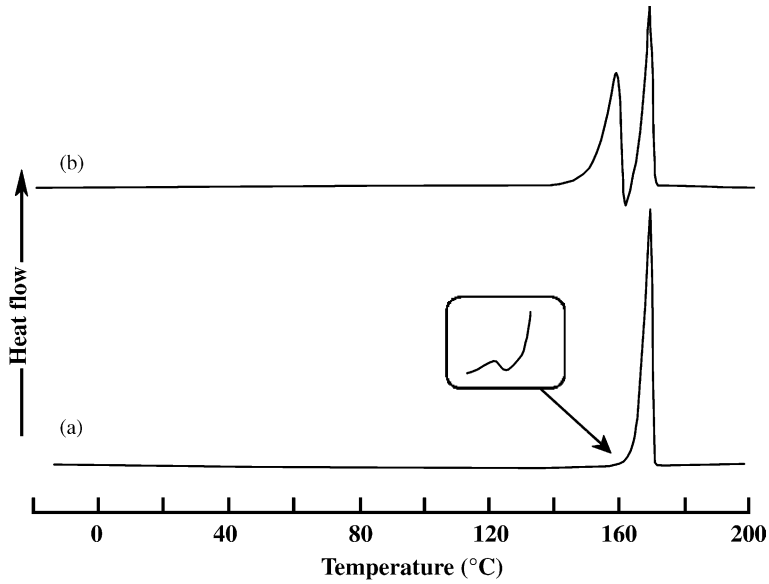

Fig. 2. DSC scans of mannitol samples after melting and ambient cooling: (a) without, (b) with 1% (w/w) of boric acid.

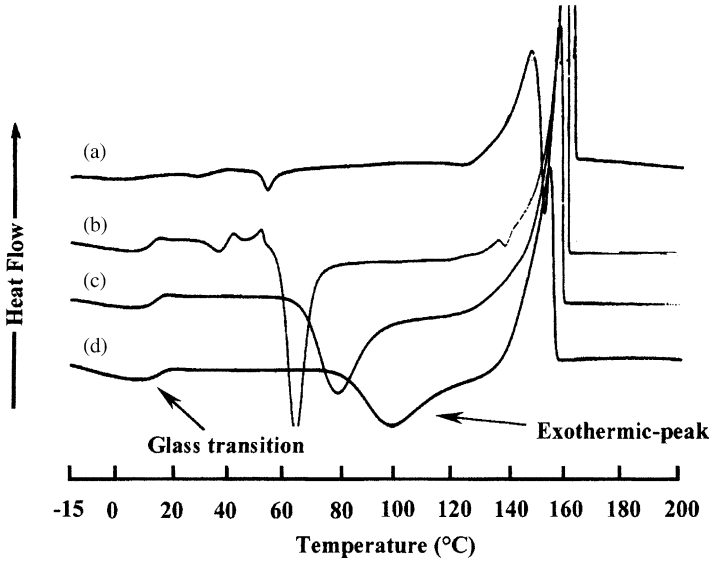

Fig. 3. DSC scans of melt-cooled mannitol samples containing various amounts of boric acid: (a) 3% (w/w), (b) 5% (w/w), (c) 7.5% (w/w) and (d) 10% (w/w) boric acid.

increased to 3% (w/w), the partially amorphous state of the solid was evident by the presence of a heat capacity change during the glass transition. At levels above 5% (w/w) boric acid, the data suggest that a totally amorphous solid was obtained under ambient conditions. The thermal peaks in the region of 25–55 °C displayed in the curve for 5% (w/w) boric acid were thought to be due to a partial phase transition. Furthermore, the magnitude of the large exothermal peak increased with increase in boric acid content was apparent. The position of these exothermal peaks was also dependent on boric acid content with transition maxima for 5, 7.5 and 10% (w/w) boric acid contents being 65, 78 and 99 °C, respectively.

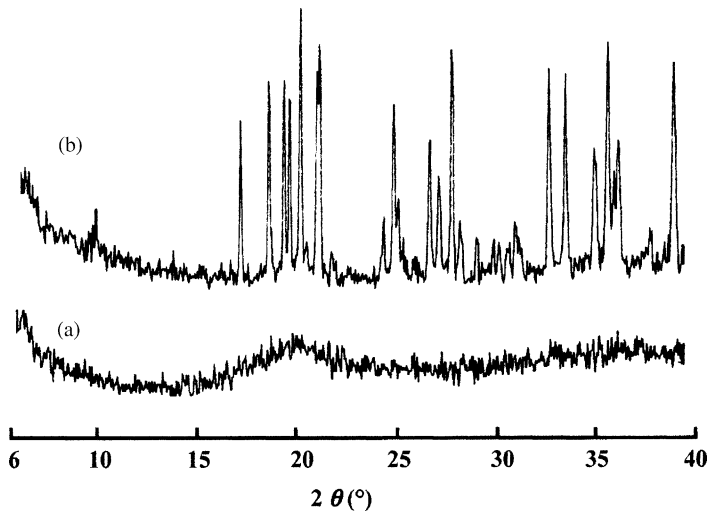

Fig. 4. X-ray diffraction patterns of melt-cooled mannitol containing 10% (w/w) boric acid at various temperatures: (a) 30 °C, (b) 120 °C.

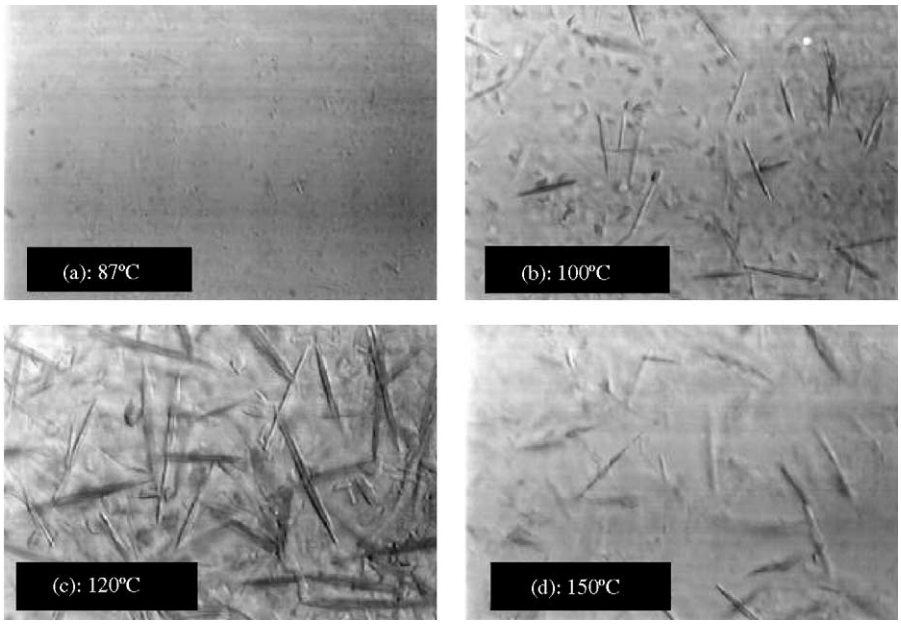

Fig. 5. Hot-stage polarisation micrographs of amorphous mannitol containing 10% (w/w) boric acid on heating to (a) 87 °C, (b) 100 °C, (c) 120 °C and (d) 150 °C.

3.2. Crystallisation of amorphous state of the mannitol/boric acid system

Temperature controlled powder X-ray diffraction measurements and hot-stage microscopic observations were performed to confirm the physical basis of these large exothermal peaks. As shown in Fig. 4, the diffraction pattern obtained at 30 °C for the 10% (w/w) boric acid sample displayed the typical halo pattern as confirmation of the amorphous state. When the sample was heated up to 120 °C, the halo pattern had disappeared and sharp diffraction peaks appeared indicating that the observed exothermal peak in the DSC curve was a crystallisation event. Hot-stage microscopy was used to visualise this thermal event (Fig. 5). Tiny crystals could be observed from about 85 °C in the microscope field with crystal growth continuing up to about 120 °C. The crystals then underwent melting at about 140 °C. No polymorphic conversions from the meta-stable to stable form were observed in the case of the sample containing 10% (w/w) of boric acid content. A trend of decreasing melting point with an increase in boric acid content was also observed.

3.3. Glass transition temperature

The effect of boric acid content on the glass transition temperature is presented in Fig. 6. It can be seen that the glass transition temperature of the sample increased with an increase in boric acid content. However, it should be noted that even in the case of 10% (w/w) boric acid content, the glass transition temperature was still below room temperature, at about 17 °C. The composition dependency of the glass transition temperature of a binary mixture can be described by the Gordon–Taylor equation (Gordon and Taylor, 1952):

$$T_{g_{mix}} = \frac{w_1 T_{g_1} + k w_2 T_{g_2}}{w_1 + k w_2}$$

where $T_{g_{mix}}$ is the glass transition temperature of the mixture, k is constant, w_1 and w_2 are weight fractions, and T_{g_1} and T_{g_2} are glass transition temperatures for component 1 and 2, respectively. The Gordon–Taylor equation assumes ideal volume mixing in binary mixture, which means that the mixture is homogeneous and that the specific volume remains constant. The good agreement between measured T_g values and

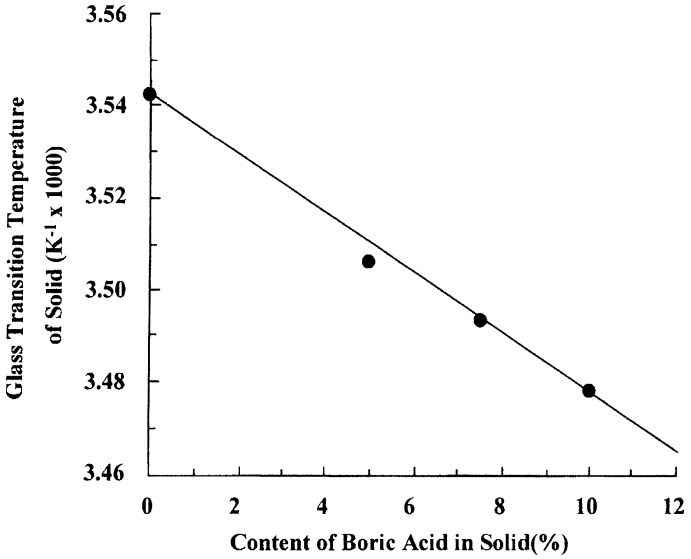

Fig. 6. Effect of boric acid content on glass transition temperature of amorphous mannitol samples.

calculated T_g values when the specific volume of each component remained unchanged suggested that the mannitol/boric acid system existed as a homogeneous mixture in an amorphous state.

3.4. Crystallisation kinetics of amorphous mannitol

Under isothermal conditions, a kinetic model was sought to explain the crystallisation of the mannitol/boric acid system. The kinetic equations tested and the results obtained are summarised in Tables 1 and 2, respectively (Avrami, 1939, 1940, 1941; Cater, 1961). On considering the *y*-intercept and correlation coefficient of the least squares line, it was found that the Avrami–Erofeev equation, with an Avrami exponent of 3, produced the equation of best fit to describe the kinetics of the observed crystallisation (Fig. 7). The Avrami–Erofeev equation is most applicable when the rate of a solid state reaction is governed by random nuclei and the exponent of 3 means that crystal growth is three dimensional.

As shown in Fig. 8, the temperature dependency of the rate constant of crystallisation of mannitol/boric acid system was analysed and the activation energy were determined from an Arrhenius plot according to the following equation:

$$\ln k = \ln A - \frac{E_a^{iso}}{RT}$$

where k is the rate constant, A is the pre-exponential factor, and E_a^{iso} is the activation energy obtained from the isothermal study. The activation energy (E_a^{iso}) obtained at levels of 0, 5 or 7.5% (w/w) of boric acid content was calculated to be 176, 124 and 94 kJ/mol, respectively. The relationship between the content of

Table 1
The equations tested in order to explain the crystallisation of the mannitol/boric acid systems

Equations	Mechanism
Avrami–Erofeev	
$[-\ln(1-\alpha)]^n$	
$n = 1$	Random nuclei, one-dimensional growth of nuclei
$n = 2/3$	
$n = 1/2$	Random nuclei, two-dimensional growth of nuclei
$n = 1/3$	Random nuclei, three-dimensional growth of nuclei
$n = 1/4$	
Jander	
$[-\ln(1-\alpha)^{1/3}]^2$	Three-dimensional diffusion

Table 2
Fit parameters for the crystallisation of amorphous mannitol/boric acid systems

Equations	Intercept	Correlation
0% (w/w) boric acid at 16 °C		
Avrami–Eroféev		
$n = 1$	−1.8085	0.928
$n = 2/3$	−0.6426	0.982
$n = 1/2$	−0.1937	0.996
$n = 1/3$	0.2101	0.996
$n = 1/4$	0.4043	0.989
Jander	−0.2900	0.917
5% (w/w) boric acid at 50 °C		
Avrami–Eroféev		
$n = 1$	−1.2345	0.858
$n = 2/3$	−0.6145	0.946
$n = 1/2$	−0.3249	0.982
$n = 1/3$	0.0005	1.000
$n = 1/4$	0.1824	0.998
Jander	−0.1857	0.780
7.5% (w/w) boric acid at 55 °C		
Avrami–Eroféev		
$n = 1$	−1.6620	0.925
$n = 2/3$	−0.7694	0.984
$n = 1/2$	−0.3813	0.998
$n = 1/3$	0.0011	0.995
$n = 1/4$	0.2211	0.985
Jander	−0.2589	0.875

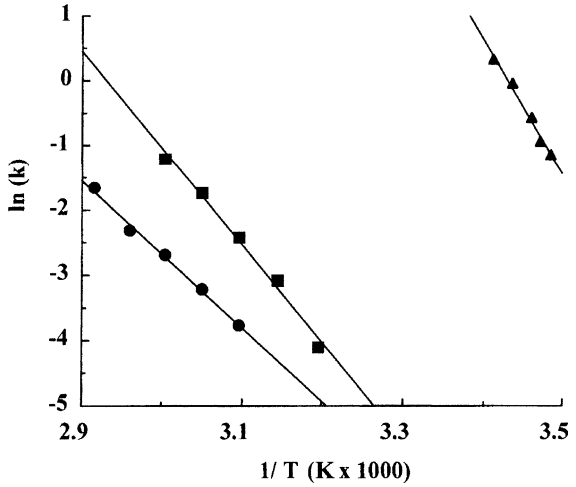

Fig. 8. Crystallisation kinetics from isothermal studies of amorphous mannitol containing various % (w/w) of boric acid: 0%: ▲; 5%: ■; 7.5%: ●.

boric acid and E_a^{iso} is shown in Fig. 9. It was found that the value of E_a^{iso} for isothermal crystallisation decreased with increasing boric acid content. Thus, the temperature dependency of crystallisation decreases with increasing boric acid content. However, the rate of crystallisation at 30 °C of the solid without boric acid (0% (w/w)) was 4000 and 7000 times higher than that of the solid with 5 and 7.5% (w/w) of boric acid, respectively. Furthermore, the rate of crystallisation

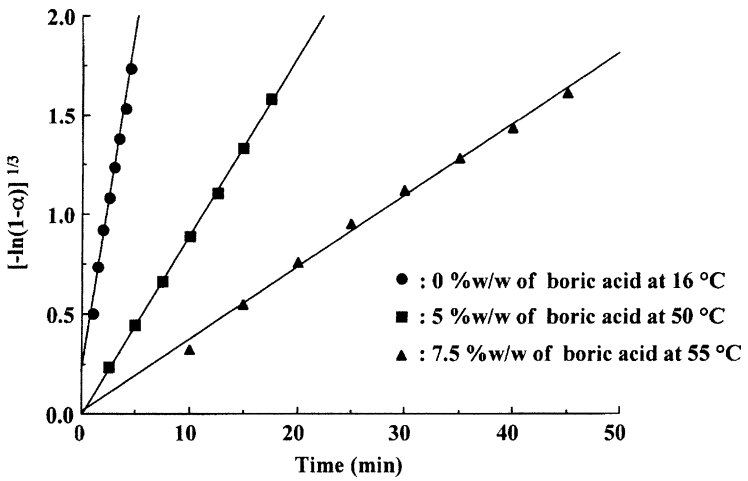

Fig. 7. Plots of $[-\ln(1-\alpha)]^{1/3}$ vs. t for the isothermal crystallisation of amorphous mannitol containing various amount of boric acid.

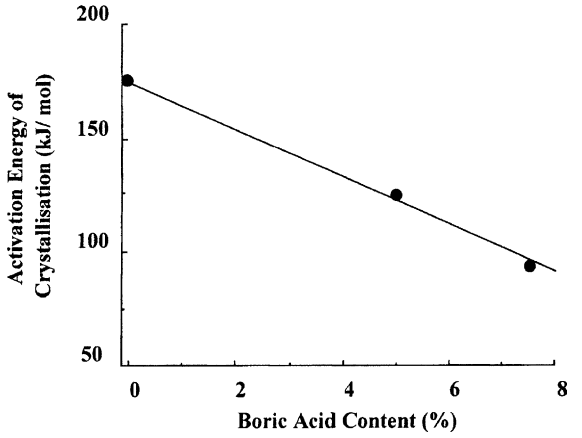

Fig. 9. Effect of boric acid content on activation energy (E_a^{iso}) from isothermal crystallisation experiments.

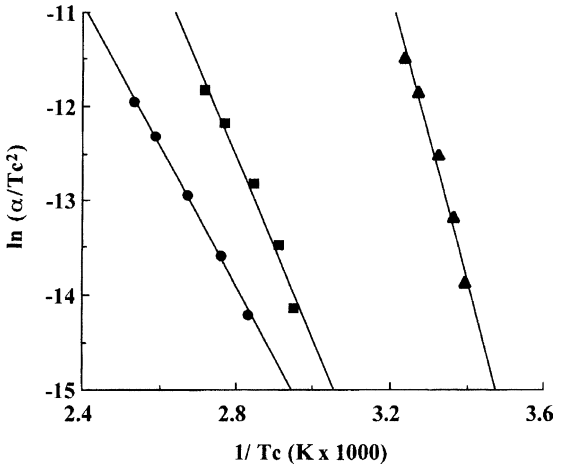

Fig. 10. Kissinger plots of the non-isothermal crystallisation of mannitol containing various % (w/w) of boric acid: 0%: ▲; 5%: ■; 7.5%: ●.

for the solid without boric acid and with 5 or 7.5% (w/w) boric acid only became equivalent at the extremely low temperatures of −57.9 and −35.4 °C, respectively.

In addition, a non-isothermal kinetic analysis was performed using the Kissinger equation (Kissinger, 1956, 1957):

$$\ln\left(\frac{\alpha}{T_C{}^2}\right) = \frac{-E_a^{\text{non-iso}}}{RT_C} + \text{constant}$$

where α is the scanning rate, T_C is the temperature of maximum rate of crystallisation, and $E_a^{\text{non-iso}}$ is the activation energy obtained by non-isothermal study. Kissinger plots of various mannitol/boric acid compositions are presented in Fig. 10. A linear relationship between the value of $\ln(\alpha/T_C^2)$ and the reciprocal of T_C was obtained and $E_a^{\text{non-iso}}$ was calculated from the slope of the least squares line. The obtained $E_a^{\text{non-iso}}$ value of the solids containing 0, 5 or 7.5% (w/w) of boric acid were 125, 81 and 62 kJ/mol, respectively. Similarly to the isothermal study, a linear relationship between boric acid content and the value of $E_a^{\text{non-iso}}$ was obtained (Fig. 11). However, when comparing the activation energies estimated by the isothermal and non-isothermal methods, the values of E_a^{iso} are approximately 1.5 times greater than that of $E_a^{\text{non-iso}}$ at all boric acid contents. Crystallisation can be considered a two-stage process; nucleation and growth of nuclei. When the values of E_a^{iso} and $E_a^{\text{non-iso}}$ are identical then the nucleation mechanism is continuous or a fixed number. A difference between the values of E_a^{iso} and $E_a^{\text{non-iso}}$ suggests that site-saturated nucleation is occurring, which means that nucleation only takes place during the very early stages of transition and the number of nuclei remains constant thereafter (Woldt, 1992). Schmitt et al. (1999) studying amorphous lactose found that lower activation energies were obtained in the presence of seeds during an isothermal study, such that the values of E_a^{iso} in the presence of seeds became approximately equal to values of $E_a^{\text{non-iso}}$

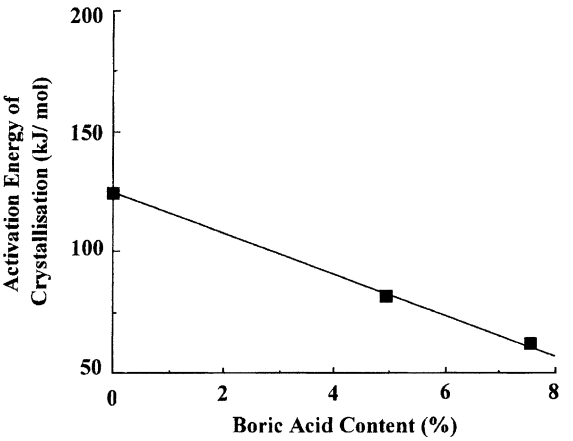

Fig. 11. Effect of boric acid content on activation energy ($E_a^{\text{non-iso}}$) obtained from non-isothermal crystallisation analysis.

obtained from a non-isothermal study. Their data indicated that, in the presence of seeds, crystallisation of lactose occurred by the growth of a fixed number of nuclei and that, without seeds, crystallisation was driven by a site-saturated nucleation mechanism. In the case of a site-saturated nucleation and three-dimensional growth, E_a^{iso} can be described as follows:

$$E_a^{iso} = \frac{{}^{N}E_a + 3{}^{G}E_a}{3}$$

where ${}^{N}E_a$ is activation energy for nucleation and ${}^{G}E_a$ is activation energy for growth of nuclei and ${}^{G}E_a$ is identical to $E_a^{non\text{-}iso}$. Therefore, ${}^{N}E_a$ can be obtained from the following equation:

$${}^{N}E_a = 3\left(E_a^{iso} - E_a^{non\text{-}iso}\right)$$

The difference between E_a^{iso} and $E_a^{non\text{-}iso}$ permits calculation of the activation energy for nucleation, ^{N}Ea, which were determined to be 152, 130 and 96 kJ/mol for 0, 5% (w/w) or 7.5% (w/w) boric acid contents, respectively.

3.5. Crystallisation inhibition mechanism

It is known that boric acid and Keggin-type heteropolyanions, that is, $H_3PW_{12}O_{40}$ and $H_4SiW_{12}O_{40}$, have an ability to selectively precipitate meta-stable forms of mannitol from aqueous solution, as demonstrated by Kim et al. (1968) and Trovão et al. (1998), respectively. Boric acid (*ortho*-boric acid) is an oxo-acid and boron, which is the central atom, has a positive charge (δ^+) and acts as an electron acceptor (Yoshimura et al., 1996). Heteropolyanions form by polymerisation of the different species of oxo-acids, whose central atoms are called hetero-atoms. They possess a very high oxidation potential and thus, are extremely strong electron acceptors, and highly positively charged (δ^+) (Himeno et al., 1999). On the other hand, mannitol is an extremely weak acid with hydroxyl groups at the C1 and C6 carbons acting as both hydrogen bond acceptors and donors. In the presence of strong electron acceptors, for example, boric acid or heteropolyanions, these hydroxyl groups act as electron donors and form a complex. Therefore, when solid mannitol is produced from aqueous solutions in the presence of boric acid or heteropolyanions, crystallisation is inhibited because the formation of hydrogen bonds, necessary for a crystalline mannitol product, is prevented by the mannitol/boric acid or mannitol/heteropolyanion molecular interaction, represented in Fig. 12. This disruption of the hydrogen bond network would explain the selective precipitation of meta-stable mannitol modifications when using boric acid or heteropolyanions. Furthermore as a result of the interaction, precipitation of mannitol in the presence of these additives might occur from higher supersaturation levels than those in the absence of additives, and thus further promote meta-stable forms. Similarly, our findings for mannitol solidification

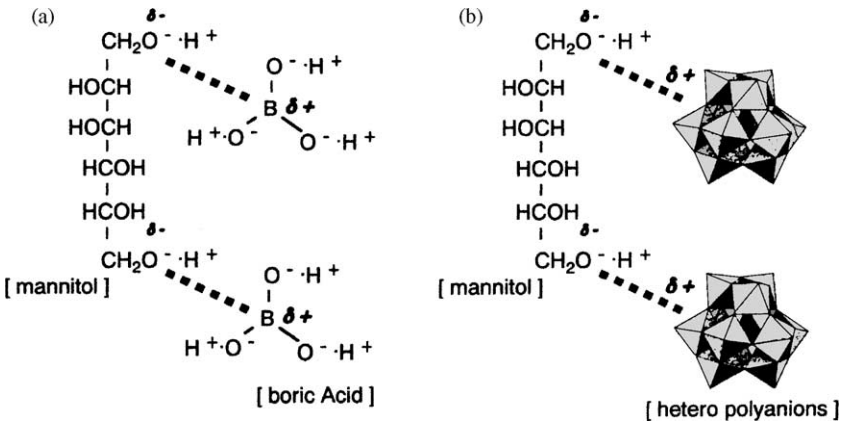

Fig. 12. Possible interaction between mannitol and additives which disrupt the hydrogen bond network of mannitol: (a) boric acid, (b) hetero polyanions.

from the melt are consistent with boric acid acting to disrupt hydrogen bonds. The results indicate that the rate of solidification was so fast, and probably faster than precipitation from solution, that, in the absence of the mannitol hydrogen bond network, an amorphous solid was the only likely outcome.

4. Conclusion

It was possible to maintain mannitol in an amorphous state using boric acid as a model additive. The mechanism of crystallisation inhibition likely includes a change in the hydrogen bond network in the presence of boric acid rather than a change in molecular mobility. Thermal characterisation of the amorphous solid under isothermal and non-isothermal conditions suggested that the crystallisation mechanism was three-dimensional growth with site-saturated nucleation. More pharmaceutically relevant additives to stabilise the amorphous state are under investigation.

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