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International Journal of Pharmaceutics 170 (1998) 63–72

international journal of pharmaceutics

Applications of pressure differential scanning calorimetry in the study of pharmaceutical hydrates. II. Ampicillin trihydrate

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Received 25 November 1997; received in revised form 27 February 1998; accepted 13 March 1998

Abstract

The dehydration of ampicillin trihydrate ($C_{16}H_{19}N_3O_4S \cdot 3H_2O$) was studied by both conventional differential scanning calorimetry (DSC) and by pressure differential scanning calorimetry (PDSC). The solid state of the anhydrous phase formed was influenced by the DSC conditions. At ambient pressure, dehydration resulted in the formation of X-ray amorphous anhydrous ampicillin, while at elevated pressures a crystalline anhydrate was obtained. These conclusions were based on variable temperature X-ray powder diffractometry (VTXRD) which was used as a complementary technique. PDSC was a reliable technique to quantify the relative amounts of ampicillin trihydrate and anhydrous ampicillin when they occur as a mixture. Grinding-induced alterations in the degree of crystallinity of ampicillin trihydrate were also quantified by PDSC. The changes in crystallinity induced after milling for just 1 min were detected and quantified with a high degree of precision. PDSC appears to be an excellent technique not only for the characterization but also for obtaining quantitative information about the solid state of pharmaceutical hydrates.

Keywords: Pressure differential scanning calorimetry (PDSC); Ampicillin trihydrate; Powder X-ray diffractometry; Hydrate; Dehydration; Degree of crystallinity

1. Introduction

Many compounds listed in the USP are capable of existing either as an anhydrate or as a hydrate (USP XXIII, 1994). Differential scanning calorimetry (DSC) is routinely used in the characterization of pharmaceutical hydrates (Ford and Timmins, 1989). Using carbamazepine dihydrate ($C_{15}H_{12}N_2O \cdot 2H_2O$) as a model compound, we demonstrated the utility of pressure differential scanning calorimetry (PDSC) in the characteriza-

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0378-5173/98/$19.00
PII S0378-5173(98)00123-9

tion of pharmaceutical hydrates (Han and Suryanarayanan, 1997). The technique had the potential to separate the dehydration and vaporization endotherms when dehydration occurred at a temperature ≥ 100°C. As a result, it was possible to determine the enthalpy of dehydration. More interestingly, the technique permitted the dehydrated water to be in intimate contact with the anhydrous phase formed, and this was found to significantly influence the solid state of the anhydrous phase formed. The overall goal of this investigation was to extend the applicability of PDSC to study complex dehydration reactions and also develop quantitative applications of PDSC.

Dehydration of carbamazepine dihydrate, the compound used in our previous study, resulted in a crystalline anhydrate (Han and Suryanarayanan, 1997). The model compound selected for this study was ampicillin trihydrate ($C_{16}H_{19}N_3O_4S \cdot 3H_2O$) which, when dehydrated under ambient pressure and high temperature, yielded an amorphous anhydrate (Shefter et al., 1973). Differential thermal analysis of ampicillin trihydrate at variable pressures is reported to result in a stable form of anhydrous ampicillin (Shishkin et al., 1972). However, the solid state of this form was not characterized.

During pharmaceutical processing and storage, a hydrate can transform to the corresponding anhydrate. The reverse transition, i.e. from an anhydrate to a hydrate is also possible (Haleblian and McCrone, 1969; Byrn, 1982; Zografi, 1988). It is well recognized that the solid state of the active ingredient (polymorphic form, state of solvation, degree of crystallinity) can not only influence the physicochemical and mechanical properties of a solid but can also affect the in vivo performance of the dosage form (Byrn et al., 1995; Giron, 1995; Khankari and Grant, 1995). It is therefore important and necessary to develop analytical techniques that can detect and quantify such transitions. Conventional techniques for water content determination such as Karl Fischer titrimetry and thermogravimetric analysis (TGA) determine the total water in the solids. In other words, these techniques may not distinguish between lattice water (sometimes referred to as chemically bound water) and sorbed water (also referred to as physically bound water). Earlier work in our laboratory demonstrated the utility of X-ray powder diffractometry (XRD) for the quantitative analyses of mixtures consisting of the anhydrous and hydrated forms of a compound (Suryanarayanan, 1989). However, this method necessitates consideration of the numerous sources of error in quantitative X-ray powder diffractometry. PDSC is potentially a simpler alternative method for such quantitative analyses.

The degree of crystallinity of solids has received considerable attention in the pharmaceutical literature (Suryanarayanan and Mitchell, 1985; Byrn et al., 1995). While numerous techniques are available to quantify crystallinity of solids, solution calorimetry (Pikal et al., 1978), isothermal microcalorimetry (Sebhatu et al., 1994) and moisture sorption techniques (Saleki-Gerhardt et al., 1994) appear to be sensitive indicators of changes in the crystallinity of solids. The compounds used in these studies have been anhydrous solids with just one exception (Sebhatu et al., 1994). We have attempted to demonstrate the utility of PDSC as a sensitive indicator of changes in crystallinity of hydrates.

This study had the following objectives: (i) to demonstrate the applicability of PDSC in the characterization of pharmaceutical hydrates; (ii) to use PDSC to quantify the proportions of the anhydrous and hydrate forms of a compound when they occur as a mixture; (iii) to monitor the alterations in the degree of crystallinity of hydrates by PDSC.

2. Materials and methods

2.1. Materials

Ampicillin trihydrate and anhydrous ampicillin purchased from Sigma Chemical Company (St. Louis, MO) were used as received.

2.2. Methods

2.2.1. Thermal analysis

A conventional differential scanning calorimeter (Model 910, TA Instruments), or a pressure

differential scanning calorimeter (Model 910 cell enclosed in a specially designed pressure chamber, TA Instruments) and a thermogravimetric analyzer (Model 951, TA Instruments) were connected to a thermal analysis operating system (Thermal Analyst 2000, TA Instruments). About 3 mg of sample was weighed into an aluminum pan, the pan was crimped nonhermetically, and subjected to DSC or PDSC. The experimental details including the calibration procedure were presented earlier (Han and Suryanarayanan, 1997).

2.2.2. Powder X-ray diffractometry (XRD) and variable temperature powder X-ray diffractometry (VTXRD)

The experimental details were presented earlier (Han and Suryanarayanan, 1997).

2.2.3. Quantification of ampicillin trihydrate in anhydrous ampicillin–ampicillin trihydrate mixtures

2.2.3.1. Standard curve. Mixtures of ampicillin trihydrate and anhydrous ampicillin were prepared wherein the weight fraction of ampicillin trihydrate ranged from 0 to 1. In order to eliminate the errors associated with mixing of solids, the appropriate amounts of ampicillin trihydrate and anhydrous ampicillin were directly weighed into the DSC pans. The pans were crimped nonhermetically, and subjected to PDSC at 300 psi. The heating rate was 10°C min^{-1} and the samples were heated up to 170°C. The enthalpy of dehydration (ΔH_d) values (endotherm at ~ 100°C) were determined and plotted as a function of the weight fraction of ampicillin trihydrate in the mixtures to yield the standard curve.

2.2.3.2. Quality control samples. Next, new mixtures of known composition were prepared wherein the weight fraction of ampicillin trihydrate ranged from 0.1 to 0.9. The enthalpy of dehydration was experimentally determined by PDSC and the weight fraction of ampicillin trihydrate was calculated using the standard curve. Quality control mixtures were also subjected to TGA up to 125°C and the weight loss was determined. The weight loss observed in each quality control sample divided by the weight loss observed for pure ampicillin trihydrate yielded the weight fraction of ampicillin trihydrate in that sample.

As mentioned earlier, the appropriate amounts of ampicillin trihydrate and anhydrous ampicillin were directly weighed into the DSC and TGA pans. As a result, replicate analyses were not possible at all concentrations of ampicillin trihydrate (Table 2). In such cases, the weight fraction of ampicillin trihydrate in the three samples was kept as close to each other as possible.

2.2.4. Milling

About 700 mg of ampicillin trihydrate was milled in a ball mill (Spex Mixer/Mill, Spex Industries, Metuchen, NJ) using a sample holder and ball made of agate. The milling time ranged from 1 to 10 min. The milled samples were subjected to PDSC and TGA.

2.2.4.1. Determination of the width of the 12.2°2θ peak (d-spacing of 7.24 Å) of ampicillin trihydrate. The powder was filled into an aluminum holder by the side-drift technique (Phadnis et al., 1997) and exposed to Cu Kα radiation (45 kV × 30 mA) in a wide-angle powder X-ray diffractometer (Model D500, Siemens). The instrument was operated in the step scan mode in increments of 0.01°2θ. The angular range was 11–13°2θ and counts were accumulated for 2 s at each step. The full width at half maximum (FWHM), expressed in degrees 2θ was determined using a commercially available software package (Shadow, Version 4, Materials Data, Inc.).

2.2.5. High-performance liquid chromatography (HPLC)

The USP method for the assay of ampicillin was followed (USP XXIII, 1994). The HPLC system consisted of an autoinjector (Model # 79855A, Hewlett-Packard), a solvent delivery pump (Model # 79852A, Hewlett-Packard), a reversed-phase MC18 column (ES Industries) and a variable wavelength UV detector (Model # 79853C, Hewlett-Packard).

3. Results and discussion

3.1. Characterization of anhydrous ampicillin and ampicillin trihydrate

The XRD pattern of anhydrous ampicillin matched that of anhydrous ampicillin reported in the literature (Shefter et al., 1973; PDF-2, 1996). When it was subjected to DSC, an exotherm was observed in the temperature range of 199–202°C which is attributed to decomposition (Ivashkiv, 1973). A weight loss of 0.2% was observed when anhydrous ampicillin was heated in the TGA up to 125°C. The water content determined by Karl Fischer titrimetry (CA-05 Moisture Meter, Mitsubishi) was 0.2%. The TGA and Karl Fischer titrimetric results were in good agreement and indicated a very low sorbed water content.

The XRD pattern of ampicillin trihydrate was identical to that of ampicillin trihydrate reported in the literature (PDF-2, 1996). When heated in the TGA up to 125°C, a weight loss of 13.3% was observed, which agreed with the theoretical weight loss of 13.4% for complete dehydration. The water content determined by Karl Fischer titrimetry was 13.5%, which was in good agreement with the weight loss observed in the TGA.

DSC at ambient pressure revealed two overlapping endotherms in the temperature range of 50–140°C (Fig. 1a). Based on previous reports and the VTXRD studies (discussed in Section 3.3), the endotherms were attributable to dehydration and vaporization processes (Shefter et al., 1973). Ampicillin trihydrate is reported to melt with decomposition either between 202 and 204°C or between 215 and 216°C (Ivashkiv, 1973). This was outside the temperature range of our studies. There appear to be no reports on the solid-state stability of ampicillin at lower temperatures. Ampicillin trihydrate was heated up to 120°C (at ambient pressure) and subjected to HPLC. There was significant drug decomposition. Therefore there are three events in the temperature range of the two overlapping endotherms—dehydration of ampicillin trihydrate, vaporization of water and partial decomposition of anhydrous ampicillin.

3.2. Pressure differential scanning calorimetry

3.2.1. Effect of pressure

The DSC profile of ampicillin trihydrate obtained in a pressure cell at ambient pressure was similar to that obtained in a conventional cell (Fig. 1b). The DSC curves at elevated pressures (100–600 psi), showed pronounced differences from those obtained at ambient pressure (Fig. 1c–g). An endotherm was observed at ~100°C followed by an exotherm at ~150°C.

In order to characterize these thermal events, ampicillin trihydrate was heated at 300 psi right past the first endotherm (~105°C). The sample was removed and subjected to HPLC analysis. Based on the ampicillin content (determined as anhydrous ampicillin), the dehydration was complete and there was no drug decomposition up to this temperature. When the solid was subjected to XRD, the powder diffraction pattern

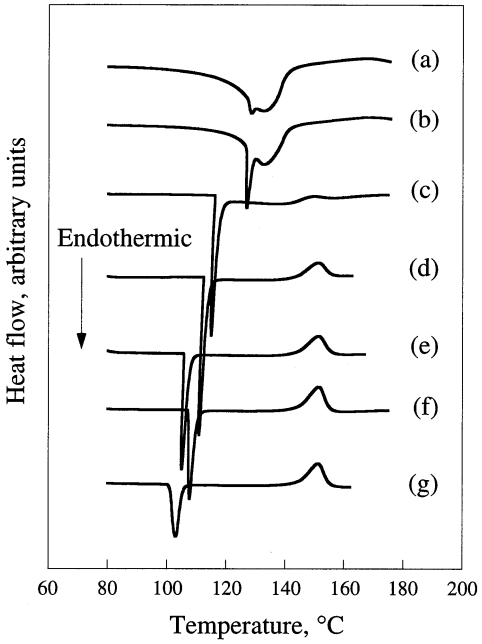

Fig. 1. DSC of ampicillin trihydrate at different pressures. (a) At ambient pressure in a conventional DSC cell, (b) at ambient pressure in a PDSC cell, (c) 100 psi, (d) 200 psi, (e) 300 psi, (f) 400 psi, and (g) 600 psi.

Table 1
Effect of pressure and heating rate on the enthalpy and temperature of dehydration of ampicillin trihydrate

Effect of pressure[a]			Effect of heating rate[b]		
Pressure (psi)	Enthalpy of dehydration (ΔH_d) (J g^{-1})	Temperature of dehydration (°C)	Heating rate (°C min^{-1})	Enthalpy of dehydration (ΔH_d) (J g^{-1})	Temperature of dehydration (°C)
100	83.0 ± 0.4	116.3 ± 6.7	2.5	76.4 ± 1.9	115.4 ± 2.1
200	77.0 ± 1.0	109.4 ± 1.5	5.0	79.2 ± 3.0	107.3 ± 1.1
300	77.7 ± 1.6	108.0 ± 2.5	10.0	77.7 ± 1.6	108.0 ± 2.5
400	77.3 ± 1.0	107.2 ± 1.7	15.0	76.5 ± 0.5	108.2 ± 3.1
600	74.3 ± 2.7	103.6 ± 1.0	20.0	75.1 ± 1.9	109.3 ± 2.3

[a] The heating rate was maintained constant at 10°C min^{-1}.
[b] The pressure was 300 psi.
[c] Mean $\pm$ S.D.; $n = 3$.

matched that of anhydrous ampicillin reported in the literature (PDF-2, 1996). A second HPLC analysis, carried out after heating a fresh sample past the exotherm ($\sim$155°C), revealed that a substantial fraction of the ampicillin had undergone decomposition. Therefore, the endotherm at $\sim$100°C is attributed to dehydration and the exotherm to decomposition.

At 100 psi, the endotherm due to vaporization of water (boiling point of water = 170°C at 100 psi) overlapped with the decomposition exotherm. As a result, only a small exotherm was observed. At higher pressures (200–600 psi), a pronounced decomposition exotherm was observed, and in these samples the boiling temperature of water was outside the temperature range of the experiment. The enthalpy and temperature of dehydration were determined as a function of pressure (Table 1). There appeared to be no systematic effect of pressure on the enthalpy and temperature of dehydration.

3.2.2. Effect of heating rate

These experiments were carried out at a constant pressure of 300 psi because the dehydration endotherm was very sharp and unambiguous determination of the peak area was possible (Fig. 1). While the enthalpy of dehydration appeared to be unaffected by the heating rates, the dehydration temperature at a heating rate of 2.5°C min^{-1} was higher than that at other, more rapid, heating rates (Fig. 2; Table 1). At heating rates $\geq$5°C min^{-1}, the temperature and enthalpy of dehydration were unaffected by the heating rate.

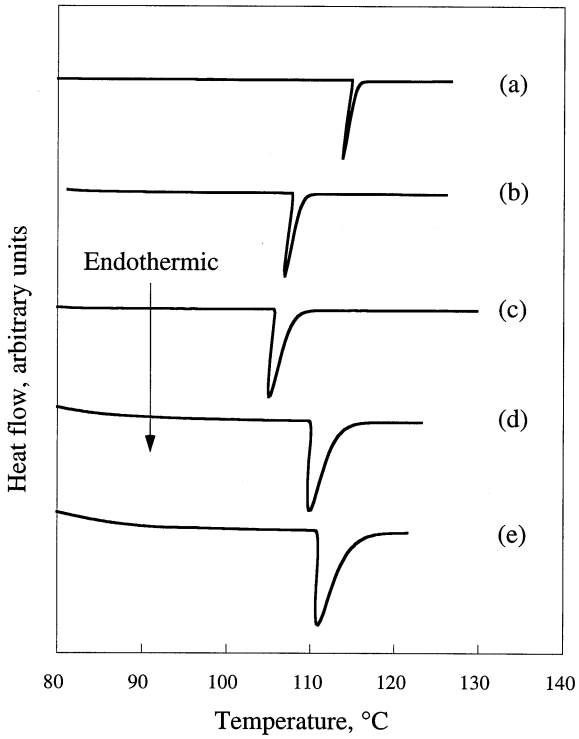

Fig. 2. DSC of ampicillin trihydrate at different heating rates. (a) 2.5°C min^{-1}, (b) 5°C min^{-1}, (c) 10°C min^{-1}, (d) 15°C min^{-1}, and (e) 20°C min^{-1}. These experiments were carried out at a constant pressure of 300 psi.

Another interesting observation from the PDSC profiles was that the dehydration endotherms were very sharp and a pronounced self-cooling effect was observed, particularly up to heating rates of 10°C min^{-1} (note the shape of the endotherms in Fig. 2a to 2c). This self-cooling effect was not observed when the samples were subjected to DSC at ambient pressure (Fig. 1a and b). In the ambient pressure DSC, as soon as the dehydration process is initiated, the water liberated on dehydration is expected to vaporize immediately, leaving behind an amorphous anhydrous phase (further discussed in Section 3.3). Its advancement may be the rate limiting step in the dehydration process. However, at elevated pressures, a substantial fraction of the water liberated on dehydration is expected to exist in the liquid state. Since this will be in intimate contact with the anhydrous phase, it may facilitate the rapid formation of crystalline anhydrate. Therefore the dehydration process could occur very rapidly over a narrow temperature range explaining the shape of the dehydration endotherm.

3.3. Role of water on the solid state of anhydrous phase formed after dehydration

In dehydration processes, the liberated water can significantly influence the solid state of the anhydrous phase formed. Variable-temperature powder X-ray diffractometry (VTXRD), a technique wherein X-ray diffraction patterns are obtained while a substance is subjected to a controlled-temperature program, is a useful technique to study this influence. VTXRD studies are usually performed in open holders wherein the water liberated on dehydration is readily removed. In an effort to simulate the PDSC conditions, we had also used a specially fabricated 'sealed' holder (Han and Suryanarayanan, 1997). This permitted VTXRD studies at elevated (albeit uncontrolled) pressures.

When carbamazepine dihydrate was subjected to VTXRD at ambient pressure, a poorly crystalline intermediate phase was formed which then transformed to anhydrous γ-carbamazepine. VTXRD of carbamazepine dihydrate in a sealed holder resulted in the formation of highly crystalline anhydrous β-carbamazepine, which then transformed to anhydrous γ-carbamazepine (Han and Suryanarayanan, 1997). The sealed holder enabled the water liberated on dehydration to be in intimate contact with the anhydrous phase. The presence of this water appeared to have a significant influence on the solid state of the anhydrous phase. It facilitated the nucleation and growth of β-carbamazepine which was the stable phase at the dehydration temperature.

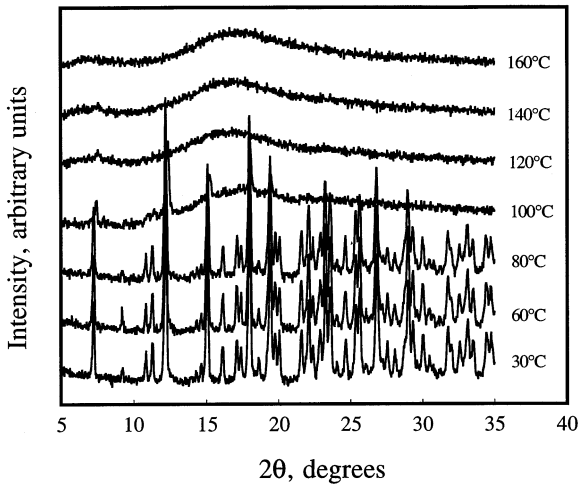

Fig. 3. VTXRD of ampicillin trihydrate wherein the sample was heated in an open copper holder and XRD patterns were obtained at selected temperatures.

When ampicillin trihydrate was subjected to VTXRD at ambient pressure (open holder), there was no change in the X-ray diffraction pattern up to 80°C (Fig. 3). At 100°C, there was a pronounced decrease in the crystallinity of the sample and at temperatures $\geq$120°C, the sample was X-ray amorphous. We had earlier observed that heating ampicillin trihydrate to 120°C (at ambient pressure) resulted in significant drug decomposition (Section 3.1). Shefter et al. (1973) dehydrated ampicillin trihydrate isothermally at 68, 74 and 83°C, and in all cases, observed that the dehydrated phase was X-ray amorphous.

VTXRD of ampicillin trihydrate in a sealed holder did not reveal any pronounced changes in the XRD pattern up to $\sim$140°C (Fig. 4). Above this temperature, the disappearance of ampicillin trihydrate and the appearance of a new crystalline

phase became evident. The formation of this new crystalline phase appeared to be complete by 150°C. In addition to the trihydrate and the anhydrate, the existence of ampicillin monohydrate has also been reported (Ivashkiv, 1973). However, there are no literature reports of ampicillin monohydrate formation from the trihydrate. The XRD pattern of ampicillin monohydrate has also not been reported.

In order to rule out drug decomposition, the sample heated up to ~150°C in the sealed holder, was subjected to HPLC. There was no detectable drug decomposition. Moreover, the assay value indicated that ampicillin existed as an anhydrate under these conditions. Two polymorphic forms of anhydrous ampicillin have been reported and the XRD pattern of this new phase did not match that of either of the polymorphs (Shefter et al., 1973). Therefore, we believe that this is a new polymorphic form of anhydrous ampicillin which has so far not been reported in the literature. Interestingly, the XRD pattern of the anhydrate formed in the PDSC matched that of anhydrous ampicillin obtained from Sigma (PDF-2, 1996). It is possible that there is only a modest pressure buildup in the sealed XRD holder which facilitates the formation of the new polymorph of anhydrous ampicillin.

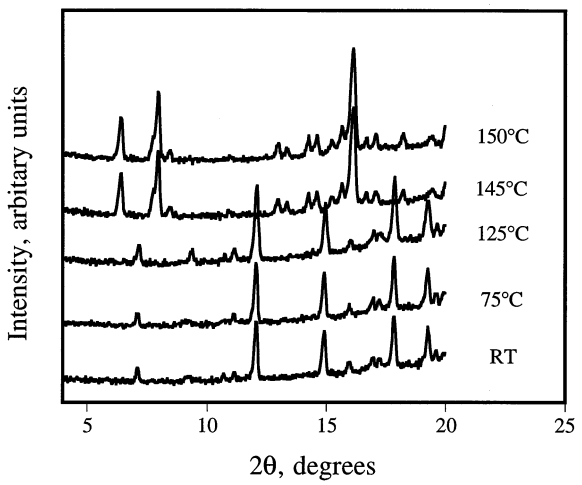

Fig. 4. VTXRD of ampicillin trihydrate wherein the sample was heated in a 'sealed' aluminum holder and XRD patterns were obtained at selected temperatures.

The relationship between solid state and chemical stability is of great significance in solid product development. Amorphous compounds can be substantially more reactive and therefore less stable than their crystalline counterparts (Pikal et al., 1978). This is also evident in case of ampicillin. When ampicillin trihydrate was dehydrated under ambient pressure, the anhydrous phase formed was X-ray amorphous (Fig. 3). There was also significant decomposition of this phase at 120°C, as revealed by HPLC. On the other hand, dehydration of ampicillin trihydrate under elevated pressures resulted in a crystalline anhydrate which was stable at least up to 150°C (Fig. 4).

3.4. Quantification of ampicillin trihydrate in a mixture with anhydrous ampicillin

The experimental details are presented in Section 2.2.3. Mixtures containing varying weight fractions of ampicillin trihydrate and anhydrous ampicillin were prepared and subjected to PDSC (at 300 psi). When the enthalpy of dehydration (ΔH_d) values were plotted as a function of the weight fraction of ampicillin trihydrate, a linear relationship was observed. The equation of the line was:

$$\text{Enthalpy of dehydration (J g}^{-1}) = 0.124 + 79.4 \times (\text{weight fraction of ampicillin trihydrate}) \quad (r^2 = 0.998)$$

Irrespective of the mixture composition, the dehydration endotherm occurred at ~100°C. Therefore, it was not necessary to correct for the temperature dependence of the dehydration enthalpy. Using this standard curve, the ampicillin trihydrate content in the quality control samples was determined (Table 2). This table also contains the ampicillin trihydrate content in the quality control samples determined by TGA. When PDSC was the analytical technique, the experimentally determined weight fraction ranged between 95.8 and 103.8% of the true weight fraction, while with TGA it ranged between 92.0 and

Table 2
Accuracy in the analysis of ampicillin trihydrate in the quality control mixtures

True weight fraction of ampicillin trihydrate	Experimentally determined weight fraction of ampicillin trihydrate		Percent of true weight fraction		Coefficient of variation (%)	
	PDSC	TGA	PDSC	TGA	PDSC	TGA
0.113	0.117	0.122	103.8	108.0	—	—
0.118	0.122	0.117	103.4	99.2	—	—
0.125	0.128	0.119	102.4	95.2	—	—
0.285	0.273	0.257	95.8	90.2	—	—
0.294	0.292	0.310	99.4	105.4	—	—
0.299	0.305	0.275	102.3	92.0	—	—
0.565	0.567	0.531	100.4	94.0	5.8	6.2
0.694	0.706	0.686	101.7	98.9	7.3	4.0
0.903	0.882	0.892	97.7	98.8	2.6	7.0

108.0% of the true weight fraction. The relative error in each determination, expressed in percent, is presented in Fig. 5. The relative error by PDSC was consistently $< 5\%$ (except in one case). It is clear from the results presented in Table 2 and Fig. 5 that PDSC is more reliable than TGA for the determination of ampicillin trihydrate content in the mixtures.

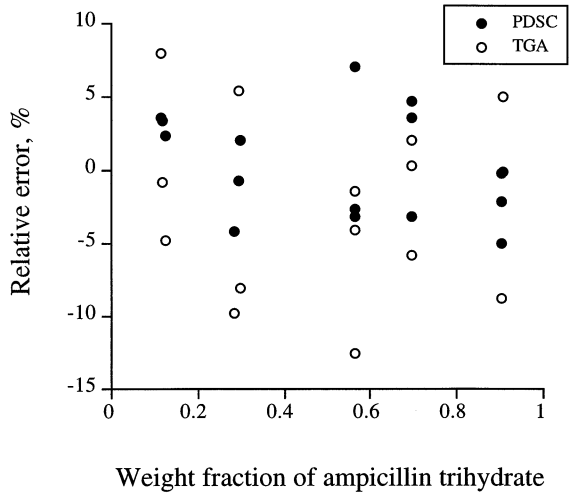

Fig. 5. The relative error in the analysis of ampicillin trihydrate in the quality control mixtures.

3.5. Degree of crystallinity of ampicillin trihydrate

The degree of crystallinity has an important bearing on the properties of pharmaceutical solids. Pharmaceutical processes such as milling and compression can bring about alterations in the crystallinity of solids. In case of hydrates, mechanical processing can also cause dehydration (Lefebvre et al., 1986; Irwin and Iqbal, 1991).

We were interested in studying the effect of milling on the solid state of ampicillin trihydrate. If milling causes disruption in the crystal lattice of ampicillin trihydrate (i.e. decrease in crystallinity), this is expected to result in a decrease in the dehydration enthalpy (ΔH_d). Therefore, ampicillin trihydrate was milled for time periods ranging from 1 to 10 min and the samples were subjected to PDSC and TGA. The percent crystallinity from PDSC was calculated as follows:

$$\text{Degree of crystallinity} = \frac{\Delta H_d \text{ of the milled sample}}{\Delta H_d \text{ of the untreated sample}} \times 100$$

and the results are presented in Table 3. This method implicitly assumes that the 'as is' (i.e. untreated) ampicillin trihydrate is 100% crystalline. Since the validity of this assumption has not been completely tested, we do not wish to place much emphasis on the numerical values of

Table 3
Crystallinity of ampicillin trihydrate as a function of milling time

Milling time (min)	% Crystallinity by PDSC	FWHM of 12.2°2θ peak (°2θ)	% Trihydrate by TGA
0.0	100.0 ± 0.2	0.450 ± 0.005	100.2 ± 3.3
1.0	96.5 ± 0.5	0.465 ± 0.001	100.3 ± 1.9
2.5	94.2 ± 0.3	0.485 ± 0.004	97.3 ± 3.2
5.0	89.7 ± 0.3	0.486 ± 0.016	97.6 ± 5.2
10.0	78.1 ± 1.2	0.546 ± 0.004	101.1 ± 2.9

the degree of crystallinity. However, it is obvious that PDSC is capable of detecting alterations in the crystallinity induced by milling for even as short a time period as 1 min.

This table also contains the ampicillin trihydrate content determined by TGA. These results suggest that milling does not cause dehydration of ampicillin trihydrate. However, the TGA results must be interpreted with caution. For the sake of argument, let us assume that milling also causes dehydration of ampicillin trihydrate. The anhydrous phase formed under ambient conditions is expected to be a poorly crystalline phase. Such phases will have a strong tendency to sorb moisture (Zografi, 1988). Therefore, the water liberated on dehydration might be immediately sorbed by the solid. Under such a circumstance, the water is present in two states in the solid–lattice water (as ampicillin trihydrate) and sorbed water (associated with the amorphous phase). TGA may indicate the total water in the solid and is not necessarily capable of discriminating between these two types of water.

If milling causes a decrease in the crystallinity of ampicillin trihydrate, this is expected to cause an increase in the width (measured as full width at half maximum (FWHM)) of the X-ray peaks of ampicillin trihydrate. This approach was successfully used to monitor the grinding induced alterations in the crystallinity of calcium gluceptate (Suryanarayanan and Venkatesh, 1990). In the case of ampicillin trihydrate, there was a progressive increase in the width of the 12.2°2θ peak as a function of the grinding time (Table 3). Thus XRD provided direct proof of the decrease in crystallinity as a function of the grinding time.

4. Conclusion

Using ampicillin trihydrate as a model compound, we have demonstrated the influence of dehydration conditions on the solid state of the anhydrous phase formed. Dehydration at ambient pressure resulted in an X-ray amorphous anhydrate. However at elevated pressures, where the dehydrated water remained in contact with the anhydrous phase, a crystalline anhydrous phase was observed. The crystalline anhydrate exhibited much better thermal stability than its amorphous counterpart. The relative amounts of ampicillin trihydrate and anhydrous ampicillin when they occur as a mixture were quantified by both PDSC and thermogravimetric analysis, and the former was observed to be a more reliable technique. PDSC was also a sensitive indicator of the degree of crystallinity of ampicillin trihydrate.

Acknowledgements

JH was partially supported by the International Student Work Opportunity Program of the University of Minnesota. We thank Laura Connor and Dr Murti Vemuri of Rhone-Poulenc Rorer for their assistance in the HPLC experiments.

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