

[Chem. Pharm. Bull.]  
33(4)1660-1668(1985)]

## Effect of Grinding on the Transformations of Polymorphs of Chloramphenicol Palmitate<sup>1)</sup>

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(Received July 30, 1984)

The effect of grinding in an agate centrifugal ball mill on the transformations of polymorphs of chloramphenicol palmitate (CPP) were studied by means of X-ray diffraction analysis, infrared (IR) spectroscopy and differential scanning calorimetry (DSC). Three kinds of the polymorphs of CPP were reported, that is, forms A, B and C.

There was no change in the X-ray diffraction profile of form A (stable form) on grinding for 600 min, but its profile became broader than that of intact form A. There was no change in the X-ray diffraction profile of form B (meta-stable form) on grinding for 130 min, but form B was transformed into form A by grinding for more than 140 min. Form B ground for 140 min contained about 35% form A; when ground for 150 min, it contained about 80% form A, and when ground for more than 150 min, it contained a constant level of about 80% form A. The X-ray diffraction profile of form C (meta-stable form) suggested that form C was transformed into form B by grinding for 20 min; thereafter the product was transformed into form A by grinding for 160 min. Form C ground for 4 min contained about 60% form B; when ground for 20 min, it contained about 100% form B, and ground for 160 min, it contained about 80% form A. These results suggest that the transformations of polymorphs of CPP by grinding are form C→form B→form A.

The melting point (mp) and the heat of fusion ( $H$ ) were measured by DSC. The mp and the  $H$  of form A obtained by grinding were about 2.5°C and about 2.5 kcal/mol lower than those of intact form A, respectively, and the solubility in 50% (v/v) aqueous isopropyl alcohol was about twice that of intact form A.

**Keywords**—chloramphenicol palmitate; polymorphism; grinding; transformation; X-ray diffraction analysis; IR spectrum; thermal analysis; solubility

### Introduction

Three polymorphs of chloramphenicol palmitate (CPP) have been reported; forms A, B, and C.<sup>2-4)</sup> The meta-stable forms B and C of CPP are suitable for clinical use; and it is required that the content of stable form A should be less than 10% in the United States Pharmacopeia XX (1980) and the British Pharmacopoeia 1980. Kelbaek *et al.*<sup>5)</sup> reported that particle size was an important factor affecting the bioavailability of CPP. Suzuki *et al.*<sup>2)</sup> reported that micro-fine particles of form B of CPP could be obtained by the freeze-drying method. In the previous paper<sup>6)</sup> the authors reported the effect of grinding on the physicochemical properties of cephalexin powder. In the present study, the change of the physicochemical properties of polymorphs of CPP caused by mechanical treatment was investigated.

### Experimental

**Materials**—CPP used in the present study was as follows.

**Form A:** The bulk powder of CPP (Sankyo, bulk powder) was dissolved in ethanol, and undissolved drug was filtered off. The saturated CPP solution was evaporated to dryness under atmospheric pressure at 35°C.

**Form B:** Form B was obtained by recrystallization from ethanol at room temperature, and was air-dried.

Form C: The bulk powder of CPP was dissolved in methanol and the undissolved drug was filtered off. The saturated CPP solution at about 70 °C was rapidly cooled at about -20 °C, and the crystals of form C were collected by filtration, and air-dried.

**Mechanical Treatment**—A sample of CPP (5 g) was ground in an agate centrifugal ball mill (capacity 350 ml; diameter and number of balls, 10 mm × 20, 15 mm × 10 and 20 mm × 4) using a grinding apparatus (Fritsch Co., Ltd.) at 200 rpm.

**X-Ray Diffraction Analysis**—The X-ray diffraction profiles were measured at room temperature with a type JDX 7E diffractometer (Nihon Denshi Co., Ltd.). The measurement conditions were as follows; target, Cu; filter, Ni; voltage, 20 kV; current, 10 mA; receiving slit, 0.2 mm; time constant, 2 s; counting range,  $6 \times 10^3$  cpm; scanning speed, 1 °/min. About 80 mg of the sample was loaded with a micro spoon into a glass holder without introducing a preferred orientation of the crystals.

**Infrared (IR) Spectra**—The IR spectra were measured as a mull in Nujol on an IR-2 infrared spectrophotometer (Nihon Bunko Co., Ltd.).

**Thermal Analysis**—The differential scanning calorimetry (DSC) curves were measured with the DSC instrument (Shimadzu Seisakusho Co., Ltd., SC-20B). The measurement conditions were as follows: sample weight, about 5 mg; sample cell, an aluminum crimp cell; N<sub>2</sub> gas flow, 30 ml/min; heating rate, 10 °C/min. The heat of fusion (*H*) and the melting point (mp) were corrected by measurement of indium as a standard sample.

**Measurement of Dissolution Curve**—A sample (500 mg) of CPP was rapidly placed in 200 ml of 50% (v/v) aqueous isopropyl alcohol solution in a 1000 ml round-bottomed flask (JP X; Dissolution Test) maintained at 25 ± 0.2 °C. The solution was stirred with a propeller having three blades at the constant rate of 200 rpm. Samples were taken with a glass syringe at suitable time intervals and were immediately filtered through a 0.45 μm membrane filter (Millipore, HAWPO 01300). The filtrate was suitably diluted for assay with a spectrophotometer (Hitachi Seisakusho Co., Ltd., type 139) at 264 nm.

## Results and Discussion

### Physicochemical Properties of Polymorphs of CPP

Figure 1 and Table I show the X-ray diffraction profiles and the main diffraction peak angles of polymorphs of CPP. The X-ray diffraction patterns and main diffraction angles of forms A, B and C in the present study agreed with the data in previous papers.<sup>2-4)</sup>

Figure 2 and Table II show the IR spectra and the absorption peak wave numbers of polymorphs of CPP. The IR spectra of forms A, B and C agreed with the results in previous papers.<sup>2,3,5,7)</sup>

Figure 3 and Table III show the DSC curves, the mp and the *H* of polymorphs of CPP. Forms A and B showed an endothermic peak at 90.3 and 86.7 °C, respectively, due to fusion. Form C showed an exothermic peak at 64.5 °C due to the transformation into form B and an endothermic peak at 86.3 °C due to fusion. The transformation of form C to form B at 64.5 °C

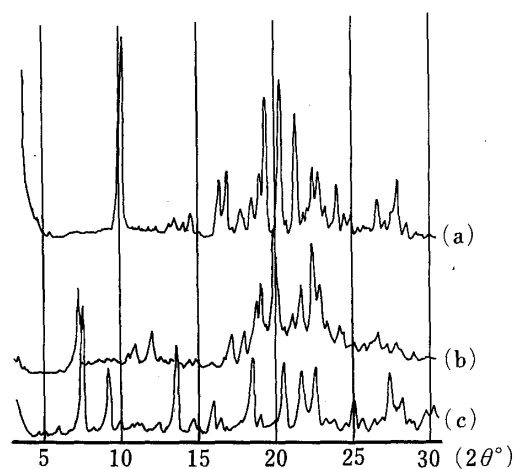


Fig. 1. X-Ray Diffraction Profiles of Polymorphs of CPP

(a) form A, (b) form B, (c) form C.

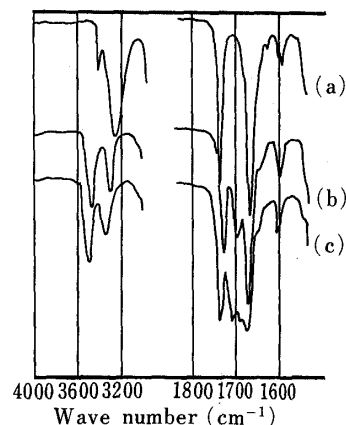


Fig. 2. IR Spectra of Polymorphs of CPP

(a) form A, (b) form B, (c) form C.

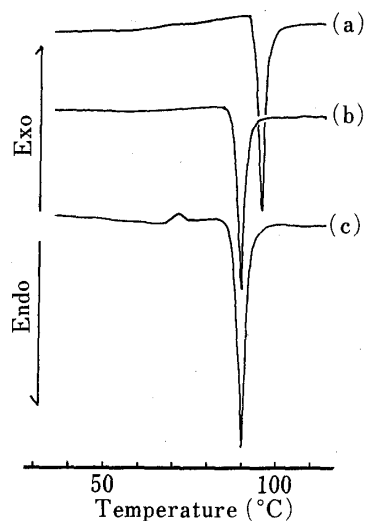


Fig. 3. DSC Curves of Polymorphs of CPP

(a) form A, (b) form B, (c) form C.

TABLE I. Main X-Ray Diffraction Peak Angles of Polymorphs of CPP

Form A		Form B		Form C	
$2\theta^\circ$	$I/I_0$	$2\theta^\circ$	$I/I_0$	$2\theta^\circ$	$I/I_0$
10.2	1.00	7.2	0.71	7.5	1.00
16.4	0.37	11.0	0.33	9.2	0.61
16.9	0.41	12.0	0.40	13.6	0.74
19.4	0.73	19.0	0.70	18.5	0.65
20.3	0.81	19.8	1.00	20.5	0.62
21.4	0.68	22.4	0.95	21.6	0.61
22.5	0.42	22.8	0.69	22.5	0.61

 $I_0$ ; the intensity of the highest diffraction peak.

TABLE II. IR Spectral Bands of Polymorphs of CPP

	Form A ( $\text{cm}^{-1}$ )	Form B ( $\text{cm}^{-1}$ )	Form C ( $\text{cm}^{-1}$ )
Amido NH stretch <sup>7)</sup>	3410	3325	3340
OH stretch <sup>7)</sup>	3270	3490	3500
Ester C=O stretch <sup>3)</sup>	1737	1727	1746
Amido C=O stretch <sup>3)</sup>	1668	1700	1707, 1687 sh
	1628 sh	1675	1675
NO <sub>2</sub> asymmetric stretch	1603	1605	1603
	1594	1596	

sh; shoulder peak.

TABLE III. Thermal Parameters of Polymorphs of CPP

Sample	Heat of fusion (kcal/mol)	Melting point ( $^\circ\text{C}$ )	Transition point ( $^\circ\text{C}$ )	Heat of transition (kcal/mol)
Form A	$15.70 \pm 0.52^a)$	90.3	—	—
Form B	$11.08 \pm 0.21^a)$	86.7	—	—
Form C	$10.87 \pm 0.23^a)$	86.3	$64.5^b)$	$0.40 \pm 0.11^a)$

<sup>a)</sup> Standard deviation ( $n=3$ ). <sup>b)</sup> Transition from C to B.

was confirmed by X-ray diffraction analysis. Tamura *et al.*<sup>3)</sup> reported that about 100% of form B of CPP was transformed into form A by heating for 1600 min at 82 °C. These results suggest that the transformations of polymorphs of CPP caused by heating are form C→form B→form A.

### Transformation of Polymorphs of CPP by Grinding

Figure 4 shows the changes of X-ray diffraction profiles of form A during grinding in an agate centrifugal ball mill. The peak intensities of form A decreased with increasing grinding time. The profile of form A ground for 10 min was very broad, but even after a prolonged grinding (600 min) the profile showed no change. The broad X-ray diffraction profile of the ground form A suggests that a part of form A was converted into a noncrystalline solid or that considerable disorder was produced in the crystal lattice of form A.

Figure 5 shows the changes of the IR spectra of form A during grinding. In the IR spectrum of form A the band at 3410  $\text{cm}^{-1}$  is due to the free or weakly hydrogen-bonded amido  $\nu_{\text{NH}}$ , and the band at 3270  $\text{cm}^{-1}$  is due to the strongly hydrogen-bonded  $\nu_{\text{OH}}$ .<sup>2,7)</sup> The bands at 1737 and 1668  $\text{cm}^{-1}$  are the ester  $\nu_{\text{C=O}}$  and the amido  $\nu_{\text{C=O}}$ , respectively.<sup>3)</sup> The IR spectra of form A ground for 10 and 600 min showed broader peaks at 3410, 3270, 1737 and 1668  $\text{cm}^{-1}$ , compared with those of intact form A, but the IR spectral patterns were the same as those of intact form A.

Figure 6 shows the changes of the X-ray diffraction profiles of form B during grinding. The X-ray diffraction profiles of form B showed no change up to 130 min of grinding time except that the diffraction peak intensities decreased slightly, but form B ground for 140 min showed diffraction peaks at  $2\theta=10.2$  and  $20.3^\circ$  attributable to form A, and the X-ray diffraction profile of form B ground for 150 min lacked the diffraction peaks at  $2\theta=7.2$ ,  $12.0$  and  $19.8^\circ$  attributable to form B.

Figure 7 shows the changes of the IR spectra of form B during grinding. In the IR spectrum of form B the band at 3490  $\text{cm}^{-1}$  is due to the hydrogen-bonded  $\nu_{\text{OH}}$ , and the band at 3325  $\text{cm}^{-1}$  is due to the intermolecular hydrogen-bonded  $\nu_{\text{NH}}$ . The band at 1727  $\text{cm}^{-1}$  is due to the ester  $\nu_{\text{C=O}}$ , and the bands at 1675 and 1700  $\text{cm}^{-1}$  are due to the amido  $\nu_{\text{C=O}}$ .<sup>3)</sup> The IR spectra of the ground form B showed no change until 130 min of grinding time, but that of the form B ground for 140 min showed the peaks at 3410 and 3270  $\text{cm}^{-1}$  attributable to form A, and the peak intensity at 1700  $\text{cm}^{-1}$  due to the amido  $\nu_{\text{C=O}}$  decreased, while the peak at 1727  $\text{cm}^{-1}$  shifted to 1737  $\text{cm}^{-1}$ . In the IR spectrum of form B ground for 150 min, the bands attributable to form B had disappeared. These results suggest that form B was transformed

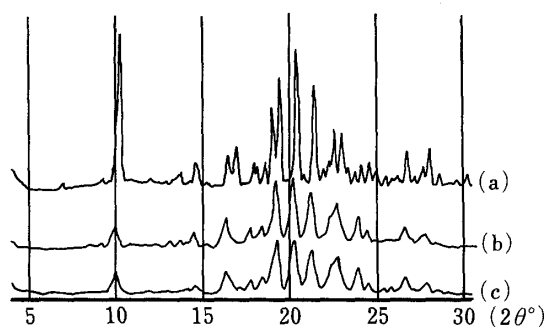


Fig. 4. Change in X-Ray Diffraction Profiles of Form A during Grinding

(a) intact form A, (b) the product ground for 10 min, (c) the product ground for 600 min.

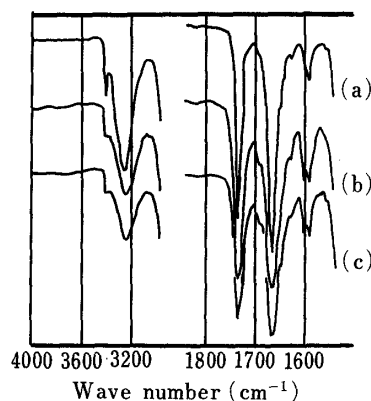


Fig. 5. Change in IR Spectra of Form A during Grinding

(a) intact form A, (b) the product ground for 10 min, (c) the product ground for 600 min.

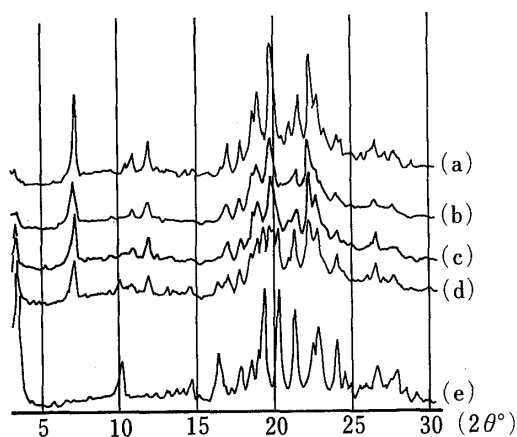


Fig. 6. Change in X-Ray Diffraction Profiles of Form B during Grinding

(a) intact form B, (b) the product ground for 120 min, (c) the product ground for 130 min, (d) the product ground for 140 min, (e) the product ground for 150 min.

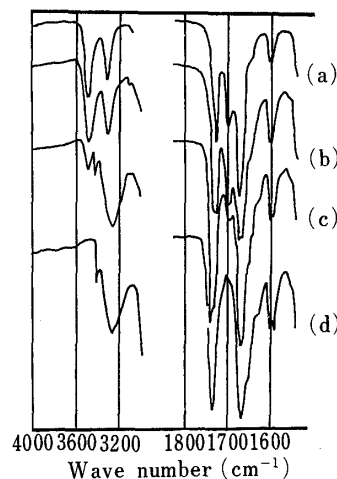


Fig. 7. Change in IR Spectra of Form B during Grinding

(a) intact form B, (b) the product ground for 130 min, (c) the product ground for 140 min, (d) the product ground for 150 min.

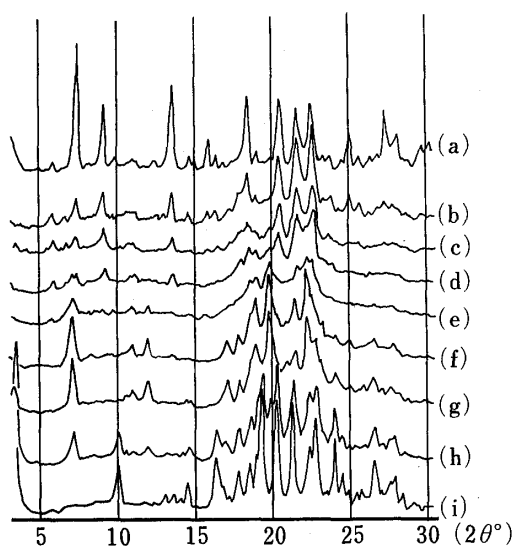


Fig. 8. Change in X-Ray Diffraction Profile of Form C during Grinding

(a) intact form C, (b) the product ground for 1 min, (c) the product ground for 2 min, (d) the product ground for 4 min, (e) the product ground for 8 min, (f) the product ground for 10 min, (g) the product ground for 140 min, (h) the product ground for 150 min, (i) the product ground for 160 min.

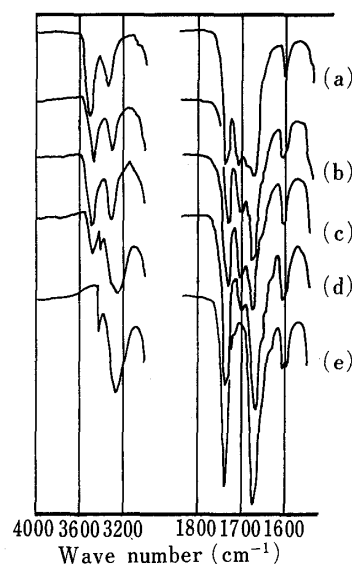


Fig. 9. Change in IR Spectra of Form C during Grinding

(a) intact form C, (b) the product ground for 10 min, (c) the product ground for 140 min, (d) the product ground for 150 min, (e) the product ground for 160 min.

into form A by grinding for more than 140 min.

Figure 8 shows the changes of the X-ray diffraction profiles of form C during grinding. In the X-ray diffraction profiles of form C ground for 1–10 min the diffraction peak intensities attributable to form C decreased while the peaks attributable to form B appeared and their intensities increased with increasing grinding time. After grinding for 10 min, form C was transformed into form B. Further, form C ground for 150 min showed the diffraction peak at  $2\theta = 10.2^\circ$  attributable to form A. In the X-ray diffraction profile of form C ground for 160 min, the diffraction peaks attributable to form B had disappeared.

Figure 9 shows the changes of the IR spectra of form C during grinding. In the IR spectrum of form C ground for 10 min the bands at 3500 and 3340  $\text{cm}^{-1}$  due to the amido  $\nu_{\text{NH}}$  and the hydroxyl  $\nu_{\text{OH}}$  shifted to 3490 and 3325  $\text{cm}^{-1}$ , respectively, and the peak at 1707  $\text{cm}^{-1}$  due to the amido  $\nu_{\text{C=O}}$  shifted to 1700  $\text{cm}^{-1}$ . The peak at 1675  $\text{cm}^{-1}$  increased with increasing grinding time, suggesting that form C was transformed into form B. Form C ground for 150 min showed bands at 3410, 3270, 1737 and 1668  $\text{cm}^{-1}$  attributable to form A, and after grinding for 160 min, the bands attributable to form B had disappeared. These results suggest that the transformations of polymorphs of CPP during grinding are form C  $\rightarrow$  form B  $\rightarrow$  form A. This is the same as in transformation caused by heating.

#### Determination of Polymorphs of CPP by the X-Ray Diffraction Method

Armando *et al.*<sup>4)</sup> used a nonlinear calibration curve to determine the amount of form A or B in their mixtures by the X-ray diffraction method, but we obtained a good linear calibration curve with Armando's data when we plotted the content of form A against the natural logarithm of the X-ray diffraction intensities ratio at  $2\theta = 20.3$  and  $19.8^\circ$ , as shown in Fig. 10(A). The X-ray diffraction profiles of standard mixtures of forms A and B and forms B and C were measured, and Fig. 10 shows the plots of the ratio of the peak heights at  $2\theta = 19.8$  and  $20.3^\circ$  versus the content of form A and the ratio of the peak heights at  $2\theta = 7.5$  and  $7.2^\circ$  versus the content of form B. The plots each gave a straight line with correlation coefficients of  $r = 0.9987$  and  $0.9975$ , respectively.

Figure 11 shows the change of crystalline form of forms A and B during grinding. In grinding of form A, the ground product showed a decreasing content of form A with increasing grinding time, since the X-ray diffraction peaks become broader than those of intact form A, but there was no peak attributable to form B. From this result it seemed that a part of form A was converted into a noncrystalline solid or that considerable disorder was produced in the crystal lattice, so that the apparent content of form A decreased.

In grinding of form B the products ground for 5–130 min were transformed into form A to extents of less than about 5%, but the product ground for 140 min contained about 30% form A, while the product ground for 150 min contained about 80% form A, and the values stayed approximately constant at 80% on further grinding. It seems that the nucleation rate is the rate-determining step of the transformation of form B to form A. This is further discussed elsewhere.

Figure 12 shows the change of crystalline form of form C during grinding. The product ground for 2 min contained about 50% form B, and about 100% of form C was transformed into form B after 18 min of grinding. The product ground for 140 min showed no further change and was form B, but the product ground for 150 min contained about 50% form A, and the product ground for 160 min contained about 80% form A. The values of products ground for more than 160 min stayed approximately constant at about 80% form A. These

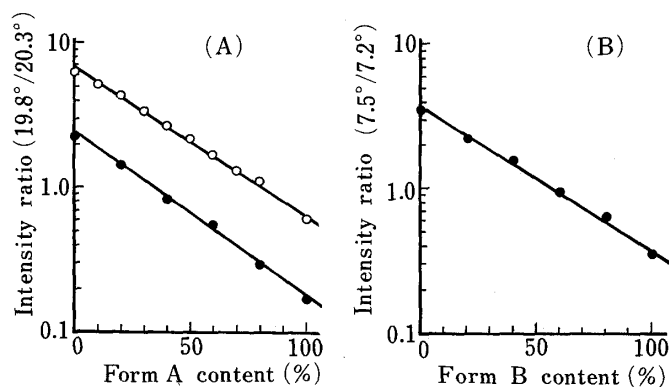


Fig. 10. Relation between Contents of Polymorphs of CPP and X-Ray Diffraction Intensity Ratios

(A) relation between forms A and B, (B) relation between forms B and C.

●, experimental data; ○, data from Armando *et al.*<sup>4)</sup>

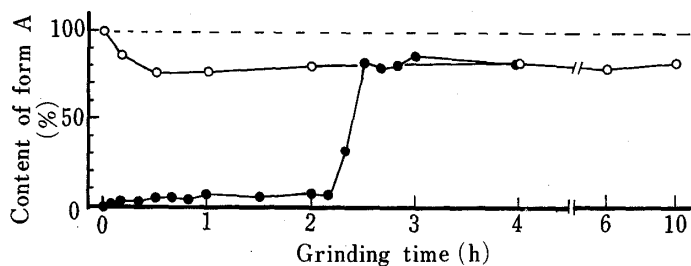


Fig. 11. Effect of Grinding on Crystalline Form of Forms A and B  
○, form A; ●, form B.

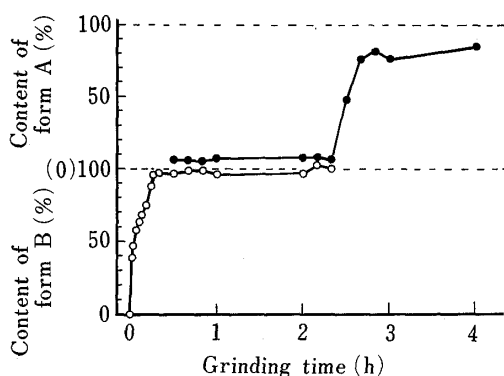


Fig. 12. Effect of Grinding on Crystalline Form of Form C  
○, content of form B; ●, content of form A.

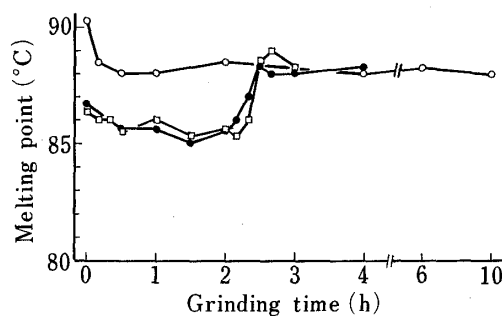


Fig. 13. Change in Melting Points of Polymorphs of CPP during Grinding  
○, form A; ●, form B; □, form C.

results suggest that the transformation of polymorphs of CPP by grinding are form C→form B→form A, the same as in the case of transformation by heating, and form C is unstable on grinding.

#### Change of Melting Point and Heat of Fusion of Polymorphs of CPP by Grinding

Figure 13 shows the relation between the mp of forms A and B and grinding time. The mp of intact form A was 90.3°C; the mp of form A ground for 20 min decreased by about 2.5°C, and its value thereafter stayed approximately constant at about 87°C, even after prolonged grinding. The mp of intact form B was 86.7°C, and the mp of form B ground for 30–120 min decreased by about 1°C. The mp of form B ground for more than 130 min increased due to the transformation into form A, and the value thereafter stayed approximately constant at about 88.0°C, even after prolonged grinding.

The transition point and the mp of intact form C were 64.5 and 86.3°C, respectively. Since about 100% of form C was transformed into form B after 18 min of grinding, the mp of the product ground for 20–150 min was about 85.5°C and stayed constant, but the mp of form C ground for 160 min was about 88°C due to transformation into form A, and its value stayed approximately constant at 88°C, even after prolonged grinding. The mp of form A obtained by grinding was about 2.5°C lower than that of intact form A, and the mp of form B ground for 30–120 min was about 1°C lower than that of intact form B. These results suggest that parts of forms A and B might have been converted into noncrystalline solid or that many disordered regions were produced in the crystal lattice.

Figure 14 shows the change of the  $H$  of polymorphs of CPP during grinding. The  $H$  of form A ground for 30 min decreased by about 2.5 kcal/mol, and remained approximately constant at about 13.5 kcal/mol, even after prolonged grinding.

In grinding of form B, the  $H$  of form B ground for 60–120 min decreased by about

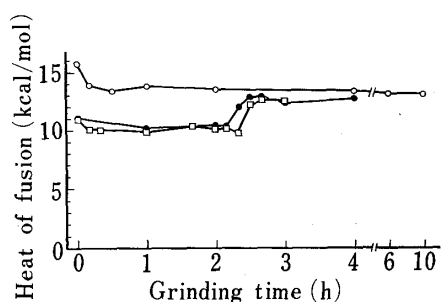


Fig. 14. Change in Heat of Fusion of Polymorphs of CPP during Grinding

○, form A; ●, form B; □, form C.

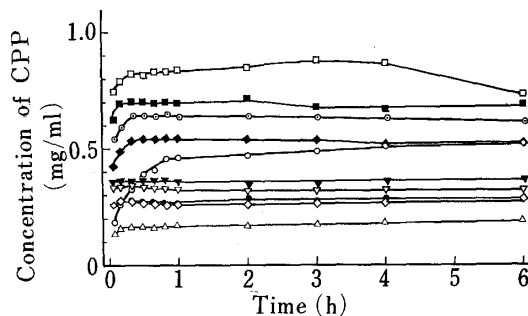


Fig. 15. Change in Dissolution Curves for Polymorphs of CPP in 50% (v/v) Aqueous Isopropyl Alcohol at 25°C after Grinding

△, intact form A; ▽, the ground form A for 10 min; ▾, the ground form A for 600 min; ○, intact form B; ⊙, the ground form B for 60 min; ●, the ground form B for 180 min; □, intact form C; ■, the ground form C for 20 min; ◆, the ground form C for 60 min; ◇, ground form C for 180 min.

1 kcal/mol, and the  $H$  of form B ground for 150 min increased due to transformation into form A, then stayed approximately constant at about 13 kcal/mol, even after prolonged grinding.

In grinding of form C, the  $H$  of form C ground for 5–150 min decreased by about 1 kcal/mol, but that of form C ground for 160 min increased due to transformation into form A, and the values stayed approximately constant at about 13 kcal/mol, even after prolonged grinding. The  $H$  of form A obtained by grinding for more than 30 min was about 2.5 kcal/mol smaller than that of intact form A, and the  $H$  of form B obtained by grinding for 60–120 min was about 1 kcal/mol smaller than that of intact form B. These results suggest that a part of forms A and B might have been converted into noncrystalline solid or that many disordered regions were produced in the crystal lattice, and the enthalpy increased.

#### Change of the Dissolution Curves of Polymorphs of CPP by Grinding

Figure 15 shows the changes of the dissolution curves of polymorphs of CPP on grinding in 50% (v/v) aqueous isopropyl alcohol at 25°C. Form A ground for 10 or 600 min was about twice as soluble as intact form A, and the solubilities of intact form A and the products ground for 10 and 600 min were 0.185, 0.320 and 0.371 mg/ml, respectively.

Form B ground for 60 min was about 30% more soluble than intact form B (0.505 mg/ml). The solubility of form B ground for 180 min decreased to 0.285 mg/ml due to transformation into form A but its value was about 50% larger than that of intact form A.

Form C was the most soluble form in all samples (its solubility was 0.855 mg/ml). The solubility of form C ground for 20 min decreased to 0.695 mg/ml due to transformation into form B, but its value was about 50% larger than that of intact form B. The dissolved amount of form C ground for 180 min decreased to 0.265 mg/ml due to transformation into form A but its value was about 40% larger than that of intact form A. The dissolved amount of forms A and B obtained by grinding were larger than those of intact forms A and B, respectively. These results suggest that the ground forms A and B might contain noncrystalline solid or regions of disordered crystal lattice.

#### Conclusion

The meta-stable forms of CPP (forms B and C), which are therapeutically active, were transformed into stable form A during grinding in an agate centrifugal ball mill for 150 and



160 min, respectively, and the solubilities of the ground products decreased. These findings suggest that the dissolution rates of forms B and C might decrease due to transformation into form A when forms B and C are ground in order to obtain small particles and increase bioavailability. On the other hand, the finding that the dissolved amount of forms A and B obtained by grinding for suitable times were larger than those of intact forms A and B, respectively, suggests that those ground products might contain noncrystalline solid or regions of disordered crystal lattice. Care seems to be necessary when drugs are treated mechanically during formulation.

**Acknowledgement** The authors wish to express their gratitude to Mrs. Y. Tobe and Mr. K. Narushima for their assistance in the experimental work. The authors are also grateful to Sankyo Co., Ltd. generous gifts of materials.

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