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RESEARCH PAPER

Physicochemical Characterization of Glybuzole Polymorphs and Their Pharmaceutical Properties

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ABSTRACT

Systematic polymorphic screening tests were performed using 11 kinds of solvents and 6 kinds of preparation methods, and the three specific modifications of glybuzole (forms I and II and amorphous form) were identified by X-ray diffractometry and differential thermal analysis (DTA). The physicochemical properties of forms I and II and amorphous forms were measured using X-ray diffractometry, differential scanning calorimetry (DSC), thermogravimetry (TG), scanning electron microscopy (SEM), solubility tests, and others. The solubilities of all modifications in JP XII, first and second fluid (pH 1.2 and 6.8, respectively) were evaluated at 37°C. Forms I and II and the amorphous form showed almost equivalent solubilities. Forms I and II were stable polymorphic forms at 0% and 75% relative humidity (RH), respectively, at 40°C for 2 months, but the amorphous form was not stable. The crystallization rates of the amorphous form at 0% and 75% RH at 40°C were estimated by X-ray diffraction analysis based on the Jander equation, and the rate at 0% RH was 364 times slower than that at 75% RH.

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INTRODUCTION

Since validation studies are of primary importance in industrial manufacturing to ensure the quality of practical pharmaceutical preparations (1), pharmaceutical properties such as average particle size, crystalline form, crystal habit, and dissolution rate of bulk drug and excipient powders must be checked during the manufacturing process. However, metastable crystalline solids such as polymorphic, hydrated, and amorphous forms eventually undergo transformation into stable crystalline solids. Therefore, the choice of crystalline form of the bulk powder for pharmaceutical preparations, especially drugs insoluble in water, is important because the changes in the physicochemical properties of bulk powders of drugs during storage affect the bioavailability of preparations through their effects on the dissolution rate. There have been many reports (2–4) concerning the stability of polymorphic forms of the bulk powders of pharmaceutical preparations under conditions of high humidity and temperature because their transformations are accelerated under these conditions.

Glybuzole is widely used as an antidiabetic drug, but there have been no reports concerning polymorphic or amorphous forms of this agent. Therefore, its polymorphic forms were investigated using various preparation methods, such as recrystallization, freeze-drying, and/or evaporation, and the physicochemical stability of each form was examined under high humidity conditions to provide basic information for a preformulation study.

MATERIALS AND METHODS

Materials

Bulk glybuzole powder was obtained from Kyowa Hakkou Kogyou Company, Tokyo, Japan. All other chemicals were analytical grade.

Screening Test for Polymorphic Forms of Glybuzole

After systematic polymorphic screening tests using 11 kinds of solvents and 6 kinds of preparation methods (discussed below), three specific modifications of glybuzole (forms I and II and an amorphous form) were identified by powder X-ray diffractometry, differential thermal analysis (DTA), and thermogravimetry (TG); the results are summarized in Table 1. Based on the fact that the proton nuclear magnetic resonance (NMR) spectrum of the amorphous form was identical to that of the bulk powder, it is suggested that no impurities were mingled with the amorphous form after heating the sample.

Table 1

Methods of Recrystallization and Resultant Crystal Forms

Methods and Solvents	Crystal Form
Recrystallization I	
Ethanol, *n*-propanol, *n*-isobutanol, chloroform, acetone	Form I
Methanol, benzene	Form II
Toluene	Forms I + II
Recrystallization II	
Methanol, toluene	Form I
Ethanol, *n*-propanol, *n*-butanol, chloroform, acetone	Form II
Recrystallization III	
Methanol, ethanol, *n*-propanol, acetone	Form I
Recrystallization IV	
Methanol, ethanol, *n*-propanol, *n*-butanol, isobutanol, acetone	Form I
Freeze-drying	
Benzene	Form I
Dioxane	Form II
Heating	
Heated at 190°C for 30 min	Amorphous form

1. Recrystallization I: A hot saturated solution of the drug was allowed to stand at room temperature. The separated crystals were then filtered off and dried in vacuo at room temperature for 24 hr.
2. Recrystallization II: A hot saturated solution of the drug was allowed to stand at 5°C. The separated crystals were then filtered off and dried in vacuo at room temperature for 24 hr.
3. Recrystallization III: A saturated solution of the drug was dropped into distilled water at room temperature. The separated crystals were then filtered and dried in vacuo at room temperature for 24 hr.
4. Recrystallization IV: A saturated solution of the drug was poured into distilled water and stirred for 1 hr at room temperature. The separated crystals were then filtered and dried in vacuo at room temperature for 24 hr.
5. Freeze-drying: A dioxane solution of the drug (1% w/v) was lyophilized using an Eyela freeze dryer (model FD-5, Tokyo Rikakikai Co., Tokyo, Japan).

6. Heating: The bulk powder was heated at 190°C for 30 min. Samples of each modification were prepared for characterization by the following methods:

Form I: A hot saturated ethanolic solution of the drug was allowed to stand at room temperature. The separated crystals were then filtered and dried in vacuo at room temperature for 24 hr.

Form II: A hot saturated ethanolic solution of the drug was allowed to stand at 0°C. The separated crystals were then filtered off and dried in vacuo at room temperature for 24 hr.

Amorphous form: After the bulk powder had been heated at 190°C for 30 min, the molten product was cooled in the atmosphere for 3 hr to room temperature. The melt product was then ground by agate pestle and mortar.

X-Ray Powder Diffraction Analysis

Diffractograms were measured at room temperature with an X-ray diffractometer (XD-3A, Shimadzu Co., Kyoto, Japan). The operating conditions were as follows: target, copper; filter, nickel; voltage, 30 kV; current, 5 mA; receiving slit, 0.1 mm; time constant, 1 sec; counting range, 2000 counts per second; and scanning speed, 4°2θ/min.

Thermal Analysis

The DTA and TG were performed in open pans using DTG-30 and TG-30 instruments (Shimadzu Co.) at a sensitivity of 25 mV and ±5 mg, respectively. The DSC was performed using a closed-pan system with a Type 3100 instrument (Mac Science Co., Tokyo). The heating rates for DTA were 10°C, 20°C, 30°C, and 50°C per minute, and those for TG and DSC were 10°C/min and 20°C/min, respectively.

Measurement of the Crystal Content

Known amounts of standard mixtures were obtained by physically mixing form I with the amorphous form with a spatula in a glass beaker with a 2-cm inner diameter. The sample powder was loaded with a spatula into a glass holder and held gently by a glass plate to minimize preferred orientation of the crystals. The calibration curves for measuring the crystal content of form I were obtained based on the area intensity of the X-ray diffraction peaks at 8.7° (2θ) attributable to form I with 100% crystallinity. The plots showed a good linear correlation and were always reproducible over three standard samples. Each crystallinity value was reproducible within 5% error and was taken as an average of two independent sample measurements.

Nuclear Magnetic Resonance Spectroscopy

The proton NMR spectra of an approximately 3% solution of the sample in fully deuterated acetone were recorded at 200 MHz (model XL-200, Varian). The proton NMR spectra of samples were compared with that of the bulk powder. No impurity was detected in the sample modifications.

Physicochemical Stability

Samples were spread over a glass plate for powder X-ray diffraction analysis, then stored at 40°C at 0% and 75% relative humidity (RH) (various saturated salt solutions) in desiccators. The samples were removed from the desiccators at various times for X-ray powder diffraction analysis. The amount of the polymorphic form transformed was determined by X-ray powder diffraction as described above.

Solubility Determination

The drug concentration profiles of three of the modifications were investigated in JP XII fluids, first (pH 1.2) and second (pH 6.8). The sample powder containing 1 g glybuzole was introduced into 100 ml of dissolution medium at 37°C ± 0.5°C. The test solution was stirred with a paddle (JP XII) at 200 rpm. Aliquots (2 ml) of the solution were withdrawn through an 0.8-mm membrane filter at appropriate time intervals using a syringe and were suitably diluted with dissolution medium. Drug concentrations were determined at 272 nm in an ultraviolet (UV) spectrophotometer. The drug concentration was reproducible within 5%, and each stated value was an average of two independent measurements. The solubility was evaluated from the mean drug concentrations at the plateau of the drug concentration-time profile 2 hr after contact between the solid and the solution medium.

Scanning Electron Microscopy

The samples were coated with gold in an ionsputter JFC-1100, (Jeol Datum Co., Tokyo, Japan). The scanning electron microscopy (SEM) photographs at a magnification of 750X were taken with a model JSM-5200LV

scanning electron microscopy (Jeol Datum Co., Tokyo, Japan).

RESULTS

Powder X-Ray Diffraction Pattern of the Solid of Modifications

Figure 1 shows the powder X-ray diffraction profiles of three modifications. The characteristic diffraction peaks of form I were 8.7°, 11.7°, and 17.5° (2θ), and those of form II were 7.9°, 8.5°, and 25.7° (2θ). In contrast, the amorphous form had no diffraction peak and showed a halo pattern.

Thermal Behavior of the Solid Modifications

Figure 2 shows the DSC of the modifications. Form I gave an endothermic peak at 167.4°C due to melting and showed no weight loss on the TG curve, suggesting a stable form at this temperature. Form II showed an endothermic phenomenon at 116.8°C, an endothermic peak at 166.6°C due to melting, and no weight loss on the TG

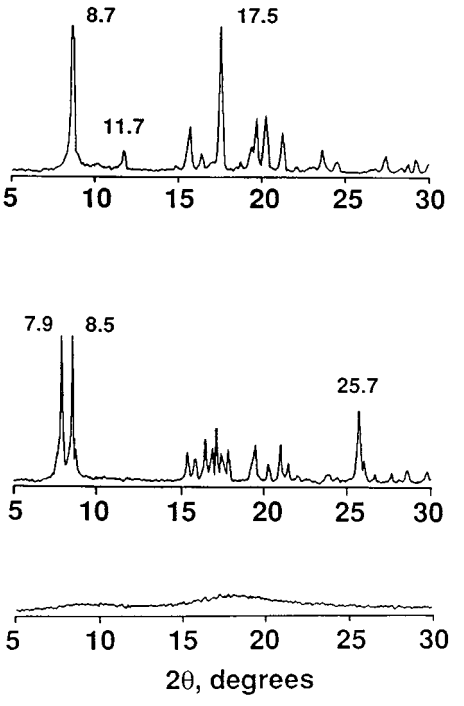

Figure 1. Powder X-ray diffraction profiles of three kinds of glybuzole modifications. Upper is form I, middle is form II, and lower is the amorphous form.

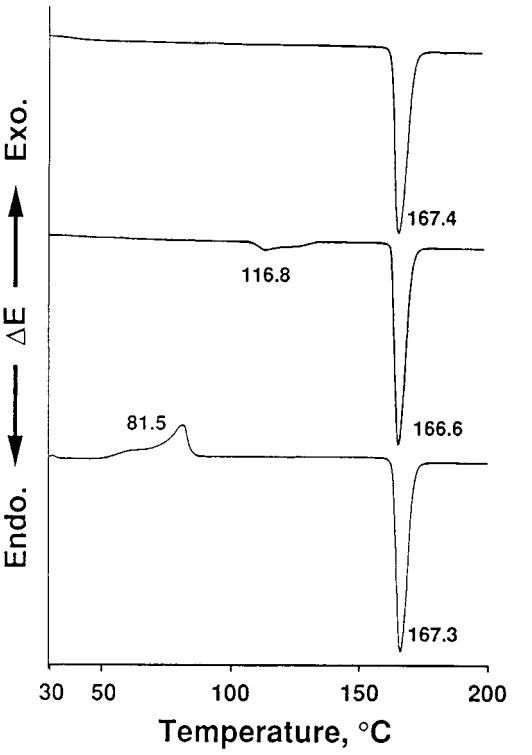

Figure 2. Differential scanning calorimetric curves of glybuzole modifications. Upper is form I, middle is form II, and lower is the amorphous form.

curve. The X-ray diffraction profile of form II after heating at 140°C for 5 min was identical to that of form I.

On the other hand, the amorphous form showed an exothermic peak at 81.5°C, suggesting a crystallization process, and an endothermic peak at 167.3°C due to melting, and no weight loss on the TG curve. The X-ray diffraction pattern of the solid obtained after heating the amorphous form at 100°C for 5 min was identical to that of form I, indicating that the exothermic peak at 81.5°C was due to crystallization of form I.

Morphological Characterization of the Solid Modifications

Figure 3 shows SEM photographs of the modifications. Distinct morphological differences were evident among these samples. Form I particles were larger platy crystals about 100 mm long. Form II consisted of aggregated particles with primary particles that were less than 50 μm in diameter, while the amorphous form consisted of aggregated particles with primary particles that were less than 10 μm in diameter.

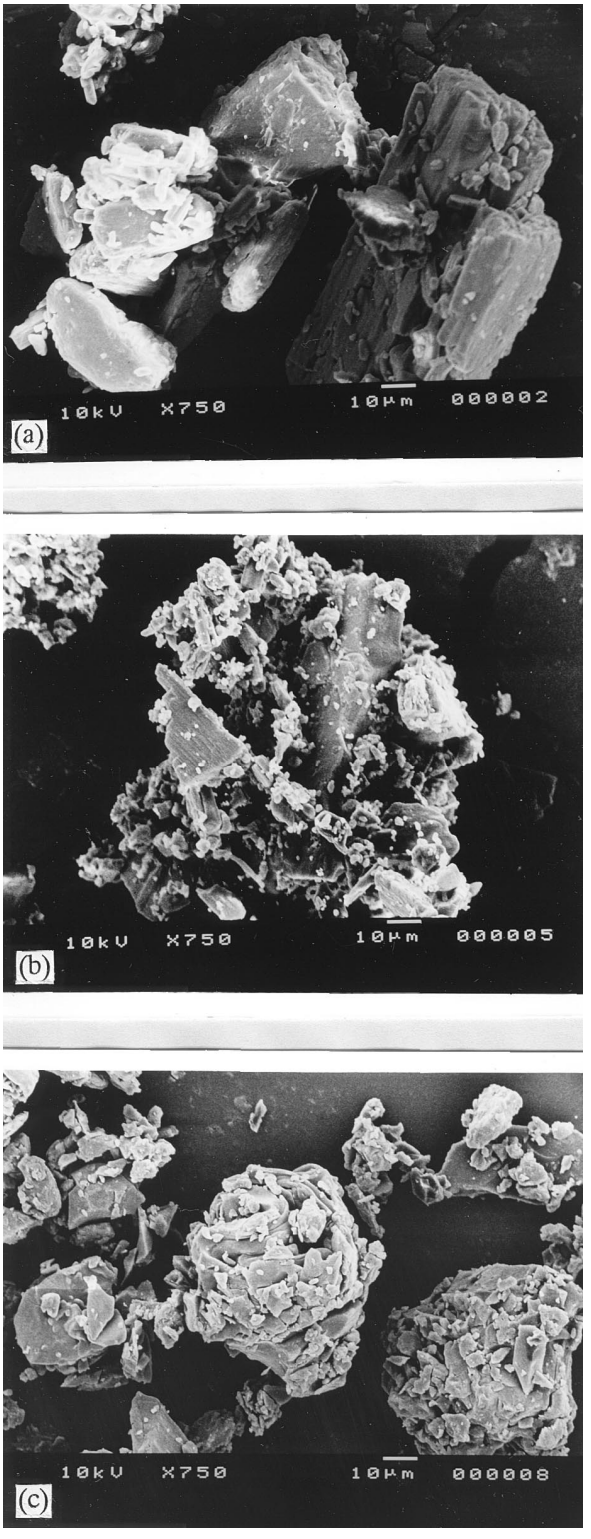

Figure 3. SEM photographs of glybuzole modifications: (a) form I, (b) form II, (c) amorphous form.

Physicochemical Stability of Glybuzole Modifications

The X-ray diffraction profiles of forms I and II did not change after storage at 40°C for 2 months. indicating that both were stable polymorphic forms at 0% and 75% RH, respectively, at 40°C. Therefore, the physicochemical stability of form II was estimated by the Kissinger method (Eq. 1) (5):

$$d\{\ln (\Phi/T_m^2)/d(1/T_m)\} = -E/R \quad (1)$$

where Φ is the heating rate, T_m is the temperature at the maximum of the DSC peak due to transformation, E is the activation energy, and R is the gas constant.

Figure 4 shows the Kissinger plot for the transformation of form II to form I. Since the plot gave a straight line, the activation energy of the transformation was calculated from the slope to be 194 kJ/mol by the least-squares method.

In contrast, the X-ray diffraction profiles of the amorphous form after storage at 0% and 75% RH, 40°C, showed several diffraction peaks (Fig. 5). These results suggested that the amorphous form was unstable and transformed into form I. The diffraction peak intensity of the amorphous form at 75% RH increased more rapidly than that at 0% RH, indicating that high humidity accelerated crystalline growth from the amorphous solid.

To clarify the mechanism of this effect, the crystalline transformation of the amorphous form was analyzed kinetically as previously described (5–9) based on the Jander equation:

$$g(x) = k(1 - (1 - x)^{1/3})^2 \quad (2)$$

where x is the weight fraction of polymorphic form, t is time, and k is the hydration rate constant.

Figure 6 shows the Jander plots for the crystalline transformation of the amorphous form of glybuzole at

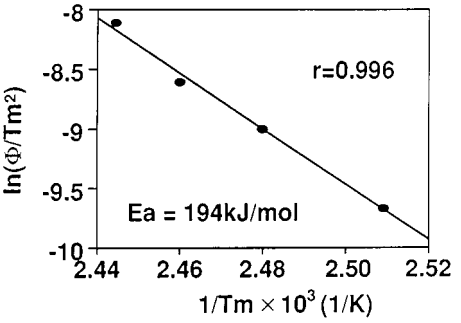

Figure 4. Kissinger plot for glybuzole polymorph form II.

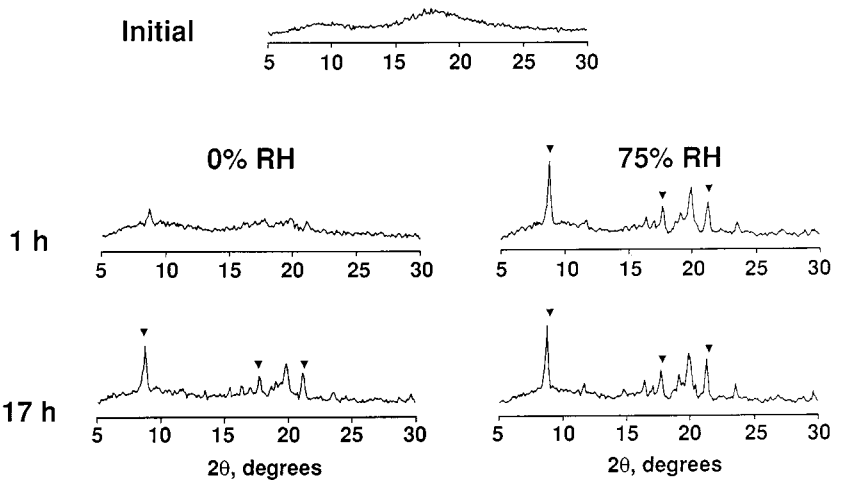

Figure 5. Change in X-ray diffraction profiles of the amorphous form stored at 0% and 75% RH at 40°C. ▼ represents the diffraction peak due to form I.

0% and 75% RH and at 40°C. The kinetic parameters for crystallization of the amorphous form were estimated by the least-squares method, and the crystalline rate constants of the glybuzole amorphous form at 0% and 75% RH were evaluated as 4.39×10^{-4} and 1.60×10^{-4} hr^{-4}, respectively.

Solubility of the Modifications in First and Second Fluids (JP XII)

The solubilities of the modifications were determined at 37°C in first fluid (pH 1.2) and second fluid (pH 6.8). The drug concentration profiles of forms I and II had almost the same patterns at pH 1.2 and pH 6. 8. The X-ray diffraction profiles of the residues measured after the solubility showed no crystalline change. After the drug concentration had reached an equilibrium, the solubilities of forms I and II at pH 1.2 were evaluated as 0.104 ± 0.001 ($n = 5$) and 0.108 ± 0.003 mg/ml ($n = 5$), respectively. Although the solubility of form II at pH 1.2 was slightly higher than that of form I (Student's t test, $p < .05$), both forms at pH 6.8 had solubilities of 2.92 ± 0.02 and 2.92 ± 0.03 mg/ml, respectively, which were not significant different ($p < .05$). In contrast, since X-ray diffraction analysis indicated that the amorphous form transformed into form I during the solubility measurement, the apparent solubilities at pH 1.2 and pH 6.8 were 0.110 ± 0.001 and 3.05 ± 0.00 ($n = 3$), respectively, similar to those of form I.

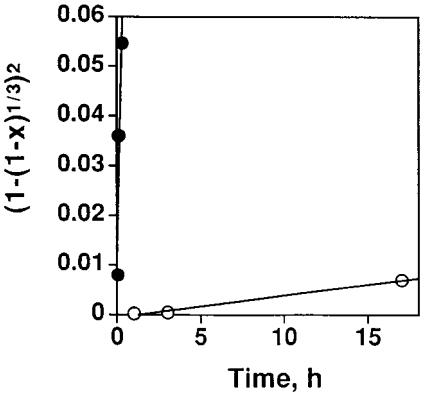

Figure 6. Jander plots for the crystalline transformation of the amorphous form of glybuzole at 0% and 75% RH at 40°C. ●, at 75% RH; ○, at 0% RH.

DISCUSSION

Identification of Glybuzole Polymorphic Forms

After systematic polymorphic screening tests, three specific modifications of glybuzole (forms I and II and amorphous form) from various kinds of organic solvents were identified by various analytical methods, indicating that form II and the amorphous form were new glybuzole modifications. However, since the preparation of bulk powder is the final process in the production of pharmaceutical preparations and since it is not easy to remove residual solvent from the solids, it is undesirable to use

toxic solvents for recrystallization to produce the bulk crystalline powder. Therefore, ethanol was selected as a solvent for safety conditioning in humans and for the proposed simple preparation methods for polymorphic forms I and II.

Physicochemical Stability of Glybuzole Polymorphic Forms

In order to evaluate the kinetic stability of forms II to form I, the activation energy of this transformation was estimated to be 194 kJ/mol by the Kissinger method. Since the activation energies of phenobarbital polymorphs (6) due to the transformations of form A to form B and form B to form F were 257 and 892 kJ/mol, respectively, the present results were roughly consistent with the activation energy (5) of polymorphic form A of phenobarbital to form B. The result of the X-ray diffraction profile of form II after heating indicated that the endothermic peak at 116.8°C was due to polymorphic transformation of form II to form I.

On the other hand, the result of the stability test of forms I and II after storage at 40°C for 2 months indicated that both were kinetically stable polymorphic forms at 0% and 75% RH, but the amorphous form was unstable during the storage condition. The crystallization rate of the amorphous form at 0% RH, based on the Jander equation, was 364 times slower than that at 75% RH. This result suggests that the atmospheric water vapor pressure accelerated the crystallization process of the amorphous form of glybuzole.

The solubility of form II was almost the same as that of form I, which is the conventional bulk crystalline form. In contrast, the amorphous form was unstable in water and transformed during the solubility test, giving almost the same apparent solubility as form I.

These results indicated that the same physicochemical properties of glybuzole bulk powder depend on the polymorphic form. Therefore, for selection of a polymorphic form of the active bulk powder suitable for production of high-quality pharmaceutical preparations, it will be necessary to perform additional preformulation and formulation studies.

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