Preparation of Amorphous and Polymorph Piretanide and Their Physicochemical Properties and Solubilities

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We prepared piretanide polymorphs by a new recrystallization procedure maximizing the solubility difference based on the pH difference of the solution. A new polymorph, form C, was formed at an acid–base molar ratio of $\geqq 1:1$, and an amorphous form at $<0.975:1$. At the molar ratio of HCl being 0.975—1, a mixture of form C and the amorphous form was generated. By X-ray powder diffractometry, the amorphous form showed no diffraction peak, and form C was significantly different from forms A (bulk material, BM) and B (polymorph). FT-IR spectra of form C and the amorphous form were significantly different from those of forms A and B at around 1700 and 3200—3400 cm^{-1}. The DSC curve of the amorphous form showed exothermic peaks at 136 and 209 °C and endothermic peaks at 207 and 225 °C (corresponding to the melting point of BM), whereas that of form C showed an exothermic peak at 143 °C and endothermic peaks at 132 and 224 °C (corresponding to the melting point of BM). By the dispersed amount method, the solubilities of the amorphous form and form C were almost the same as that of form B and 2.0- and 1.4-fold higher than that of form A (8.3 mg/100 ml) in JP XII first fluid (pH 1.2) and were about 4.5-fold higher than those of forms A and B (about 200 mg/100 ml) in JP XII second fluid (pH 6.8) at 37 °C.

Key words piretanide; X-ray powder diffractometry; solubility; polymorph; amorphous

Generally, there are several means of preparing polymorphs, including recrystallization under rapid or slow cooling, rapid cooling of a drug melt,[1] compressing, grinding,[2] freeze-drying[3] and spray-drying.[4] The amorphous form commonly has higher solubility than the crystalline forms due to its high chemical potential and is more bioavailable. However, it is unstable. The amorphous form may transform to the stable form in solution,[5] and this would affect its bioavailability. Pharmaceutical drugs differ highly in solubility. Some substances are soluble in water, but practically insoluble in organic solvent. Some have characteristic solubility depending on pH. Solubility must influence bioavailability. Piretanide (3-(aminosulfonyl)-4-phenoxy-5-(1-pyrrolidinyl)-benzoic acid; IUPAC) (Fig. 1) has widely been used as a diuretic or antihypertensive drug. It is practically insoluble in water at a low pH, but it is soluble in water in a basic environment (6.5 mg/100 ml at pH 1, more than 6 g/100 ml at pH 9).[6] In this study, we prepared piretanide polymorphs (amorphous form and form C) according to the solubility of piretanide at various pH values in solution, and investigated its physicochemical properties and solubilities.

Experimental

Materials A piretanide bulk sample (lot No.: L023, L024) was obtained from Hoechst Aktiengesellschaft, Germany. The amorphous form and form C were obtained by recrystallization at different pH values.

A brief outline of the preparative procedure is as follows:

Form C: The drug (10g of piretanide bulk material) was dissolved in 1000 ml of 0.1 N NaOH at room temperature (pH: about 12.5), then 200 ml of 0.5 N HCl was added with stirring at 700 rpm (pH: about 3.3). The separated crystals were collected by filtration and dried *in vacuo* over phosphoric anhydride for 24 h at room temperature.

Amorphous Form: The drug (10 g of piretanide bulk material) was dissolved in 1000 ml of 0.1 N NaOH at room temperature (pH: about 12.5), then 190 ml of 0.5 N HCl was added with stirring at 700 rpm (pH: about 4.0). The separated crystals were collected by filtration and dried *in vacuo* over phosphoric anhydride for 24 h at room temperature.

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X-Ray Powder Diffraction Analysis X-ray powder diffraction profiles were taken at room temperature with an X-ray powder diffractometer (MXP^3, Mac Science Co.). The operating conditions were as follows : target, Cu; filter, Ni; voltage, 30 kV; current, 10 mA; receiving slit, 0.15 mm; scanning speed 5° 2θ/min.

Thermal Analysis Differential scanning calorimetry (DSC) and thermogravimetry (TG) were performed with an 8085E2 DSC–TG instrument (Rigaku Denki Co.). The operating conditions in the open-pan system were as follows: sample weight, *ca.* 5 mg; heating rate, 10 °C/min; N_2 gas flow rate, 20 ml/min.

Fourier-Transform Infrared (FT-IR) Spectroscopy FT-IR spectra were taken by the Nujol mulls method using an FT-IR spectrophotometer (JIR5500, JEOL Co.).

Elemental Analysis Elemental analyses of the samples were performed for atoms of C, H and N with a CHN Corder (Perkin-Elmer 240C) and for S by means of oxygen flask combustion.

Scanning Electron Microscopy (SEM) SEM photographs of samples were taken with a scanning electron microscope (JSM-5000 JEOL Co.) at a magnification of × 500, 1500 and 2000.

Water Determination The water content of the two forms was determined using an Aquacounter (AQ-5, Hiranuma Co.).

Solubility Study Using the Dispersed Amount Method Various pH dissolution media were prepared as follows:

JP XII first fluid (pH 1.2): 2.0 g of NaCl was added to 7.0 ml of HCl, and the mixture was diluted with water to 1000 ml.

JP XII second fluid (pH 6.8): 118 ml of 0.2 N NaOH was added to 250 ml of 0.2 M KH_2PO_4 and the mixture was diluted with water to 1000 ml.

pH 5 buffer[7]: 32 ml of 2.5 M NaCl, 20.0 ml of 1.0 M sodium acetate and 40.0 ml of 0.35 M acetic acid made up to 1000 ml.

The dissolution profiles of the amorphous form and form C were investigated in the JP XII second fluid (pH 6.8) at 37 °C. An excess (2500 mg) of the sample was introduced into 250 ml of medium in a

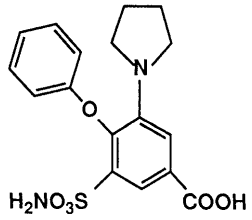

Fig. 1. Structure of Piretanide

1000-ml round-bottomed flask with a plastic cover. The flask was fixed on the sample holder in a thermostatically regulated water bath maintained at 37±0.5 °C, and stirred by a paddle at 200 rpm. Aliquots (10 ml) of the solution were withdrawn at appropriate intervals with a syringe through a 0.45-μm membrane filter. The filtrate was diluted and the concentration of the drug was measured at 275 nm using a spectrophotometer (type 430B, Jasco Co.).

The dissolution profiles of the amorphous form and form C were investigated in JP XII first fluid (pH 1.2) and in a pH 5 buffer at 37 °C. The procedure was as described except for the sample amount (500 mg).

Results and Discussion

Characterization of Amorphous Form and Form C

a) X-Ray Powder Diffractometry Figure 2 shows the X-ray powder diffraction profiles of the amorphous form and form C, for reference forms A and B. There were seven main diffraction peaks at 5.4, 8.4, 10.3, 11.1, 19.7, 20.7 and 21.7° (2θ) in form A and seven main diffraction peaks at 10.4, 13.3, 15.7, 18.4, 20.1, 23.2 and 24.3° (2θ) in form B, whereas the crystals showed a different profile. Form C, having a different crystal form, had nine main diffraction peaks at 10.5, 12.2, 15.6, 19.1, 19.9, 22.0, 22.8, 25.6 and 30.6° (2θ). The amorphous form did not exhibit a diffraction peak, but instead displayed a halo.

b) Thermal Analysis (DSC and TG) Figure 3 show the DSC and TG curves of the amorphous form and form C prepared by recrystallization, for reference forms A and B. The DSC curve of the amorphous form showed exothermic peaks at 136 and 209 °C and endothermic peaks at 207 and 225 °C, corresponding to the melting point, whereas that of form C showed an exothermic peak at 143 °C and an endothermic peak at 132 °C, as well as a subsequent endothermic peak at 224 °C.

Taking into consideration the peaks (except the melting peak) in the DSC curves of the amorphous form and form C, after heating at 150 °C for 30 min, these samples showed the same profile as form B by X-ray powder diffraction. This finding indicated that form C and the amorphous form may be transformed into form B by heating.

c) IR Spectra Figure 4 shows the IR spectra of the amorphous form and form C, for reference forms A and B. The IR spectrum of piretanide has been explained by Matsubara, *et al.*[6] as follows. The absorption band at 3415 cm^{-1} is attributable to the OH group in carboxylic acid, the bands at 3360 and 3265 cm^{-1} to the NH_2 group in sulfonic acid, and those at 1694 and 1679 cm^{-1} to the C=O group in carboxylic acid. In this figure, peaks at round 1700 cm^{-1} in the amorphous form and form C shifted to a higher wave number compared to those in forms A and B. The two peaks were attributable to the OH group and the NH_2 group which form one peak with a shoulder curve at around 3386 cm^{-1} in the amorphous form and form C.

d) SEM Observation Figure 5 shows SEM photo-

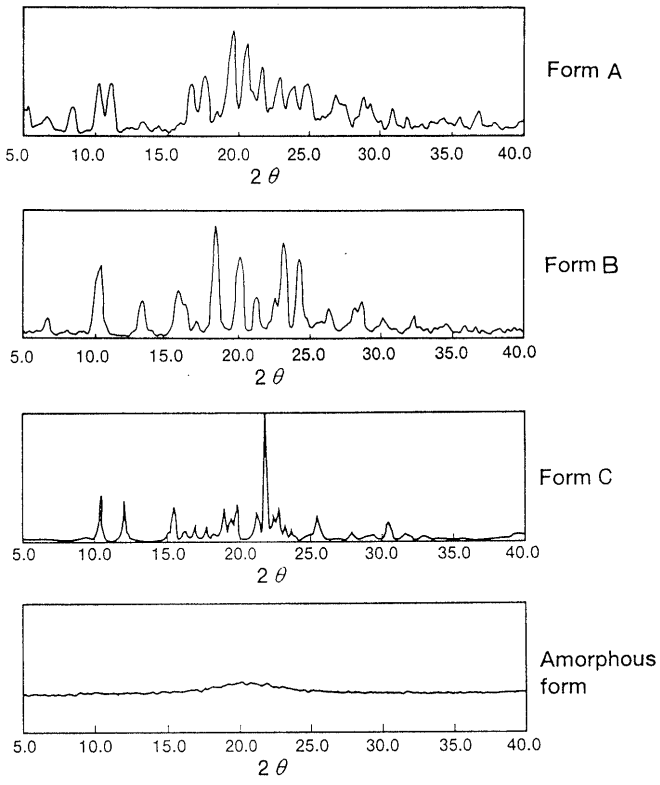

Fig. 2. X-Ray Powder Diffraction Profiles of Piretanide Polymorphs (Amorphous Form and Forms A, B, C).

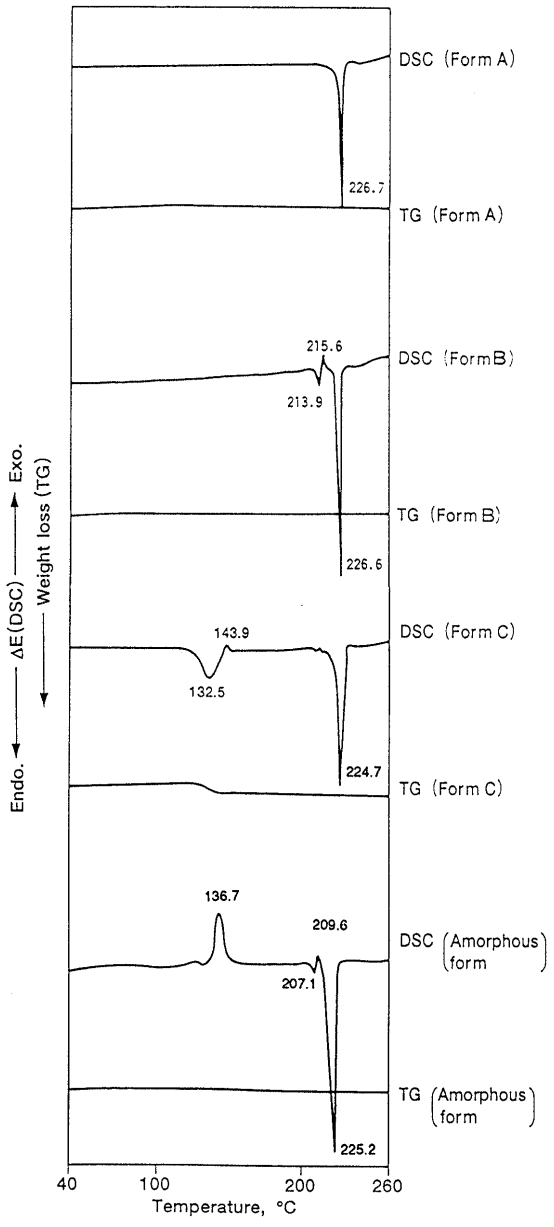

Fig. 3. DSC and TG Thermograms of Piretanide Polymorphs (Amorphous Form and Forms A, B, C).

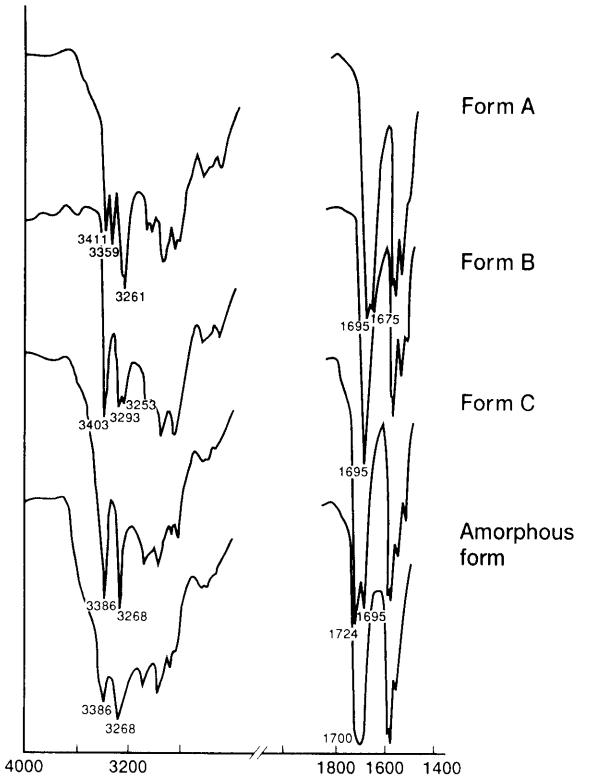

Fig. 4. FT-IR Spectra of Piretanide Polymorphs (Amorphous Form and Forms A, B, C)

graphs of the amorphous form and form C. Distinct morphological differences were evident. The amorphous form consisted of aggregated secondary particles of about 150 mm in diameter with primary particles less than 1—2 mm. Form C consisted of aggregated secondary particles of about 100 mm in diameter with primary scaly crystals being less than 10 mm long and less than 1 mm wide.

e) Water Determination and Elemental Analysis Table 1 shows the water content of the amorphous form and form C. The amorphous form contained $2.35 \pm 0.31\%$ water and form C contained $2.50 \pm 0.14\%$ water, respectively. This indicated that each form has about 0.5 mol of water per mol of piretanide according to the calculation of molecular weight ratio between piretanide and water.

The results of the elemental analysis of the amorphous form and form C are summarized in Table 2. The observed values (Amorphous form, C: 54.94%, H: 5.10%, N: 7.55%, S: 8.58%, Form C, C: 54.82%, H: 5.05%, N: 7.46%, S: 8.44%) were in good agreement with the calculated values (C: 54.99%, H: 5.10%, N: 7.55%, S: 8.63%) for the hemihydrate of the amorphous form and form C. According to these results obtained by two analytical procedures, the two forms have about 1 mol of water per 2 mol of piretanide.

Solubility of Amorphous Form and Form C with Dispersed Amount Method Table 3 shows the solubilities of the amorphous form and form C by the dispersed amount

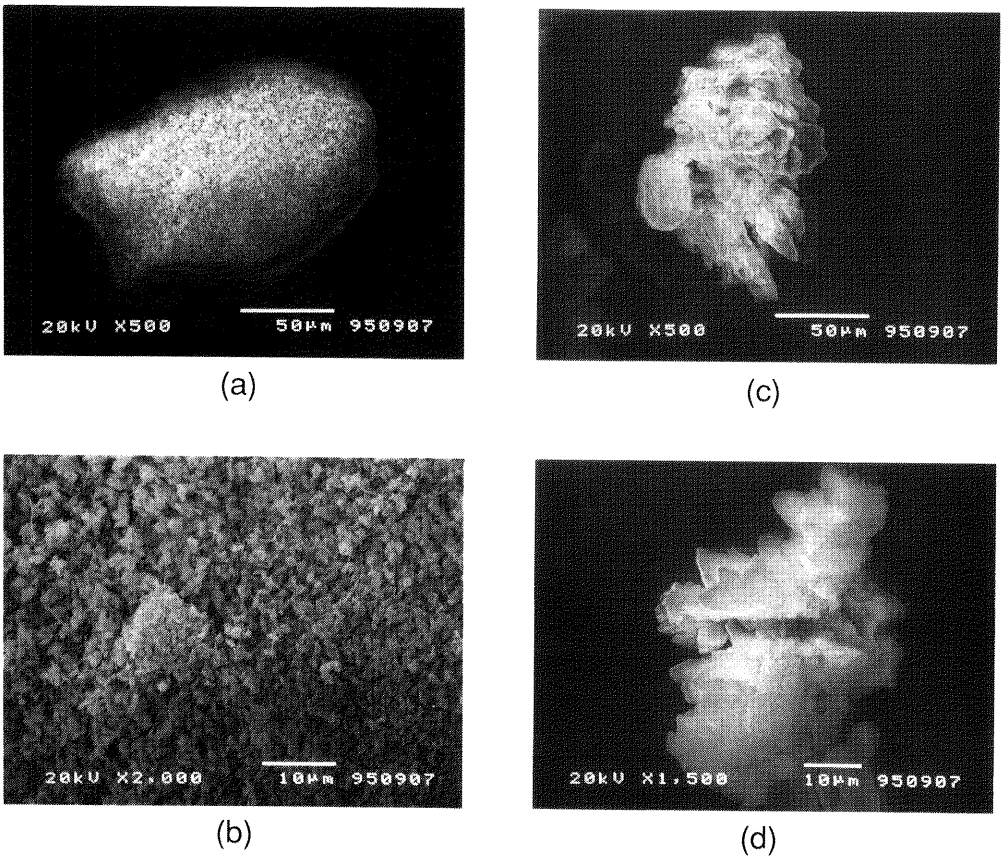

Fig. 5. Scanning Electron Photomicrographs of Piretanide Amorphous Form and Form C
(a) Amorphous form (× 500), (b) amorphous form (× 2000), (c) form C (× 500), (d) form C (× 1500).

Table 1. Water Contents of Piretanide Amorphous Form and Form C

	Amorphous form	Form C
Water content (%)	2.35±0.31	2.50±0.14

$n=3$.

Table 2. Elemental Analysis of Piretanide Amorphous Form and Form C

			Elemental analysis (%)			
			C	H	N	S
Amorphous form	$C_{17}H_{18}N_2O_5S \cdot 1/2H_2O$	Calcd	54.99	5.12	7.55	8.63
		Found	54.82	5.05	7.46	8.44
Form C	$C_{17}H_{18}N_2O_5S \cdot 1/2H_2O$	Calcd	54.99	5.12	7.55	8.63
		Found	54.94	5.10	7.55	8.58

Table 3. Solubilities of Piretanide Polymorphs (Amorphous Form and Forms A, B, C) in pH 1.2, 5 and 6.8 at 37 °C

Solvent	Solubilities (mg/100 ml)			
	Form A	Form B	Form C	Amorphous form
JP XII first fluid (pH 1.2)	8.3	13.3	11.9	16.9
pH 5 buffer	42.3	64.7	49.5	66.0
JP XII second fluid (pH 6.8)	193.8	195.4	927.3	901.3

method in JP XII first fluid (pH 1.2), pH 5 buffer and JP XII second fluid (pH 6.8), at 37 °C. The solubilities of the amorphous form were 16.9, 66.0 and 901.3 mg/100 ml, respectively. Those of form C were 11.9, 49.5 and 927.3 mg/100 ml, respectively. The solubilities of the amorphous form and form C were almost the same as that of form B and about 2.0 and 1.4-fold higher than that of form A in the JP XII first fluid (pH 1.2) at 37 °C. In a pH 5 buffer at 37 °C, the solubility of the amorphous form was similar to that of form B, and those of forms C and A were similar. The two forms were about 4.5-fold more soluble than forms A and B in the JP XII second fluid (pH 6.8) at 37 °C. After the dissolution experiment, the X-ray powder diffraction patterns of crystallized precipitate of the amorphous form coincided with those of intact form C, but no conversion of form C was observed.

Conclusion

In this study, we identified a novel polymorphic amorphous form and form C of piretanide by means of a new recrystallization procedure using the difference in solubility at various pH values. The amorphous form can be obtained in gram order by this procedure. Their structures were confirmed by X-ray powder diffractometry, DSC, TG, FT-IR spectroscopy, elemental analysis and SEM. The solubilities of the amorphous form and form C were not very different from those of forms A and B in acid (JP XII first fluid, pH 1.2 and pH 5 buffer) at 37 °C. However, the two forms were 4.5-fold more soluble than forms A and B at neutral pH (JP XII second fluid, pH 6.8) at 37 °C. Therefore, the two forms may be used as bulk powders since they have greater bioavailability than forms A and B.

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