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Interconversion Studies of Betamethasone Acetate Polymorphs

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ABSTRACT The polymorph interconversions of Betamethasone Acetate (BA) were studied under various pharmaceutical conditions, such as grinding, heating and suspending in water, based on differential scanning calorimetry, thermogravimetric analysis, and X-ray powder diffraction. There existed enantiotropic relationships between the three polymorphs of BA, which were named form II, Iα, and Iβ work, respectively. It was concluded that form II was the most stable form when suspended in water.

KEYWORDS Polymorphs, Betamethasone acetate, Transformation, Solubility, Enantiotropy

INTRODUCTION

The importance of polymorphism in drug development has been widely reported. It is shown that the physical stability and biological availability of drugs are greatly affected by their polymorphism and solvation. Among these study of polymorphs, the transformation between the polymorphs is a very complicated aspect in the development of a dosage form, since the presence of transformation during the processes or in the final product often leads to unconducted drug release or/and stability, especially in suspensions.

Due to use of a wrong polymorph of a drug in suspension, a phase conversion from the metastable to stable polymorph may occur. This produces crystal growth and caking which resulted in the decrease of the physical stability.

It is thus crucial to understand the mechanism and kinetics of phase transformation and the factors that may influence those in pharmaceutical manufacturing. In addition, right understanding of the thermodynamic stability relationships between the polymorphs is especially important which often aids in the proper selection of the desired drug crystalline modification.

Betamethasone acetate (BA) was selected as the drug for study since it was rather difficult in the formulation of suspension combined with betamethasone phosphate sodium due to the polymorphism. In our former study the physical stability of BA suspensions decreased during storage and it was observed that the crystal growth invariably occurred with subsequent sedimentation and caking. Associated crystal growth formulation problems include adverse changes in syringeability, dispersibility, and irritability to the

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patient upon administration. So, selection of the polymorphic form of BA to maintain the physical stability of the suspension becomes very important and has posed many problems to formulators.

Nurnberg and Seiller reported that BA had three polymorphic forms (form II, Iα, Iβ) and a hydrate (Nurnberg & Seiller, 1988). They studied the interconversions between the polymorphs under grinding and heating conditions (Nurnberg & Seiller, 1988). However, they didn't find a suitable method to produce the pure form II. In our previous study, we successfully obtained the pure form II (Ke et al., 2003). Furthermore, the transformation relationships between polymorphs of BA were studied in our present work which was a little different from the result of Nurnberg and Seiller (1988). Especially, the polymorphic transition in water was studied, too. It is helpful to the BA suspension formulation combined betamethasone phosphate sodium.

This work has the following objectives: 1) to study the transformations between the polymorphs of BA under various pharmaceutical conditions, such as grinding, heating, and reacting with water; 2) to determine the free energy relationships (i.e., enantiotropy or monotropy) between the polymorphs. These objectives lead to a suitable choice of crystal form for BA suspension formulation.

MATERIALS

Betamethasone acetate (BA) was purchased from Shanghai Hualian Pharmaceutics Co. Ltd.; all the reagents were of analytical grade.

METHODS

Preparation and Polymorph Characterization of Samples

According to the result of Nurnberg and Seiller (1988), there were three anhydrous phases that were designated form II, Iα, Iβ and a hydrate with one water molecule. The preparation methods were reported in our previous paper (Ke et al., 2003). Briefly, the form II was prepared by slow cooling of a hot, saturated acetone solution; the form Iα was obtained by heating the acetone solution at 70°C till the solid produced; the hydrates were derived from the form Iα upon grinding with water at room temperature for 1 h; and the form Iβ was the transformation of the hydrate when heated in an oven maintained at 160°C for 15 min.

The melting point of the form II, Iα, and Iβ was 150–156°C, 210–212°C, 222–224°C, respectively. The hydrate with one water molecule in the lattice lost the 3.97% weight between 60–100°C according to the TGA profile.

Equilibrium Solubility Determination

The equilibrium solubility of different forms of BA in distilled water were determined in the temperature range of 30 to 44°C. Excess amount of BA crystals were added to 100 ml distilled water, the suspension was equilibrated with shaking at 80 strokes/min at the designated temperature (±0.2°C) in a shaking water bath (CHZ82, Changzhou Guohua Scientific Company, Changzhou, China). Aliquots were drawn and filtered through 0.45-um filters at designated times and assayed by UV spectrometer (UV-9200, Beijing Ruili Analytical Instrument Corp., Beijing, China) at the wavelength of 254 nm. Equilibrium solubility was judged to have been attained when the variation of three consecutive readings did not differ by >4%.

No change in the polymorphic identity, determined by X-ray powder diffraction (XRPD), was observed in the solid phases recovered from the solubility study.

Phase Transformation Studies

Grinding

Samples of 0.5 g of each polymorph were subjected to the ball-milling in an agate centrifugal mill (QM—3P, Nanjing University Instrument Factory, Nanjing, China) and were withdrawn at the time of 30, 60, and 120 min for detection. Samples of 0.1 g of each polymorph with 20 ml distilled water were ground for 60 min. The mixture was filtered and dried at 40°C under vacuum.

Heating

The samples of each polymorph were placed and heated at different temperatures for various time intervals, after cooling the samples were detected by differential scanning calorimeter (DSC) or thermogravimetric analysis (TG).

Suspension in Water

The suspensions were prepared by suspending each polymorph powder in distilled water and stirring for some time. The suspension was maintained at the fixed temperature in the water bath. A portion of the suspension was taken and isolated by filtration and vacuum drying (1–2 mm Hg, at 40°C) at intervals.

X-Ray Powder Diffraction

X-ray powder diffraction (XRPD) diffractograms were recorded for BA polymorphs on an automated diffractometer using CuKα radiation and a scan speed of 5°/min (MO3XHF$_{22}$, MAC Science, Japan). The powder was ground in a ball mill for 5 min using a sample holder and ball made of agate. The samples were screened through a 250-μm sieve before being packed into standard aluminum sample holders. This eliminated the preferred orientation of crystals during analysis. The powder patterns were recorded under the following conditions: 40 kV; 40 mA; range 4°–40° (2θ).

Thermal Analysis

The thermal behavior was studied by heating ±3 mg of the powdered samples at the rate of 5°C/min from 30°C to 250°C with a differential scanning calorimeter (DSC204, Netzsch Company, Germany). Thermogravimetric analysis (TGA) thermograms were recorded with a Netzsch TG system(TG209, Netzsch Company, Germany). Mass loss from ±10 mg samples at the heating rate of 10°C/min under the nitrogen purge was recorded.

RESULTS AND DISCUSSION

Solubility

The apparent solubility of BA polymorphs determined at 30–44°C was shown in Table 1.

TABLE 1 **The Solubility (μg/ml) of BA Polymorphs in Distilled Water**

Crystal forms	30°C	37°C	44°C
Hydrate	26.83±2.40	31.56±2.08	35.01±2.54
Form II	37.27±2.69	49.24±5.40	58.86±5.20
Form Iα	46.80±3.48	56.44±4.57	70.66±4.13
Form Iβ	52.26±4.24	67.25±4.34	83.96±4.06

Each value represents the mean±S.E. ($n=3$).

The solubility of the different crystal forms of BA increased with the temperature. The solubility of the hydrate was lower than that of the other crystal forms, and the form Iβ was the most soluble crystal forms.

Transformation During Milling

The X-ray diffraction patterns of crystallined and ground BA samples were detected. The broader crystalline peaks indicated that the crystallinity of three forms all decreased with time during the grinding and gradually turned to the amorphous state. The form Iβ was completely transferred to amorphous phase after 2 h, while the form Iα and form II were partly transformed to the amorphous phase. No interconversion between three forms was found during grinding. During grinding with water, the form Iα and Iβ transformed to the hydrate, while the form II was very stable without any change even when ground with water for 2 h.

Temperature-Induced Transformation

The polymorphs of BA showed the different thermal behavior. The form II completely converted to the form Iβ when heated at 160°C for 15 min. The form Iα at 95°C for 7 days transformed to the form Iβ partially, but the same treatment did not cause any change of the form Iβ.

The conversion of the hydrate was related with the temperature, which was shown in Fig. 1. When the hydrate was kept at 40°C for 72 h under vacuum, it didn't change, which can be deduced from the TG diagram (Fig. 1a), 3.97% weight loss was just the water content of the hydrate; when the hydrate was kept at 60°C for 48 h, it lost the part of water, because the weight loss in Fig. 1b was only 1.11%, which was below the normal value (3.97%); when the hydrate was kept at 105°C for 0.5 h, it converted the amorphous phase, which behaved a broad peak at 122.0°C in Fig. 1c (Ke et al., 2003); when the hydrate was kept at 150°C for 0.5 h, it completely transformed to the form Iβ, which can be inferred from only one sharp peak at 223°C in Fig. 1d. So, we concluded that the stability of the hydrate was related with the temperature; it was stable at room temperature, but gradually lost water above 60°C and converted to the amorphous phase, which could be

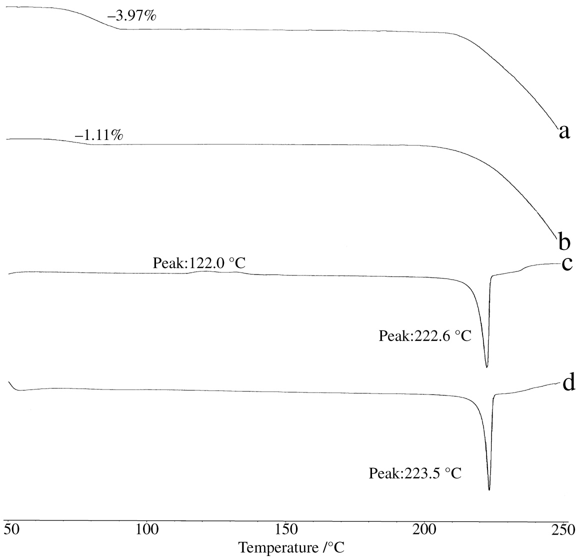

FIGURE 1 Thermal Diagrams After Hydrate Heated at Some Temperature for Some Time. a. 40°C, 72 hour, b. 60°C, 48 hour, c. 105°C, 0.5 hour, d. 150°C, 0.5 hour. The Upper Two Curves are TG Diagrams, and the Lower Two Curves are DSC Diagrams.

transformed to the form Iβ with continuing heating. These transformations were also proved by XRPD.

According to the above results, it seemed that the form Iβ was the most stable phase at higher temperature.

Transformation When Suspended in Water

The results of DSC showed that the form II was stable without any change when suspended in water at different temperatures. XRPD gave further evidence.

The transformation of the form Iα suspended in water at 100°C can be viewed from the change of characteristic peak intensity of XRPD in Fig. 2. We chose the unique characteristic peaks of $2\theta = 12.64° \pm 0.06°$ and $2\theta = 16.96° \pm 0.04°$ as the representative of the form Iα and II. With time, the peak intensity of the form Iα decreased, while the form II gradually increased. It showed the form Iα turned to the form II when suspended in water. Another noticeable phenomenon was the content of the form II showed a sharp increase at about 6 h. It seemed that the transformation was restricted by the nucleation rate of the form II. Once the crystal seeds of the form II appeared, the transformation rate of the form Iα would be accelerated. This phenomenon suggests that, at the initial stage of the conversion, the formation of the form II nuclei is very slow, but will be accelerated by the formed nuclei themselves.

The form Iβ suspended in water changed to the form Iα and II according to the results of XRPD (the characteristic peak of the form Iβ is $2\theta = 13.42° \pm 0.06°$). Figure 3 shows the form Iβ transferred to the form Iα and II after 1 h in water at 80°C. Afterwards, the peak intensities of the form Iβ and Iα gradually decreased, while those of form II increased. According to the above solubility test, the form Iβ has the highest solubility and the form II has the lowest solubility. Theoretically, the form Iβ will directly undergo gradual dissolution-recrystallization to the form II in water due to the difference of solubility. However, the form Iα was found at the initial period of transformation. This phenomenon can be explained by Ostwald's step rule (Bauer et al., 2001; Grant, 1999). In all processes, it is not the most stable state which is initially formed, but the least stable state lying nearest in free energy to the original state. From the solubility results, the form Iα was less stable and nearer to the form Iβ in free energy, so the form Iα was formed first with subsequent transformation to the form II.

Another noticeable phenomenon was that the form II in the initial period of transformation must come from the form Iβ, while not from Iα. This can be concluded with the comparison of Fig. 2 and Fig. 3. It suggested that the form Iβ converted to the form II and Iα at the same time when suspended in water. With the further transformation of the form Iα to the form II, the final crystal must be the form II due to its lower solubility.

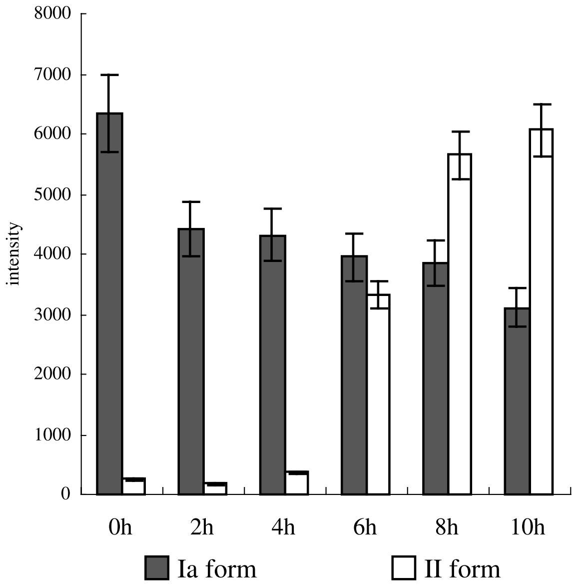

FIGURE 2 XRPD Result of Form Iα Suspended in Water at 100°C. Each Value is the Mean±S.E. of Three Determinations.

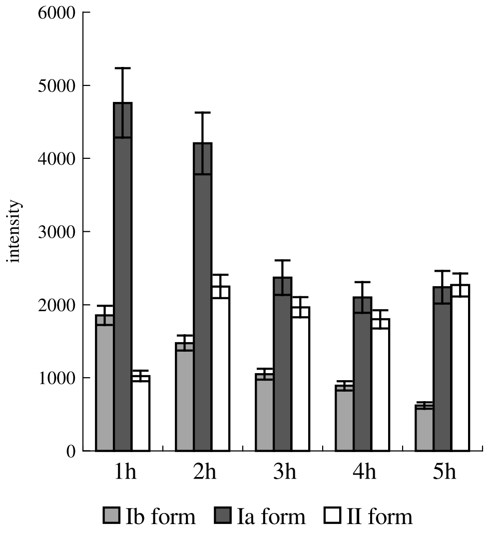

FIGURE 3 XRPD Result of the Form Iβ Transformation When Suspended in Water at 80°C. Each Value is The Mean ± S.E. of Three Determinations.

The result of DSC showed that the hydrate was stable without any change when suspended in water at 60°C for 3 h. But when temperature was above 60°C, even in water, the hydrate was transferred to the form II. It showed the temperature was an important factor that affected the stability of the hydrate in which the water molecules were combined with the BA molecules.

According to the above results, it can be concluded that the form II is the most stable form in water without any transformation while the form Iα and Iβ were unstable in water. They can change to the form II. The temperature affected the stability of the hydrate in water. Below 60°C the hydrate was stable, or it would transfer to the form II. The interconversion relationship of different forms of BA was shown in Fig. 4.

The interconversion relationships of BA as studied above are a little different from the results of Nurnberg and Seiller (1988). According to the report of Nurnberg and Seiller, the form Iα would convert to Iβ at the initial stage of dry-grinding and the form Iβ with the following crystallinity decreased would turn to the amorphous phase. The transition of the form Iα to the form Iβ in dry-grinding was not detected in our XRPD study. Their hydrate was prepared by suspending the form Iβ in water and the hydrate in our study can only be prepared when grinding the form Iβ with water.

Stability Relationships Between the BA Polymorphs

Burger and Ramberger have established useful thermodynamic rules for determining whether a system is enantiotropic or monotropic based on the

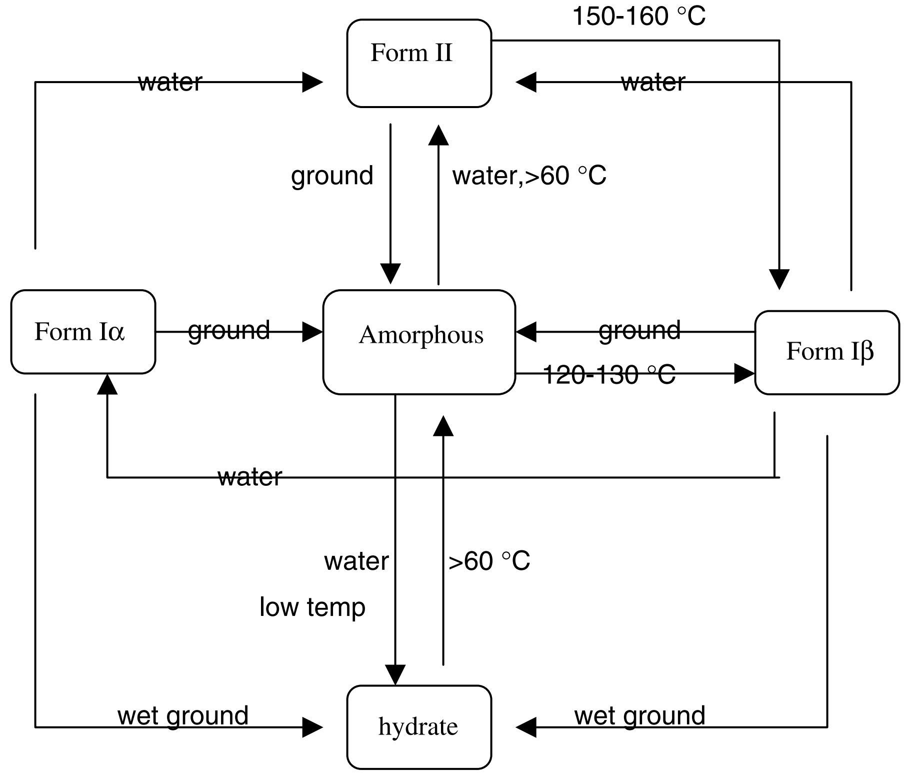

FIGURE 4 Phase Interconversion of Various Polymorphs of BA.

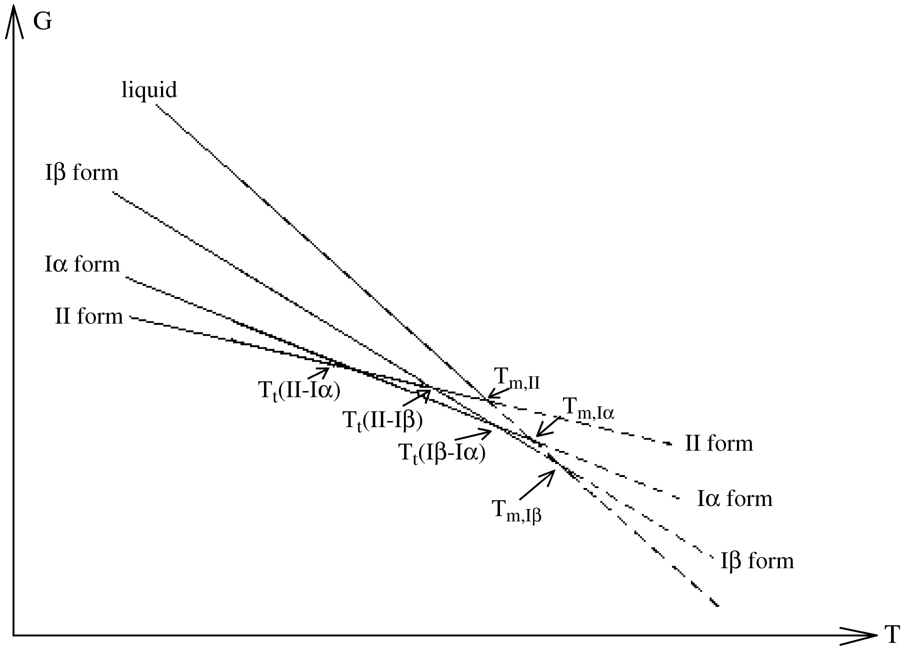

FIGURE 5 Schematic Free Energy Versus Temperature Diagram of BA Polymorphs (Form II, Form Iα and Form Iβ). T_m: Melting Point, T_t: Transition Temperature.

thermodynamic measurements (Burger & Ramberger, 1979a). They further concluded there was a relationship between thermodynamic stability and solubility of two modifications. That is, if that modification which has the higher melting point (Tf) is the less stable or the more easily soluble at a certain temperature (T0), there must be a transition point between these temperatures, and the two forms are enantiotropic (Burger & Ramberger, 1979b).

According to the rule and the solubility data, the three polymorphs of BA exhibit enantiotropic relationships. Based on these analyses, a schematic free energy versus temperature diagram was made in Fig. 5. In this figure, the transition temperature is estimated according to the interconversion relationships.

CONCLUSIONS

In this article, we studied the interconversions between the different polymorphs of BA. Comparing to the studies reported by Seiller and Nürnberg (1990), we not only successfully obtained the pure form II, but also developed the transformation methodology on grinding, heating, and suspending in the water. As learned from the Nurnberg's article, the hydrate of BA was the proper form for the suspension. However, our current investigation strongly recommended the form II to be used in pharmaceutical preparations, especially in suspension formulation.

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