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International Journal of Pharmaceutics 288 (2005) 245–252
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Influence of dehydration temperature on water vapor adsorption, dissolution behavior and surface property of ampicillin

Kunikazu Moribe[a], Arpansiree Wongmekiat[a], Yuki Hyakutake[a], Yuichi Tozuka[a], Toshio Oguchi[b], Keiji Yamamoto[a,*]

[a] *Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*
[b] *Department of Pharmacy, University Hospital, University of Yamanashi, 1110 Shimokato, Tamaho-cho, Nakakoma-gun, Yamanashi 409-3898, Japan*

Received 19 July 2004; received in revised form 2 October 2004; accepted 5 October 2004
Available online 30 November 2004

Abstract

Several specimens of anhydrous ampicillin were prepared by heating the ampicillin trihydrate at 100, 120, 140 and 160 °C. The effects of dehydration temperature on water vapor adsorption, dissolution behavior and surface property were investigated. The water vapor adsorption of anhydrous ampicillin was studied at 89% relative humidity, 40 °C and the water vapor adsorption rate was found to decrease with increase of dehydration temperature. Dissolution profiles of the various anhydrous specimens were investigated in 96% ethanol at 35 °C by the static disk method. The anhydrous form prepared at higher dehydration temperature exhibited faster dissolution rate. Solid phase transformation from the anhydrous form to the trihydrate form occurred during the dissolution test. The rate of phase transformation during the dissolution test decreased with increasing dehydration temperature. Topographic difference of the anhydrous forms prepared at 100 and 160 °C was not observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM); however, difference of the microstructural properties was apparently observed by the AFM phase image. Surface free energy study revealed that when ampicillin was dehydrated at high temperature, the sample surface became more hydrophobic resulting in less interaction force with water and slow water sorption rate. From the results, we concluded that the polarity of sample surface induced by dehydration of ampicillin would affect the phase transformation and dissolution behavior.

Keywords: Ampicillin; Dehydration temperature; Water vapor adsorption rate; Dissolution rate; Atomic force microscopy; Surface property

1. Introduction

Hydrates are molecular complexes that have water molecules incorporated into their crystal lattice (Haleblian, 1975) and are classified as

* Corresponding author. Tel.: +81 43 290 2938; fax: +81 43 290 2939.
E-mail address: yamamotk@p.chiba-u.ac.jp (K. Yamamoto).

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doi:10.1016/j.ijpharm.2004.10.001

pseudo-polymorph. Ampicillin, caffeine, theophylline, pranlukast and carbamazepine are well-known examples of pharmaceutical materials that exist in both hydrate and anhydrous forms. Hydrates can be formed when drug substances are contacted with or exposed to moisture during the drug manufacturing process, e.g., wet granulation, and storage. Since transformation between hydrate and anhydrous forms is usually reversible, hydrates can also transform to anhydrous form by heating or drying. The physicochemical and biological properties, e.g., chemical stability, solubility, dissolution rate and bioavailability of drug, are significantly different between the hydrate and anhydrous form (Khankari and Grant, 1995; Kobayashi et al., 2000). Recently, Reutzel-Edens et al. (2003) reported that stable anhydrous form of LY334370 HCl showed six times slower dissolution rate than dihydrate form, indicating that the characterization of solid-state properties is essential requirement for the pharmaceutical formulation development.

In the pharmaceutical manufacturing, the drying process is often performed in the final stage and the hydrate–anhydrate transformation could occur during the process. In addition, the physicochemical properties of drug substances are significantly influenced by the conditions of drying process. Although there are numerous researches studied on anhydrous and hydrate forms (Suihko et al., 1997; Kajiwara et al., 1999; Ito et al., 1997; Han et al., 1998; Rustichelli et al., 2000; Stephenson and Diseroad, 2000; Richards et al., 2002; Cooper et al., 2003; Kobayashi et al., 2003), the researches concerning the physicochemical properties of the anhydrous forms obtained under various heating conditions have not much been attempted (Ono et al., 2001, 2002; Airaksinen et al., 2004). In the present study, ampicillin was chosen as a model drug. Ampicillin is widely used as an antibiotic drug and is known to exist in both hydrate and anhydrous forms. There are two hydrate forms of ampicillin, which are monohydrate and trihydrate, while anhydrous ampicillin has three polymorphic forms, which are amorphous form, forms I and II (Shefter et al., 1973). The purpose of this study was to investigate the influence of dehydration temperature on water vapor adsorption, dissolution behavior and surface property of ampicillin anhydrous forms prepared by various dehydration temperatures.

2. Materials and methods

2.1. Materials

Ampicillin trihydrate was purchased from Sigma–Aldrich, Japan. Anhydrous forms of ampicillin were prepared by heating the ampicillin trihydrate under a vacuum at 100, 120, 140 and 160 °C for 4 h.

2.2. Powder X-ray diffraction (PXRD) measurement

Powder X-ray diffraction was carried out on a Rigaku Miniflex diffractometer (Tokyo, Japan). Measurements were performed at 30 kV voltage, 15 mA current, a scanning speed of $5^\circ\ \mathrm{min}^{-1}$, a Ni filter and a radiation source of Cu Kα.

2.3. Dissolution study by the static disk method

The dissolution profiles of the trihydrate and the anhydrous forms prepared by heating at various temperatures were measured in 96% ethanol at 35 °C by static disk method as described in the previous report (Ito et al., 1997). A disk of 10 mm diameter was prepared by compressing 300 mg of sample at 100 $\mathrm{kg/cm}^2$ for 1 min. The compressed disk was put in the thin metal columnar sinker of suitable size for that only one surface of the disk contacted with the dissolution media. The disk was placed at the bottom of the dissolution flask with in 500 ml of 96% ethanol at 35 °C and was rotated at 100 rpm with a paddle. Then, 5 ml of the solution was withdrawn at definite intervals and the concentration of ampicillin in the solution was measured by UV spectrophotometer (Shimadzu UV-160, Japan) at a wavelength of 346 nm. It was confirmed by PXRD analysis that no polymorphic transition took place during disk preparation for any sample.

To confirm the phase transformation from the anhydrous form to the trihydrate form occurring during the dissolution test, 300 mg of the anhydrous forms prepared at 100 and 160 °C was separately dispersed in 50 ml of 96% ethanol at 25 °C. Each suspension was stirred for 30 min by magnetic stirrer and passed through a membrane filter (0.8 μm). Powder X-ray diffraction measurement was performed to powder residue on the membrane filter.

2.4. Water vapor sorption behavior

To investigate the water vapor sorption behavior, the anhydrous forms were stored in a desiccator containing saturated aqueous solution of KNO_3 (89% relative humidity (RH)) at 40 °C. The weight changes of the samples were monitored for 48 h.

2.5. Scanning electron microscopy (SEM)

The surfaces of ampicillin trihydrate and anhydrous forms prepared by heating at 100 and 160 °C were observed by scanning electron microscope (Hitachi S-2150, Japan).

2.6. Atomic force microscopy (AFM)

Atomic force microscope (Seiko Instrument SPI4000/SPA400, Japan) was also used to observe the surfaces of the anhydrous forms prepared at 100 and 160 °C. The measurement was performed by the dynamic force mode and a stiff silicon cantilever was oscillated closer to the sample than in noncontact mode. The advantage of tapping the surface is improved lateral resolution on soft samples. Surface roughness was monitored by the angle of reflection of the laser from upper side of the cantilever using laser light detector. The AFM topography image, which reflects topographic features of the surface, was obtained from the amplitude change of the cantilever oscillation. The AFM phase image, which reflects the probe motion, was obtained from the phase shift relative to a driving oscillator. The phase difference of the sample surface was expressed in a contrast between light and shade.

2.7. Surface free energy calculated from wetting measurement

A wetting of the sample was measured using Tensiometer (Kruss K121, Germany) in terms of the liquid penetration rate by capillary method. The contact angle was calculated by Washburn equation using the penetration rate and the surface free energy was calculated by the following equation as reported by Chung et al. (2003).

$$\frac{1+\cos\theta}{2}\frac{\gamma_L}{\sqrt{\gamma_L^d}} = \sqrt{\gamma_s^p}\sqrt{\frac{\gamma_L^p}{\gamma_L^d}} + \sqrt{\gamma_s^d} \quad (1)$$

where γ_L is a surface free energy of the liquid (mJ/m^2), γ_S a surface free energy of the solid (mJ/m^2), θ a contact angle (degree), and the superscripts, d and p, show the dispersive and polar components of the surface free energy, respectively.

Particle size of the sample was in the range of 125–250 μm. Approximately 300 mg of sample was loaded in the sample holder and the measurement was performed after tapping 500 times. Solvents used in the measurement were *n*-hexane, carbon tetrachloride, toluene, 2-methoxyethanol and benzene.

3. Results and discussion

3.1. Characterization of anhydrous ampicillin prepared by heating at various temperatures

The PXRD patterns of ampicillin trihydrate and anhydrous form prepared at 100 °C are shown in Fig. 1. Ampicillin trihydrate showed characteristic crystalline peaks in the PXRD pattern. The anhydrous form prepared at 100 °C showed halo pattern, indicating that the sample was amorphous after dehydration. The PXRD patterns of the anhydrous forms prepared at 120, 140 and 160 °C were also halo patterns. All dehydrated samples were in amorphous state.

3.2. Dissolution behavior

The dissolution behavior of ampicillin trihydrate and its anhydrous forms prepared at various dehydration temperatures was investigated in 96% ethanol at 35 °C. Ethanol forms an azeotrope with water when its

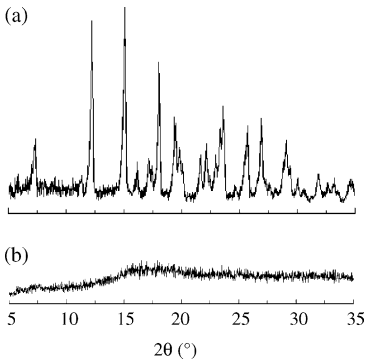

Fig. 1. Powder X-ray diffraction (PXRD) patterns of (a) ampicillin trihydrate and (b) ampicillin anhydrous form prepared at 100 °C.

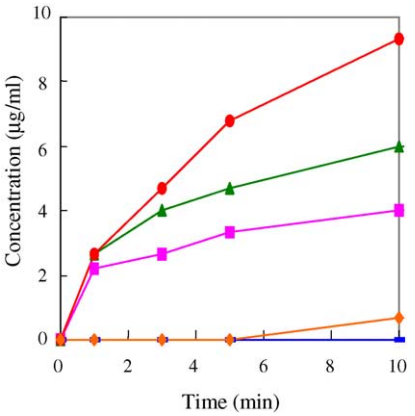

Fig. 2. Dissolution profiles of ampicillin trihydrate and anhydrous forms prepared at various temperatures in 96% ethanol at 35 °C: (-■-) hydrate, anhydrous forms prepared at (♦) 100 °C; (■) 120 °C; (▲) 140 °C and (●) 160 °C.

water content is 4.0% at 1 atm. The azeotrope retains the same composition in the vapor state as in the liquid state when distilled or partially evaporated under a certain pressure. To prevent compositional change of the solution, we used 96% ethanol as a dissolution media. Solubility of ampicillin trihydrate in water is 6 mg/ml at 25 °C.

As shown in Fig. 2, ampicillin trihydrate exhibited very poor dissolution in 96% ethanol, while all of the anhydrous forms showed faster dissolution than the trihydrate. The anhydrous form prepared at higher dehydration temperature exhibited a faster dissolution rate. To confirm the phase transformation from an anhydrous form to the trihydrate form during dissolution test, the anhydrous form prepared at 100 and 160 °C was separately dispersed in 96% ethanol and each sample was collected on a membrane filter (0.8 μm). Powder X-ray diffraction measurement was performed to the excess powder residue on the membrane filter and the results are shown in Fig. 3. The residue of the anhydrous form prepared at 100 °C showed similar PXRD pattern to that of the trihydrate, suggesting that the anhydrous form changed to the trihydrate form after dispersion in 96% ethanol. In case of the anhydrous form prepared at 160 °C, PXRD pattern exhibited that the residue after dispersed in ethanol was still remaining in an amorphous form. It was found that the anhydrous forms dehydrated at different temperatures had varied stability against crystallization. The rate of phase transformation was found to decrease with an increase in dehydration temperature. The difference in the phase transformation rate could lead to the difference in the dissolution rate of the anhydrous forms obtained. Phase transformation from the anhydrous form to the trihydrate form was further studied by water vapor adsorption investigation.

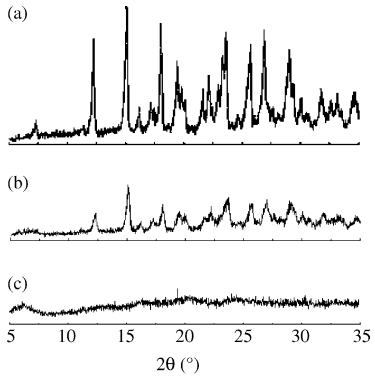

Fig. 3. PXRD patterns of (a) ampicillin trihydrate and anhydrous forms prepared at (b) 100 °C and (c) 160 °C after dispersed in 96% ethanol for 30 min at 25 °C.

3.3. Water vapor sorption behavior

The anhydrous forms prepared at various dehydration temperatures were stored in a desiccator at 89% RH, 40 °C. The isothermal hydration profiles of various ampicillin anhydrous forms are illustrated in Fig. 4. A broken line in the figure shows calculated water content of the trihydrate (water content = 13.4%). When

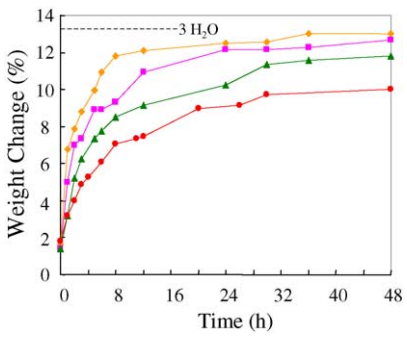

Fig. 4. Isothermal hydration profiles of ampicillin anhydrous forms prepared at various temperatures (condition: 89% RH at 40 °C). Dehydration temperature: (♦) 100 °C; (■) 120 °C; (▲) 140 °C and (●) 160 °C.

the anhydrous forms were kept at 89% RH, 40 °C, all of the anhydrous forms sorbed moisture and gradually transformed to the trihydrate form showing that percent weight change of all anhydrous forms increased with the storage time. The anhydrous form prepared at 100 °C transformed to the trihydrate after 36 h storage. On the other hand, the anhydrous form prepared at 160 °C exhibited a slow transformation rate, where complete transformation was observed after 7 days storage. It was found that water vapor sorption rate of anhydrous forms decreased with increasing dehydration temperature. We speculated that the difference in the surface properties of anhydrous ampicillin was an important factor causing the difference in the water vapor adsorption rate, the phase transformation rate and consequently the dissolution behavior of the anhydrous forms prepared at various dehydration temperatures.

3.4. SEM and AFM

Scanning electron microscope and atomic force microscope were used to observe the surfaces of ampicillin anhydrous forms prepared at different temperatures. In case of AFM, a progressive cantilever was employed to approach the surface of the sample. The cantilever was scanned through sample surface by feedback control system of atomic force signal between the sample surface and the cantilever. AFM has high resolution in an atomic molecular level; therefore, this method can be employed to observe the surface roughness that could not be observed by SEM. The AFM topography image reflects the topographic feature of the surface, while the phase image can be correlated with microstructural properties that effect the tip/sample interaction. Since the phase shift can be used to differentiate areas on a sample with such differing properties as adhesion and elasticity, the surface state could then be observed in detail by phase image.

Fig. 5 shows SEM image of the trihydrate and AFM images of the anhydrous form prepared at 100 °C. The AFM images of the anhydrous form prepared at 160 °C are shown in Fig. 6. The SEM image showed that ampicillin trihydrate crystals were in plate shape with the size around 5 μm; however, we could not obtain any

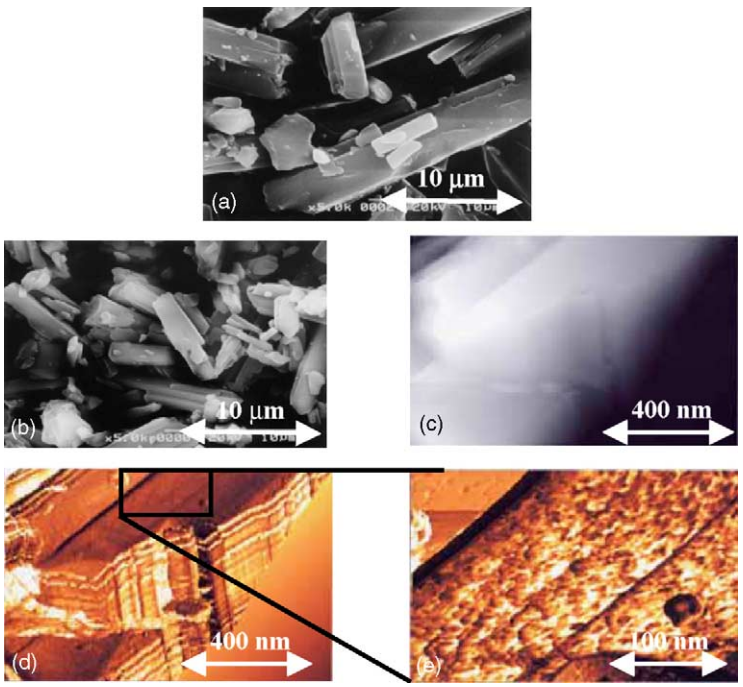

Fig. 5. Scanning electron microscope (SEM) image and atomic force microscopy (AFM) images of ampicillin trihydrate and anhydrous form prepared at 100 °C: (a) SEM of ampicillin trihydrate; (b) SEM of anhydrous form; (c) AFM topography image of anhydrous form; (d) AFM phase image of anhydrous form and (e) magnification of (d).

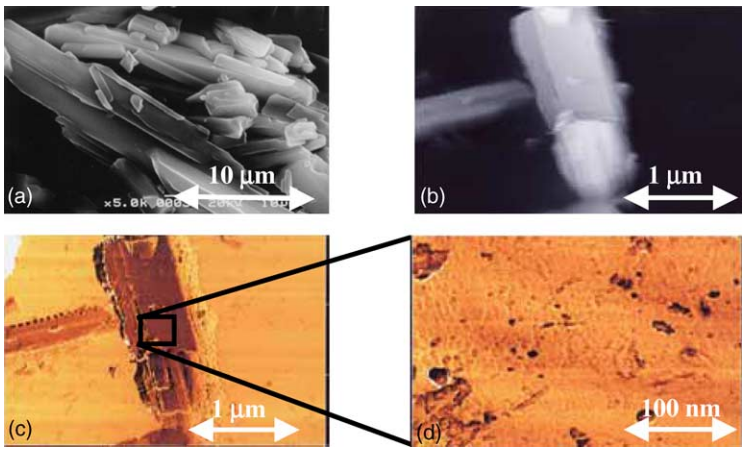

Fig. 6. Scanning electron microscope (SEM) image and atomic force microscopy (AFM) images of ampicillin anhydrous form prepared at 160 °C: (a) SEM; (b) AFM topography image; (c) AFM phase image and (d) magnification of (c).

information of the sample surface from the SEM images. The AFM topography images of the anhydrous forms did not provide much information as well. The different microstructural properties of the sample surface, however, could be observed in an AFM phase image. The magnification of the AFM phase image in Figs. 5 and 6 illustrated that the surface of the anhydrous form prepared at 100 °C showed coarse and rough contrast while that of the anhydrous form prepared at 160 °C was rather smooth. Difference of the microstructural properties would reflect on the different water sorption rate.

3.5. Surface free energy

Surface free energy of ampicillin anhydrous forms prepared at various temperatures is shown in Fig. 7. The surface free energy of the anhydrous form prepared at 100 °C was 30.8 mJ/m^2 while that of the anhydrous form prepared at 160 °C was only 20.9 mJ/m^2. It was obvious that the surface free energy of ampicillin anhydrous forms decreased when the dehydration temperature increased. The surface free energy can be divided into the polar and the dispersive components. The polar component represents interaction energy of the surface with water and includes hydrogen bonding. The dispersive component represents non-polar interaction of the surface and includes van der Waals attractive forces. The dispersive component of all anhydrous forms was rather constant in the range of 14.7–16.7 mJ/m^2. On the other hand, the polar component markedly decreased from 15.8 to 5.0 mJ/m^2 when the dehydration temperature increased from 100 to 160 °C. The ratio of polar component to the total surface free energy is expressed as percent polarity as shown in Fig. 7. The anhydrous form prepared at 100 °C showed high polarity of 51%, while the polarity of the anhydrous form dehydrated at 160 °C decreased to 24%. The above results showed that when ampicillin was dehydrated at higher temperature, the sample surface became more hydrophobic and therefore, resulting in less interaction force with water and slower water sorption rate.

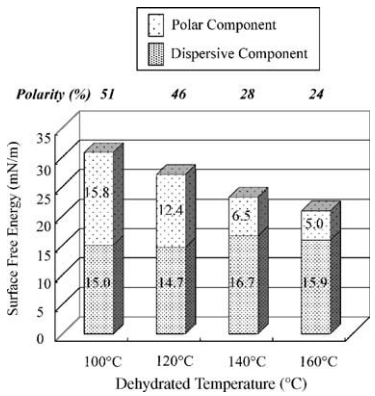

Fig. 7. Surface free energy of ampicillin anhydrous forms prepared at various temperatures.

The dehydration temperature clearly had a significant influence on the physicochemical properties of the hydrate and anhydrous forms. Airaksinen et al. (2004) investigated the effect of two drying methods, i.e., fluid bed and simulated tray drying methods on the relative amounts of different theophylline polymorphic forms remaining in dried granules. It was reported that a metastable anhydrous form of theophylline was predominantly formed after drying at 40–50 °C, while a stable theophylline anhydrous form was mainly produced when drying at the temperature above 50 °C. Our study revealed that dehydration of ampicillin trihydrate at different temperatures gave ampicillin anhydrous forms with varied physicochemical properties. We found that the anhydrous form prepared at higher dehydration temperature exhibited faster dissolution rate. Moreover, the anhydrous form prepared at higher dehydration temperature had less hydrophilic surface and it was assumed that the phase transition from anhydrous form to hydrate form during dissolution test was slow because of the less interaction with water. Hydrophilicity of the surface would be attributed to the existence of polar functional group on the surface. Accordingly, the anhydrous form prepared at higher dehydration temperature showed faster dissolution rate in 96% ethanol solution.

4. Conclusions

In this study, the water vapor adsorption, dissolution behavior and surface property of ampicillin anhydrous forms prepared at various dehydration temperatures were investigated. The anhydrous form prepared at higher dehydration temperature exhibited faster dissolution rate in 96% ethanol solution. Phase transformation from the anhydrous form to the trihydrate form occurred during dissolution test. The rate of phase transformation during the dissolution test decreased with higher dehydration temperature. The water vapor adsorption rate at 89% RH, 40 °C was also decreased when the dehydration temperature increased. Different microstructural properties of the ampicillin dehydrate seemed to significantly influence on the water adsorption rate. The surface of the anhydrous form prepared at higher temperature was more hydrophobic resulting in less interaction force with water and slower water adsorption rate.

Acknowledgements

The authors would like to thank Dr. Katsuhide Terada and Dr. Etsuo Yonemochi, Faculty of Pharmaceutical Sciences, Toho University, for the cooperation and kind advices in the surface free energy measurement. We are also thankful to Seiko Instrument Co., Ltd., Japan, for the measurement of atomic force microscopy.

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