ELSEVIER

International Journal of Pharmaceutics 139 (1996) 33–43

international journal of pharmaceutics

Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 2. Ampicillin

Haijian Zhu, David J.W. Grant*

Department of Pharmaceutics, College of Pharmacy, University of Minnesota, Weaver-Densford Hall, 308 Harvard Street SE, Minneapolis, MN 55455-0343, USA

Received 14 February 1996; revised 18 April 1996; accepted 23 April 1996

Abstract

The hydration state of a hydrate depends on the water activity, a_w, in the crystallization medium. A guideline has recently been established for the selection of an appropriate ratio of water to cosolvent in the crystallization medium of a hydrate (*Int. J. Pharm.*, 135 (1996) 151–160). This work examines the influence of a_w in organic solvent + water mixtures on the physical stability of the solid phases of ampicillin, which comprise a crystalline anhydrate, a crystalline trihydrate and an amorphous form. Saturation of the aqueous solution with ampicillin reduced a_w by less than 4%. The solubilities of the anhydrate and trihydrate were measured in different methanol + water mixtures of varying a_w at 25°C. The excess solid phase was characterized by powder X-ray diffractometry, differential scanning calorimetry, thermal gravimetric analysis and Karl-Fischer titrimetry. The crystalline anhydrate is kinetically stable for at least 5 days over the whole range of a_w in methanol + water mixtures. However, addition of seeds of the crystalline trihydrate to mixtures of ampicillin anhydrate + methanol + water at $a_w \geq 0.381$ resulted in the conversion of the anhydrate to the thermodynamically stable trihydrate. The trihydrate converted to the amorphous form at $a_w \leq 0.338$. The metastable amorphous form converted to the anhydrate at $a_w \leq 0.338$ when the suspension was seeded with the anhydrate. The metastable amorphous form took up water progressively with increasing a_w from 0 to 0.338 in methanol + water mixtures. These result suggest (a) that water activity is the major thermodynamic factor determining the nature of the solid phase of ampicillin which crystallizes from methanol + water mixtures, (b) that the system, ampicillin anhydrate $\rightleftharpoons$ ampicillin trihydrate, is in equilibrium at $0.338 < a_w < 0.381$ and at 25°C, and (c) that the anhydrate and amorphous forms may exist in metastable states. This study demonstrates the complex interplay between thermodynamic and kinetic factors in determining the prevailing solid phase of ampicillin.

Keywords: Ampicillin; Anhydrate; Trihydrate; Methanol; Water activity; Crystallization; Solubility; Seeding; Solvent-mediated transformation

* Corresponding author. Tel: +612 624 3956; fax: +612 625 0609; e-mail: grant001@maroon.tc.umn.edu.

PII S0378-5173(96)04570-X

1. Introduction

1.1. Relative stability of hydrates

The pharmaceutical reasons for studying and understanding drug hydrate equilibria and their physico-chemical basis have been presented in part I of this series (Zhu et al., 1996). The physical chemistry underlying these concepts is briefly reproduced here for convenience. The formation of hydrated crystals from anhydrous crystals (Shefter and Higuchi, 1963; Grant and Higuchi, 1990) may be represented by the following equilibrium

$$A(solid) + mH_2O \rightleftharpoons A.mH_2O(solid)$$

$$k_h = \frac{a[A.mH_2O(solid)]}{a[A(solid)]a[H_2O]^m} \quad (1)$$

where K_h is the equilibrium constant for the process shown in Eq. 1, and a[A.mH$_2$O(solid)], a[A(solid)], and a[H$_2$O] are the thermodynamic activities of the hydrate, the anhydrate and water, respectively. When a[H$_2$O] = [a[A.mH$_2$O(solid)]/{a[A(solid)]K_h}]$^{1/m}$, the anhydrate, A(solid), and water are in equilibrium with the hydrate, A.mH$_2$O(solid). When a[H$_2$O] > [a[A.mH$_2$O(solid)]/{a[A(solid)]K_h}]$^{1/m}$, the hydrate, A.mH$_2$O(solid), is more stable than the anhydrate, A(solid). The anhydrate, A(solid), is more stable than the hydrate, A.mH$_2$O(solid), in the inverse situation when a[H$_2$O] < [a[A.mH$_2$O(solid)]/{a[A(solid)]K_h}]$^{1/m}$. Corresponding relations also hold for the phase transitions between *different hydrates* of a drug. If the standard states of unit activity of A(solid) and of A.mH$_2$O(solid) are represented by their pure solid phases, Eq. 1 simplifies to Eq. 2.

$$K_h = a[H_2O]^{-m} \quad (2)$$

where m is the number of moles of water taken up by one mole of the anhydrate (or lower hydrate) in the stoichiometric equation. Thus, the hydration state of a hydrate depends on the water activity in the surrounding medium, for example, in the crystallization medium, which may consist of water and organic solvent mixture, or in the vapor phase in which the water activity can be controlled in a relative humidity chamber.

1.2. Water activity in organic solvent + water mixtures

The water activity, a[H$_2$O], which is abbreviated to a_w, in a crystallization medium can be controlled by changing the composition of an appropriate water + organic solvent mixture. The organic cosolvent should be water-miscible, such as methanol, ethanol, 2-propanol, acetone, acetonitrile or dioxane. Values of the mole fraction-based activity coefficient, γ_w, in mixtures of each of these solvents with water are known (Gölles, 1961; Gölles, 1962; Udovenko and Mazanko, 1967; Sokolova and Morachevskii, 1967; Chirikova et al., 1966; Bacarella et al., 1956). Values of a_w in mixtures of organic solvent + water can be calculated from the literature values of γ_w and the mole fraction of water, x_w, in these mixtures by Eq. 3.

$$a_w = \gamma_w \cdot x_w \quad (3)$$

Values of a_w are fitted to a polynomial in x_w, which enables a_w to be estimated at any value of x_w in the organic solvent + water mixtures employed for the crystallization of a drug. The a_w value in mixtures of organic solvent + water will be modified by the presence of the dissolved drug and a suitable means must be used to evaluate this effect.

1.3. Hypotheses to be tested

The fundamental hypotheses in the present work are that when a drug, which can form a hydrate, is crystallized from mixtures of organic solvent + water, (a) the water activity, a_w, in the mixtures is the major factor determining the nature of the anhydrate or hydrate phase that crystallizes and, (b) that the dissolved drug in the aqueous solvent mixtures influences a_w to a much smaller extent than does the organic solvent. For this concept to be useful, the organic solvent should not form a solid solvate nor a solid sol-

vate-hydrate phase with the drug. The above hypotheses have been previously tested in preliminary studies with nedocromil sodium ($C_{19}H_{15}NO_7Na_2$) in IPA + water mixtures (Khankari and Grant, 1993) and with theophylline in mixtures of methanol + water and IPA + water (Zhu et al., 1996). The work reported here is part of a continuing series of studies in which the above hypotheses are further tested using caffeine, carbamazepine and nedocromil magnesium as model compounds. In the present work, ampicillin, which exists as an anhydrate, a trihydrate and a metastable amorphous form, is crystallized from methanol + water mixtures. The main objective here is to investigate whether a_w is a major factor determining the nature of the anhydrate and hydrate phases of ampicillin which crystallize from the organic solvent + water mixtures. The second objective is to examine the effect of seeding on the anhydrate-hydrate transformation. The third objective is to study the influence of solvent composition on the solubility of the ampicillin phases. A preliminary abstract and presentation of this work has previously appeared (Zhu and Grant, 1995).

1.4. Ampicillin anhydrous and hydrated forms

Ampicillin ($C_{16}H_{19}N_3O_4S$) is a common antibiotic that is effective against a wide variety of Gram-positive and Gram-negative organisms (Doyle et al., 1961). Various hydrated forms of ampicillin have been reported, including a monohydrate, a dihydrate and a trihydrate (Grant and Alburn, 1965; Austin et al., 1965; James and Hall, 1968). However, it is probable that the trihydrate is a stable hydrate of defined composition, whereas the other forms were either amorphous or consisted of partially dehydrated trihydrate (Austin et al., 1965). Temperature is a critical factor in the crystallization of ampicillin (Austin et al., 1965), since crystallization of ampicillin from aqueous solution at temperatures below 50°C results in the formation of the trihydrate while temperatures above 60°C yield the anhydrate. The present work examines the influence of a_w at constant temperature (25°C). Ampicillin is chemically unstable in contact with aqueous solutions over prolonged periods of storage. However, reconstituted ampicillin powder for oral suspension is stable for at least 30 days when stored at 25°C (Sylvestri and Makoid, 1986). To minimize the decomposition of ampicillin, the present thermodynamic and kinetic studies with ampicillin in contact with methanol + water mixtures were limited to 5 days. During this period, no significant decomposition of ampicillin was observed using the spectrophotometric method described below.

2. Experimental

2.1. Materials

Ampicillin anhydrate and trihydrate were obtained from Sigma Chemical Company (St. Louis, MO). Imidazole was obtained from Aldrich Chemical Company (Milwaukee, WI). Mercuric chloride and boric acid were purchased from J. T. Baker Inc. (Phillipsburg, NJ). Acetic anhydride, acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ)

2.2. Spectrophotometric determination of ampicillin

Ampicillin concentration was determined by a spectrophotometric procedure which is specific for the β-lactam ring (Bundgaard, 1974). This assay involves initial acetylation of the side chain amino group of ampicillin to α-acetamidobenzylpenicillin and subsequent spectrophotometric measurement of the α-acetamidobenzylpenicillenic acid mercuric mercaptide at λ_{max} = 325 nm, which is formed by an imidazole-catalyzed rearrangement of the α-acetamidopenicillin. The standard curve, constructed using solutions of known ampicillin concentration and a UV spectrophotometer (model DU-64, Beckman), was linear in the concentration range of 10–70 mg/ml with a correlation coefficient of 0.9999.

2.3. Solubility measurements

The solubilities of ampicillin anhydrate and trihydrate were each determined in methanol + water mixtures of varying a_w values. Excess of ampicillin (1 g) was equilibrated for 5 days with 10 g of each methanol + water mixture (each containing a different mole fraction of water corresponding to a defined water activity) in a 20 ml vial at 25.0 ± 0.1°C for 5 days. Equilibration was attained by shaking the vials in a temperature-controlled water bath. Samples (0.5 ml) were withdrawn, filtered, quantitatively diluted with water and then the concentration was determined using the above UV spectrophotometric method. Equilibrium (stable or metastable) was judged to have occurred when the successive measurements differed by not more than 1%.

2.4. Powder X-ray diffraction

The powder X-ray diffraction patterns of the ampicillin solid phases were determined using an X-ray generator and goniometer (model D-500, Siemens, Germany) with Cu Kα radiation at 30 mA and 45 kV with 2θ increasing at the rate of 3°/min. Counts were accumulated for 1 s at each step. Each sample was packed into an aluminum holder and the instrument were operated between an initial and final 2θ angle of 5° and 35°, respectively, in increments of 0.05 2θ.

2.5. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (model 910, DuPont Instruments, New Castle, DE) equipped with a data station (Thermal Analyst 2000, DuPont Instruments, New Castle, DE) was used to record the thermograms of the samples. The temperature axis and the cell constant of the DSC cell were calibrated with indium (8 mg, 99.999% pure, peak maximum at 156.6°C and heat of fusion = 28.4 J/g). The sample (2–5 mg) was weighed onto an aluminum pan. A heating rate of 10°C/min with a nitrogen purge was employed throughout the study. DSC studies were carried out with crimped pans from which water vapor can escape.

2.6. Thermogravimetric analysis (TGA)

A model 951 thermogravimetric analyzer (DuPont Instruments, New Castle, DE) linked to a Thermal Analyst 2000 data station (DuPont Instruments, New Castle, DE) was used. The ampicillin solid phases were placed in aluminum pans and heated at 10°C/min under a nitrogen purge.

2.7. Karl-Fischer titrimetry (KFT)

The relative amounts of water, expressed as % w/w and as the number of moles of water per mole of anhydrous ampicillin, in the ampicillin solid phases were determined using a Mitsubishi Moisture meter (model CA-05, Mitsubishi Chemical Industries Limited, Tokyo, Japan). The sample (6–8 mg) was accurately weighed and quickly transferred to the titration vessel to measure the water content.

2.8. Relative humidity measurement

The relative humidity of the vapor phase above saturated aqueous solutions of ampicillin was measured by a Rotronic-Hygroskop BT (Rotronic Instrument Corp., Huntington, NY). At equilibrium, the relative humidity, expressed as a decimal fraction, is equal to a_w both in the vapor phase and in the solution.

2.9. Water uptake at various relative humidities

Vapor phase environments of different relative humidity (RH) values were produced by placing saturated, inorganic salt solutions in sealed desiccators. The following inorganic salt solutions were used: LiCl (15% RH, Mallinckrodt Inc, Paris, KY); CH_3COOK (20% RH, Fisher Scientific, Fair lawn, NJ); $CaCl_2 \cdot 6H_2O$ (32.3% RH, J.T. Baker Inc., Phillipsburg, NJ), $Mg(NO_3)_2 \cdot 6H_2O$ (52% RH, EM Science, Gibbstown, NJ); NH_4Cl (79.6% RH, EM Science, Gibbstown, NJ); and $CuSO_4 \cdot 5H_2O$ (98% RH, J.T. Baker Inc., Philipsburg, NJ). Ampicillin anhydrate samples were allowed to equilibrate for 6–9 months, the amount of water absorbed was analyzed by Karl-Fischer titrimetry, and the solid phase remaining was examined by powder X-ray diffraction.

3. Results and discussion

The values of a_w in methanol + water mixtures have been calculated from the literature values of γ_w (Gölles, 1961) using Eq. 3 and plotted against x_w, as in the previous work (Zhu et al., 1996). A saturated solution of ampicillin in water has a measured relative humidity of 96%, indicating that it is in equilibrium with water vapor at a_w = 0.96. Therefore, when the saturated solution (a_w = 0.96) is diluted infinitely with water (a_w = 1.00), a_w changes by only 4%. This relatively small change in a_w appear to justify hypothesis (b) that the presence of ampicillin, even at the solubility limit, has little effect on a_w in organic solvent + water mixtures.

Ampicillin anhydrate or trihydrate was added to methanol + water mixtures of various compositions. After equilibration at 25°C for 5 days to determine the solubility (Fig. 1), the excess solid was characterized by powder X-ray diffractometry, DSC and TGA. The powder X-ray diffraction patterns (Fig. 2a, b and c) enabled these solid forms of ampicillin to be characterized by comparison with the powder X-ray patterns of the anhydrate and the trihydrate reported by Shefter et al. (1973) and Brittain et al. (1988). The third form of variable water content was characterized as amorphous, as shown by a broad amorphous halo with no distinct diffraction peaks (Fig. 2c).

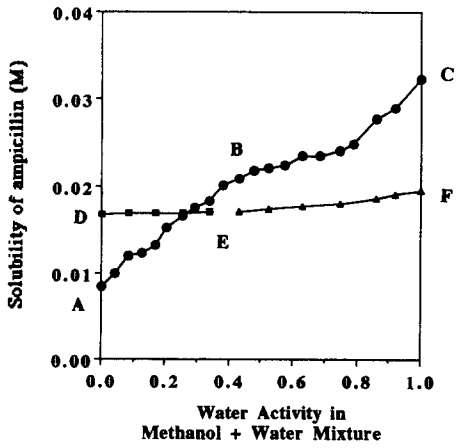

Fig. 1. ABC is the solubility profile of ampicillin anhydrate, while DE and EF are those of the phases resulting from ampicillin trihydrate, each as a function of water activity in methanol + water mixtures at 25°C. The final phases obtained are as follows: ABC anhydrate (●); DE amorphous (■); EF trihydrate (▲).

The DSC and TGA curves of the ampicillin anhydrate and trihydrate crystallized from methanol + water mixtures are consistent with those reported by Brittain et al. (1988). For the amorphous form obtained from pure methanol, the DSC curve (Fig. 2d) exhibits a broad endotherm at 117°C, indicating a low degree of cooperativity of the binding of the water molecules in the crystal structure in contrast to the narrow endotherm associated with trihydrate. For the amorphous form, the exotherm at 178°C and the endotherm at 205°C correspond to those of the anhydrate. The amorphous form possesses certain DSC features that resemble those of both the anhydrate and trihydrate forms, and may therefore be a metastable transition form during the dehydration of the trihydrate. For all the amorphous solids crystallized from methanol + water mixtures at $a_w \leq 0.338$, TGA shows a one-step dehydration from 25°C to about 125°C (Fig. 2e), while the DSC plots are similar (Fig. 2d) and the powder X-ray diffraction patterns are almost identical (Fig. 2c).

When kept for 5 days in contact with methanol + water mixtures at 25°C over the whole range of water activity, ampicillin anhydrate remained in the crystal form initially added, since powder X-ray diffractometry of the solid samples showed no changes. The metastability of the anhydrate may be attributed to the low probability of nucleating the trihydrate, which requires three molecules of water with each ampicillin molecule. From kinetic considerations, the rate of solution phase transformation in the crystallization medium depends largely on the absolute and relative solubilities of the forms at that temperature. The higher the solubilities and the greater the difference in the solubilities of the two forms the greater will be the rate of transformation (Mullin, 1993). From Fig. 1, the low solubilities (0.01–0.03 M) and the small solubility difference (0.013 M) between the anhydrate and the trihydrate at 25°C in water may explain the low rate of conversion of ampicillin anhydrate to the thermodynamically

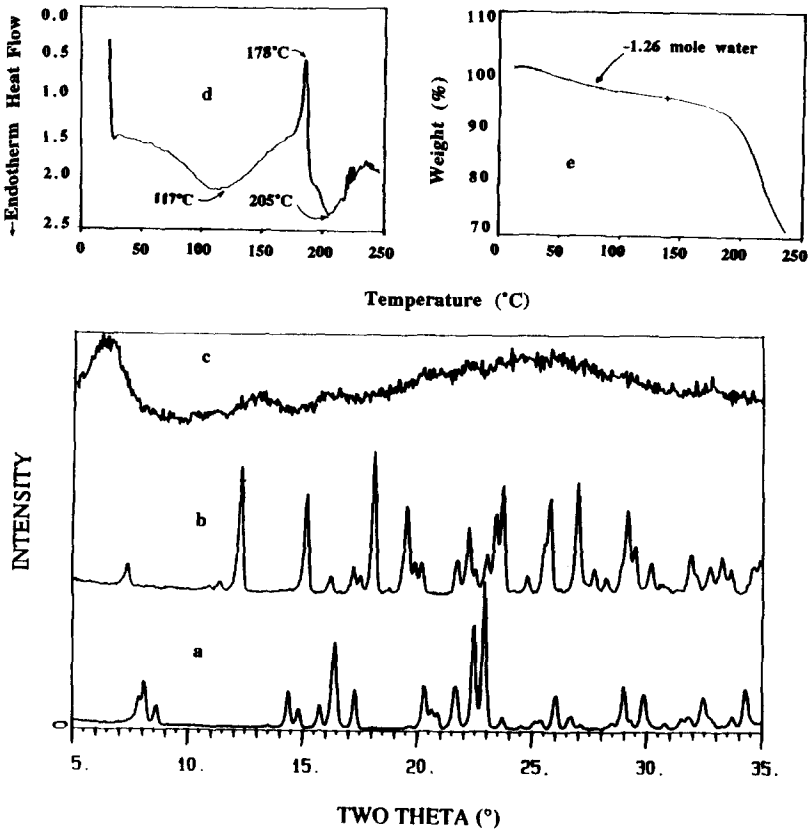

Fig. 2. Comparative powder X-ray diffraction (PXRD) and thermal analysis of the amorphous form of ampicillin: (a) PXRD of ampicillin anhydrate; (b) PXRD of ampicillin trihydrate; (c) PXRD of the amorphous form; (d) DSC curve of the amorphous form; (e) TGA thermogram of the amorphous form.

stable trihydrate, which, during the 5-day equilibration period, is evidently beyond the detection limit of powder X-ray diffractometry.

Seeding is used extensively to control both crystal form and the extent of nucleation (Byrn et al., 1994). Poole and Bahal (1968) have reported that seeding accelerates the phase transformation from ampicillin anhydrate to the trihydrate. In the present study, seeding with 5 mg of ampicillin trihydrate crystals in contact with methanol + water mixtures at $a_w \geq 0.381$ was found to result in the complete conversion of the excess anhydrate to the trihydrate. This conversion is shown in Fig. 3 in which the powder X-ray diffraction pattern changes from that of ampicillin anhydrate to the trihydrate.

The phase conversion of the anhydrate to the trihydrate was also confirmed by the decrease in the concentration of ampicillin at $a_w = 1.0$ and 0.862 in methanol + water mixtures (Fig. 4). The concentration-time profiles can be divided into three stages. In the first stage, the concentration remained approximately constant for 16 h, corresponding to an initial plateau. Dissolution of the anhydrate was very slow because the solution was only slightly undersaturated with respect to the more soluble anhydrate. In the second stage, the ampicillin concentration decreased corresponding to growth of the less soluble ampicillin trihydrate that was added as seeds. In this second stage, partial conversion is shown by the degree of the resemblance of the powder X-ray diffraction pattern (central patterns obtained 24 h and 72 h after

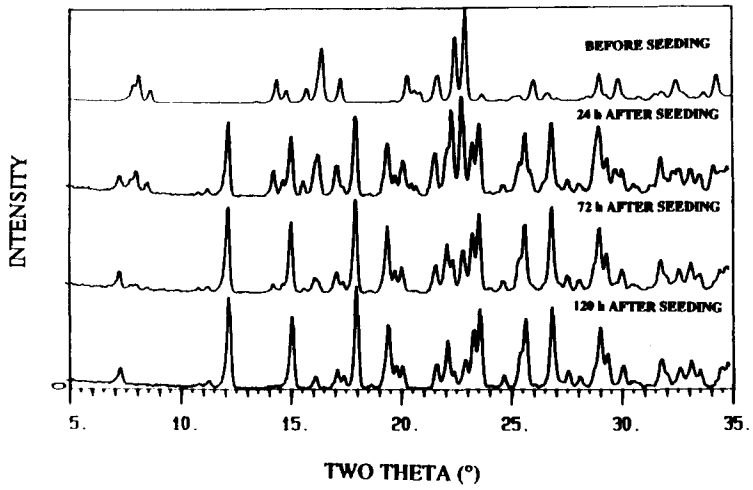

Fig. 3. Powder X-ray diffraction patterns showing the progress of the transformation of ampicillin anhydrate to the trihydrate in water (a_w = 1.0) at 25°C after seeding with the trihydrate.

seeding in Fig. 3) to those of the anhydrate and trihydrate. Dissolution of the anhydrate was then slower than recrystallization of the trihydrate. In the final stage, the trihydrate grew at a low supersaturation as the concentration approached the solubility of the trihydrate. The phase conversion appeared to be complete about 120 h after seeding, as confirmed by the lowest powder X-ray diffraction pattern in Fig. 3. However, the rate of conversion was faster at a_w = 1.0 than at a_w = 0.862, presumably because the thermodynamic driving force, namely the solubility of the anhydrate, was greater at the higher water activity (curve BC in Fig. 1). The anhydrate to trihydrate phase conversion with seeding, deduced above from the concentration profiles and powder X-ray diffraction patterns, may be a solvent-mediated process in which the anhydrate dissolves and creates the supersaturation for the growth and secondary nucleation of hydrate crystals. Rodriguez-Hornedo et al. (1992) reported a solvent-mediated phase transformation of theophylline anhydrate to the monohydrate with similar concentration-time profiles. For methanol + water mixtures at a_w = 0.338 saturated with the ampicillin anhydrate and seeded with trihydrate, no conversion was observed from either the powder X-ray diffraction pattern or concentration-time profiles (Fig. 4).

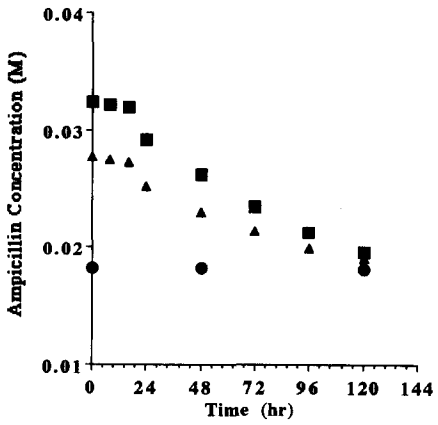

Fig. 4. Concentration-time profiles showing the conversion of the more-soluble ampicillin anhydrate to the less-soluble trihydrate at various water activities at 25°C after seeding with the trihydrate: (■) a_w = 1.0; (▲) a_w = 0.862. Curve (●) corresponds to a_w = 0.338 which is less than the equilibrium value for anhydrate ⇌ trihydrate and at which the anhydrate is stable.

The phase diagram in Fig. 5 shows the dependence of hydrate stoichiometry on water activity for ampicillin anhydrate in contact with methanol + water mixtures. Without seeding the anhydrate remained, whereas after seeding the anhydrate changed to the trihydrate at $a_w \geq 0.381$. When ampicillin trihydrate was placed in contact with methanol + water mixtures, the phase diagram

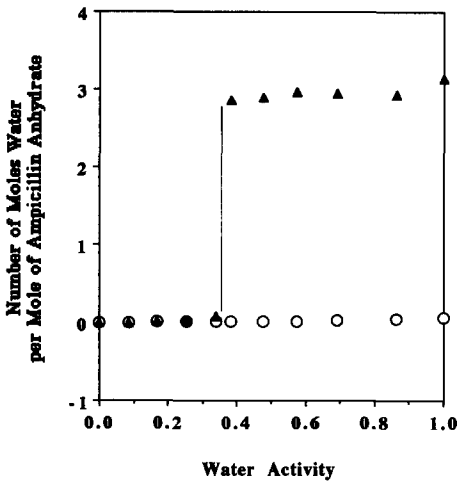

Fig. 5. Phase diagram at 25°C showing the dependence of hydrate stoichiometry on water activity in methanol + water mixtures with seeding (▲) and without seeding (○) with ampicillin trihydrate. Ampicillin anhydrate was the initial solid phase.

in Fig. 6 shows the dependence of hydrate stoichiometry on water activity. Without seeding, the trihydrate dehydrated to the amorphous form at $a_w \leq 0.338$. The water content of the amorphous form obtained increased progressively from 1.26 to 2.34 moles of water per mole of ampicillin anhydrate as a_w increased from 0 to 0.338. After seeding with the anhydrate, the amorphous form gradually changed to the anhydrate, but without seeding remained kinetically stable for 5 days. This observation indicates that equilibrium between the liquid mixture and the more stable form, anhydrate or hydrate, was readily achieved after seeding with the more stable form. These results demonstrate the validity of hypothesis (a) that water activity is the major factor determining the nature of the ampicillin phase obtained at equilibrium. Figs. 5 and 6 show that both solid phases are in equilibrium with the liquid mixture and, therefore, ampicillin anhydrate $\rightleftharpoons$ ampicillin trihydrate, at $0.338 < a_w < 0.381$. The lower value, $a_w = 0.34$, is taken to be the equilibrium water activity, while the stoichiometric number, $m = 3$, in Eqs 1 and 2. Substituting these values into Eq. 2 leads to $K_h = 25.9$. Zhu et al. (1996) have provided some additional values for comparison. The relative tendencies for transition from the anhydrate to the hydrate or from a lower hydrate to a higher hydrate are reflected in the relative values of K_h for any pharmaceutical compound. For thermodynamic reasons, these equilibrium constants apply in all liquid mixtures of organic solvent + water that are capable of providing these a_w values.

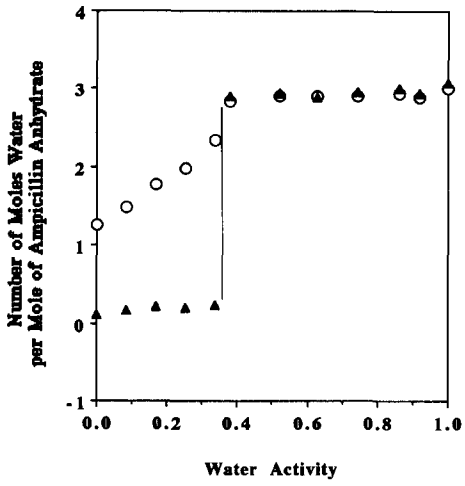

Fig. 6. Phase diagram at 25°C showing the dependence of hydrate stoichiometry on water activity in methanol + water mixtures with seeding (▲) and without seeding (○) with ampicillin anhydrate. Ampicillin trihydrate was the initial solid phase.

Table 1 shows the measured solubilities of ampicillin anhydrate and trihydrate in methanol ($a_w = 0$) and in water ($a_w = 1.0$) at 25.0°C and the nature of the final solid phase obtained without seeding. These data are expressed in mg/mL to facilitate comparison with those at 21°C reported by Marsh and Weiss (1967), who did not report the final solid phase obtained. The similarities between the corresponding values for the present work without seeding and those reported by Marsh and Weiss (1967) are notable and strongly suggest that the final phases obtained by Marsh and Weiss (1967), although not reported, correspond to those obtained in the present work. The small differences between corresponding values may be attributed to the small difference between the temperatures of measurement. When ampicillin anhydrate was equilibrated with water, the concentration of ampicillin 120 h after seeding

Table 1
Comparison of the solubility of ampicillin anhydrate and trihydrate in methanol and water

Initial solid phase	Present work Solubility (mg/mL) at 25.0 ± 0.1°C		Marsh and Weiss (1967) Solubility (mg/mL) at 21 ± 1°C	
	Methanol	Water	Methanol	Water
Ampicillin anhydrate	2.95	11.32	2.97	10.10
Final solid phase	Anhydrate	Anhydrate	Not reported	Not reported
Ampicillin trihydrate	6.75	7.85	6.65	7.56
Final solid phase	Amorphous form	Trihydrate	Not reported	Not reported

with the trihydrate (7.94 mg/mL from Fig. 4) corresponds to the solubility of the trihydrate in water (7.85 mg/mL from Table 1), indicating phase transformation of the anhydrate to the trihydrate after seeding. This phase transformation is confirmed by the change of the powder X-ray diffraction pattern from the anhydrate to the trihydrate shown in Fig. 3. The above results also suggest that characterization of the solid phase remaining after solubility studies is critical for the solubility determination of substances that are subjected to phase transformation.

When the measured solubility of ampicillin anhydrate was plotted against a_w in methanol + water mixtures, a nonlinear relationship was obtained (Fig. 1). The solubility increased from 0.00843 M to 0.0324 M with increase of a_w from 0 to 1. A reduced rate of increase was observed at $0.40 < a_w < 0.75$. Since powder X-ray diffraction of the remaining solid showed no phase transition over the whole range of a_w, the explanation for the lower rate of increase with a_w is still unknown. The measured solubility of ampicillin trihydrate in methanol + water mixtures increased gradually from 0.01674 to 0.01946 M (Fig. 1). Powder X-ray diffraction of the residual solid showed that the trihydrate changed to the amorphous form at $a_w \leq 0.338$. Between $0.338 < a_w < 0.381$ a phase transition occurs at which the amorphous form and the trihydrate are in metastable equilibrium. Similarly, Pfeiffer et al. (1970), in their studies of pseudopolymorphism of cephaloglycin and cephalexin crystallized in several solvent + water mixtures, observed a break in the solubility profile at the solvent composition at which one hydrate changes to another.

Bogardus (Bogardus, 1982; Bogardus, 1983) emphasized that knowledge of the crystal phase present at equilibrium is essential for the study of solubility. Thermodynamic consideration shows that the more stable solid phase of a compound has the lower chemical potential and hence the lower solubility in any solvent. Hence Fig. 1 shows that the anhydrate has the lower solubility and hence is more stable in methanol + water mixtures at $a_w < 0.3$ at which the solubility curves intersect, whereas the trihydrate has the lower solubility and, hence, is more stable in methanol + water mixtures at $0.381 \leq a_w < 1.0$, at which the anhydrate is metastable, all at 25°C. For kinetic reasons, the amorphous form exists in a metastable state at $a_w = 0.338$ (Fig. 1). The break at E between curves DE and EF reflects the fact that different solid phases were obtained, namely, the stable trihydrate (curve EF) or the metastable amorphous form (curve DE).

In order to compare the influence of a_w in the vapor phase with that in the aqueous phases (methanol + water mixtures), ampicillin anhydrate samples (1 g) were placed in sealed containers (desiccators) at various relative humidity (RH) values between 0% and 98%. After 6 months, the water content was measured by Karl Fischer titrimetry and the water uptake was found to increase with increasing RH, up to 0.4 mole of water per mole of ampicillin anhydrate at 98% RH. The residual solid samples were characterized by X-ray powder diffraction. The resulting diffractograms corresponded to the original anhydrous phase. After 6 months, ampicillin trihydrate seeds (20 mg) were sprinkled on each solid phase and the container was again sealed. After a fur-

ther period of 3 months, the powder X-ray diffractograms still corresponded to that of the anhydrate, indicating transformation in contact with the vapor phase is much slower than in aqueous solution (methanol + water) for kinetic reasons. Phase transformations from anhydrate to hydrate may proceed by one or both of two processes (Rodriguez-Hornedo et al., 1992): (i) a solid-solid transformation by which water molecules are incorporated into the crystal lattice as it remains in the solid state or (ii) a solvent-mediated process by which the anhydrous solid dissolves while the hydrate crystallizes from the resulting solution. A solvent-mediated process is possible if the solvent adjacent to the solid surface is supersaturated. Transition from ampicillin anhydrate to the trihydrate in methanol + water mixtures is probably solvent-mediated. In the vapor phase, the phase transition probably occurs via a solid-solid transformation. The differences between the products obtained, when a_w is controlled in the vapor phase and in water + methanol mixtures, are readily explained by a difference in the mechanism of water uptake.

Although the amorphous form is obtained when the trihydrate remains in contact with methanol + water mixtures at $a_w \leq 0.338$ for 5 days (Fig. 1), it is not obtained from the anhydrate under the variety of conditions studied (various a_w values in methanol + water mixtures or in the vapor phase). For this reason, the amorphous form is unlikely to be the intermediate transition form in the conversion of the anhydrate to the trihydrate and therefore a solvent-mediated process is proposed. Additional experiments, such as monitoring of particle size distribution, would be necessary (Rodriguez-Hornedo et al., 1992) to confirm the transformation mechanism.

To further investigate the metastability of the amorphous form in contact with the vapor phase, the water stoichiometry of the amorphous form was measured after exposure to various RH values between 0% and 98%. The amorphous form took up water progressively with increasing RH and transformed to the trihydrate after equilibration at 98% RH for 10 days. This result demonstrates that the amorphous form may undergo a solid-state transformation to the stable trihydrate while taking up significant amounts water. Similarly, Saleki-Gerhardt et al. (1995) reported that the amorphous form of raffinose crystallized at RH above 45% leading to the rapid restoration of the crystalline pentahydrate structure.

Because the trihydrate in contact with methanol + water mixtures at $a_w \leq 0.338$ for 5 days may be converted to the amorphous form (Fig. 1), and because the amorphous form may be converted either to the anhydrate after seeding or to the trihydrate under various conditions (e.g. $a_w \geq 0.98$), the amorphous form is proposed as a metastable transition form in the conversion of the trihydrate to the anhydrate.

4. Conclusions

The first hypothesis (a) that "when a drug, which can form a hydrate, is crystallized from mixtures of organic solvent + water, the water activity, a_w, in the mixtures is the major factor determining the nature of the anhydrate or hydrate phase that crystallizes" is essentially correct for ampicillin in water + methanol mixtures. The anhydrate is in equilibrium with the trihydrate at $0.338 < a_w < 0.381$ in each of these solvent mixtures. Saturation of water with ampicillin trihydrate reduces a_w by only 4%, confirming the second hypothesis (b) that "the dissolved drug in the aqueous solvent mixtures influences a_w to a much smaller extent than does the organic solvent".

Ampicillin anhydrate is thermodynamically stable at $a_w \leq 0.338$. Ampicillin anhydrate is kinetically stable when equilibrated for 5 days in methanol + water mixtures at any water activity. Seeding of ampicillin anhydrate with the trihydrate when in contact with methanol + water mixtures at $a_w > 0.381$ results in the conversion of anhydrate to the trihydrate. Ampicillin trihydrate dehydrates to the amorphous form at $a_w \leq 0.338$, but is thermodynamic stable at $a_w > 0.381$. The amorphous form of ampicillin takes up water progressively with increasing $a_w \leq 0.338$ in methanol + water mixtures but converts to anhydrate when seeded with the anhydrate.

The solubilities of the anhydrate and the trihydrate increase nonlinearly with increase of a_w in methanol + water mixtures. Ampicillin anhydrate, when in contact with the vapor phase for 6 months at 22°C at various RH values, each corresponding to a_w in the vapor phase, takes up water very slowly but progressively with increasing a_w from 0 to 0.98, reaching only 0.4 mole of water per mole of ampicillin at $a_w = 0.98$.

References

Austin, K.W.B., Marshall, A.C. and Smith, H., Crystalline modifications of ampicillin. *Nature*, 208 (1965) 999–1000.

Bacarella, A.L., Finch, A. and Grunwald, E., Vapor pressure and activities in the system dioxane-water. Second virial coefficients in the gaseous system nitrogen-dioxane-water. *J. Phys. Chem.*, 60 (1956) 573–576.

Bogardus, J.B., Unusual cholesterol solubility in water/glyceryl-1-monooctanoate solution. *J. Pharm. Sci.*, 71 (1982) 370–372.

Bogardus, J.B., Crystalline anhydrous-hydrate phase changes of caffeine and theophylline in solvent-water mixtures. *J. Pharm. Sci.*, 72 (1983) 837–838.

Brittain, H.G., Bugay, D.E., Bogdanowich, S.J. and DeVincentis, J., Spectral methods for determination of water. *Drug. Dev. Ind. Pharm.*, 14 (1988) 2029–2046.

Bundgaard, H., Spectrophotometric determination of ampicillin sodium in the presence of its degradation and polymerization products. *J. Pharm. Pharmacol.*, 26 (1974) 385–392.

Byrn, S.R., Pfeiffer, R.R., Stephenson, G., Grant, D.J.W. and Gleason, W.B., Solid state pharmaceutical chemistry. *Chem. Mater.*, 6 (1994) 1148–1158.

Chirikova, Z.P., Galata, L.A., Kotova, Z.N. and Kofman, L.S., Liquid-vapour equilibrium in the system acetonitrile-water. *Rus. J. Phys. Chem.*, 40 (1966) 493–496.

Doyle, F.P., Nayler, J.H.C., Smith, H. and Stove, E.R., Some novel acid-stable penicillins. *Nature*, 191 (1961) 1091–1092.

Gölles, F., Examination and calculation of thermodynamic data from experimental measurements. I. The numerical integration of the vapor-pressure curves of the system methanol-water. *Monatsh. Chem.*, 92 (1961) 981–991.

Gölles, F., Examination and calculation of thermodynamic data from experimental data. II. The numerical integration of the vapor-pressure curves of the system ethanol-water. *Monatsh. Chem.*, 93 (1962) 191–120.

Grant, D.J.W. and Higuchi, T., *Solubility behavior of organic compounds*, John Wiley and Sons, New York, 1990, pp. 38–39.

Grant, N.H. and Alburn, H.E., Peptide hydrate: influence of the hydration state on the properties of α-aminobenzylpenicillin. *Nature*, 207 (1965) 645–646.

Khankari, R.K. and Grant, D.J.W., Prediction of nedocromil sodium hydrate phases in isopropanol + water mixtures from the relative humidity phase diagram. *Pharm. Res.*, 10 (1993), No. 10, S-153 (PT 6106).

James, M.N.G. and Hall, D., Crystalline modifications of ampicillin I: the trihydrate. *Nature*, 220 (1968) 168–170.

Marsh, J.R. and Weiss, P.J., Solubility of antibiotics in twenty-six solvents. III. *J. A.O.A.C.* 50 (1967) 457–462.

Mullin, J.W., *Crystallization*, 3rd edition, Butterworth-Heinemann Ltd., Oxford, 1993.

Pfeiffer, R.R., Yan, K.S. and Tucker, M.A., Crystal pseudopolymorphism of cephaloglycin and cephalexin. *J. Pharm. Sci.*, 59 (1970) 1809–1814.

Poole, J.W. and Bahal, C.K., Dissolution behavior and solubility of anhydrous and trihydrate forms of ampicillin. *J. Pharm. Sci.*, 57 (1968) 1945–1948.

Rodriguez-Hornedo, N., Lechuga-Ballesteros, D. and Wu, H., Phase transtion and heterogenous/epitaxial nucleation of hydrated and hydrous theophylline crystals. *Int. J. Pharm.*, 85 (1992) 149–162.

Saleki-Gerhardt, A., Stowell, J.G., Byrn, S.R. and Zografi, G., Hydration and dehydration of crystalline and amorphous forms of raffinose. *J. Pharm. Sci.*, 84 (1995) 318–323.

Shefter, E. and Higuchi, T., Dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals. *J. Pharm. Sci.*, 52 (1963) 781–791.

Shefter, E., Fung, H. and Mok, O. Dehydration of crystalline theophylline monohydrate and ampicillin trihydrate. *J. Pharm. Sci.*, 62 (1973) 791–794.

Sokolova, E.P. and Morachevskii, A.G., Thermodynamic properties of the acetone-water system. *Vestn. Leningrad. Univ.*, 22(16), *Fiz. Khim.*, 3 (1967) 110–115.

Sylvestri, M.F. and Makoid, M.C., Stability of ampicillin trihydrate suspension in amber plastic oral syringes. *Am. J. Hosp. Pharm.*, 43 (1986) 1496–1498.

Udovenko, V.V. and Mazanko, T.F., Liquid-vapour equilibrium in the propan-2-ol-water and propan-2-ol-benzene system. *Rus. J. Phys. Chem.*, 41 (1967) 863–866.

Zhu, H., Yuen, C. and Grant, D.J.W., Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 1. Theophylline. *Int. J. Pharm.*, 135 (1996) 151–160.

Zhu, H. and Grant, D.J.W., Influence of water activity on the anhydrate and hydrate phases of ampicillin crystallized from methanol + water mixtures. *Pharm. Res.*, 12 (1995), No 9, S-196, PDD 7016.