

Elucidation of Solid-State Complexation in Ground Mixtures of Cholic Acid and Guest Compounds

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The solid-state complexation between cholic acid (CA) and either methyl *p*-hydroxybenzoate (MPB) or ibuprofen (IBP) was investigated. Powder X-ray diffractometry, IR spectroscopy and thermal analysis suggested the complex formation between CA and MPB as well as between CA and IBP by co-grinding method. The stoichiometry of CA–MPB was 1 : 1 while that of CA–IBP was 2 : 1, reflecting the effect of guest size on complex formation. The guest compounds were assumed to be included in the channel of complexes formed by hydrogen bonds among CA molecules.

Key words complexation; cholic acid; grinding; inclusion compound; methyl *p*-hydroxybenzoate; ibuprofen

During a few past decades, several attempts have been made to elucidate the molecular complexation between bile acids and chemical compounds. Deoxycholic acid (DCA), a commonly known bile acid, has an ability to form inclusion complexes with a variety of chemical compounds.¹⁾ Viewing the crystal structures,^{2–5)} we can find the guest molecules accommodated in the flexible channel formed by DCA molecules. The channel dimension can be adjusted depending on the size of guest molecules, resulting in an effective complexation with many compounds.

Recently, Miki *et al.* reported the formation of inclusion complex of cholic acid (CA).⁶⁾ Cholic acid, as depicted in Fig. 1a, shows similar chemical structure as DCA and was also characterized as a host for complexation. Numerous compounds were reported to form complexes with CA; including aliphatic, alicyclic and aromatic compounds.⁷⁾ A crystallographic study has extensively been made to clarify molecular arrangement of CA and guest compounds^{8–15)} and it could mainly be classified into two groups. The first group showed no channel (bilayer structure) as exemplified by inclusion complexes of small guest molecules, *e.g.*, methanol, ethanol and 1-propanol.^{8,9)} The second group^{10–15)} obviously showed channel structure, just like that of DCA, and should provide a more suitable space for accommodating larger guest molecules.

The complexes of bile acids have usually been prepared by co-precipitation from a solution containing the bile acids and a guest compound. This method, however, was not always applicable because some solvents were preferentially included to the guest compounds and formed stable bile acid-solvent complexes,⁷⁾ and some guest compounds were easily decomposed in solution state. As an alternative mean for solid-state complexation, the sealed-heating method has been used for complex formation between guest compounds and either cyclodextrin^{16–18)} or amylose.^{19,20)} For bile acids, we have been reported that DCA^{21–23)} and ursodeoxycholic acid (UDCA)²⁴⁾ could be formed inclusion complexes with guest compounds during grinding process. However, complex formation between CA and guest compounds by this method has not been reported so far.

The aim of the present study was to investigate the possi-

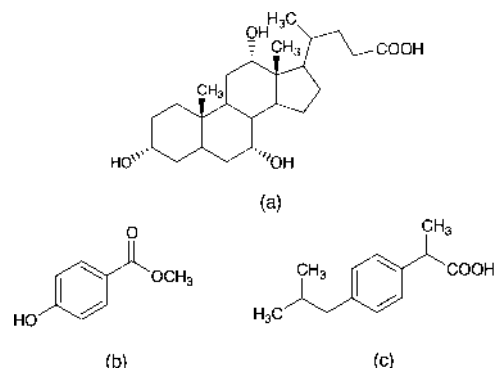


Fig. 1. Chemical Structure of Materials Used in This Study

(a) Cholic acid (CA), (b) methyl *p*-hydroxybenzoate (MPB), and (c) ibuprofen (IBP).

ble complex formation between CA and guest compounds by co-grinding. The guest compounds, including methyl *p*-hydroxybenzoate and ibuprofen, were used to investigate the effect of guest species on complex formation. In addition, the co-precipitation and sealed-heating methods were employed for comparative evaluation of complex formation.

Experimental

Materials Cholic acid and methyl *p*-hydroxybenzoate (MPB) were purchased from Nacalai Tesque (Kyoto, Japan) and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), respectively. Ibuprofen (IBP) was obtained from Kowa Co., Ltd. (Nagoya Japan). All the chemicals were of reagent grade.

Co-precipitation Method Cholic acid and guest compound were dissolved at 55 °C in various solvents which were commonly used for preparation of DCA-guest inclusion complex; including methanol, ethanol and 1-propanol. The solution was cooled down and slowly evaporated at room temperature to promote crystallization. The crystallized specimen was filtered out and checked by powder X-ray diffractometry.

Grinding Method Each physical mixture of CA and guest compound (molar ratio of 1 : 3, 1 : 2, 1 : 1, 2 : 1, 3 : 1) was blending in a vial by using a vortex mixer for 5 min. For the preparation of ground mixture, three grams of each physical mixture was loaded in a mill chamber, made of aluminum oxide, and ground using a CMT, TI-200 vibrational mill (Tochigi, Japan). In case of low temperature grinding, three grams of equimolar physical mixture was loaded in a mill chamber made of stainless steel and ground using a CMT, TI-500 ET (Tochigi, Japan). The liquid nitrogen was gradually fed until the temperature was dropped to –180 °C before grinding.

Sealed-Heating Method Equimolar physical mixture of CA and guest compound (200 mg) was filled in a 2.0 ml glass ampoule. The ampoule was

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then sealed and heated to the desired temperature and time.

Powder X-Ray Diffractometry The powder X-ray diffraction (PXRD) measurements of samples were conducted on a Rigaku (model Miniflex) powder X-ray diffractometer (Tokyo, Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage 30 kV; current, 15 mA; and scanning speed 4°/min.

Infrared Spectroscopy The Fourier-transformed infrared (FT-IR) spectra of samples were obtained as KBr pellet on a JASCO 230 FT-IR spectrophotometer (Tokyo, Japan).

Thermal Analysis The thermal gravimetry (TG) and differential scanning calorimetry (DSC) of samples were performed using DuPont TA 9900 thermal analysis system (Newcastle, U.S.A.) under a constant purge of nitrogen over a temperature range of 30–250 °C at a heating rate of 5 °C/min. Prior to running DSC analysis, the system was calibrated using indium as a reference standard.

Sublimation Experiment The sublimation curves of ibuprofen from the physical and ground mixtures were recorded on the TG module of DuPont TA 9900 thermal analysis system. About 20 mg of samples were placed on a platinum pan and measured at constant temperature of 60 °C under a vacuum.

Results and Discussion

Co-grinding of CA–MPB System Figure 2 shows the change of PXRD patterns of an equimolar physical mixture of CA and MPB during the grinding process. Characteristic diffraction peaks were observed at $2\theta=10.4$ and 12.9° for CA crystals and $2\theta=17.0$ and 25.2° for MPB crystals, respectively. The physical mixture showed the diffraction peaks due to both CA and MPB crystals. After grinding for 5 min, new diffraction peaks, not found in the PXRD patterns of CA and MPB crystals, were observed at $2\theta=11.1$, 13.5 and 20.7° . The intensities of the new diffraction peaks were increased with the prolongation of grinding time, whereas the intensities of the diffraction peaks due to CA and MPB crystals were decreased. After grinding for 15 min, the diffraction peaks of CA and MPB crystals were disappeared. Since one component grinding conducted halo pattern for CA and no change in PXRD pattern for MPB, the results suggested the formation of new crystalline complex between CA and MPB.

In order to study molecular interaction of CA and MPB in

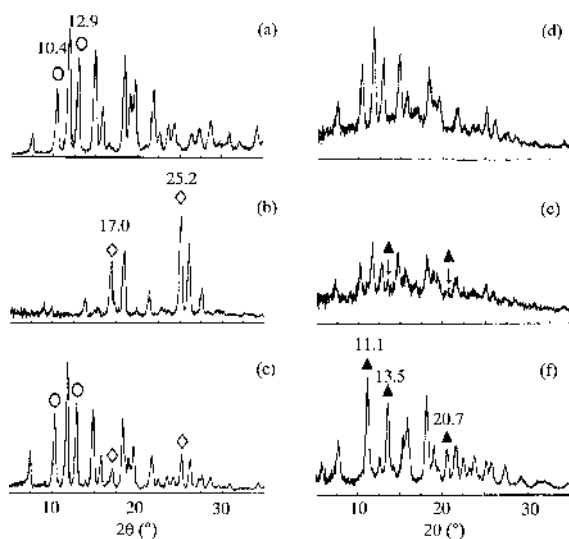


Fig. 2. Change of PXRD Patterns of Equimolar Physical Mixture of CA–MPB after Grinding

(a) CA crystals, (b) MPB crystals, (c) equimolar physical mixture of CA–MPB, (d) sample (c) after grinding for 3 min, (e) after grinding for 5 min, and (f) after grinding for 15 min.

the complex, FT-IR was employed. Figure 3 demonstrates the FT-IR spectra of the CA–MPB system. Cholic acid crystals showed OH-stretching bands at 3523 , 3325 and 3192 cm^{-1} and carbonyl stretching peak at 1716 cm^{-1} . In the spectrum of MPB crystals, OH-stretching band was appeared as a broad band around 3307 cm^{-1} , while sharp C=O stretching peak appeared at 1682 cm^{-1} and C=C benzene ring stretching appeared at 1589 cm^{-1} . The spectrum of the physical mixture was the superimposition of those of CA and MPB crystals. For the ground mixture, the OH stretching band due to CA crystals was disappeared and a new band was observed. In addition, the peak at 1716 cm^{-1} also disappeared and a new diffraction peaks was observed at 1722 cm^{-1} .

Miki *et al.*²⁵ reported that CA crystals (with no guest molecules) showed no channel structure whereas CA–guest complexes demonstrated the bilayer channel structure where guest molecules were accommodated.⁷ The channel structure was formed by the hydrogen bonds, which were not observed in CA crystals, between C=O and OH groups of adjacent CA molecules. The formation of the most suitable hydrogen bond network to accommodate MPB in the channel should be a reasonable explanation of the difference in the IR spectra of CA crystals and CA–MPB complex.

From the comparison of IR spectra of MPB crystals and the ground mixture, it was observed that C=O stretching due to MPB was shift to a higher value of 1697 cm^{-1} after complexation. The results suggested the molecular arrangement of MPB molecules in CA–MPB complex should be different from that in MPB crystals. Viewing the crystal structure of MPB crystals,²⁶ we could find the hydrogen bonds between OH group and C=O group between MPB molecules. Since the higher wave number shift of C=O group was observed in the IR spectrum of CA–MPB complex, the hydrogen bonds between MPB molecules might disappear and the MPB mol-

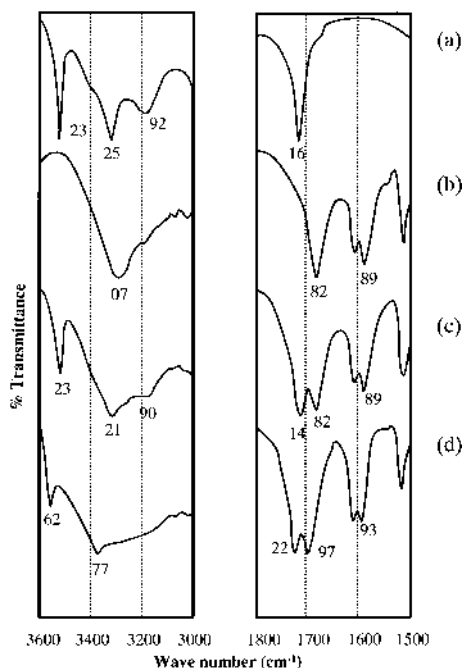


Fig. 3. FT-IR Spectra of CA–MPB Systems

(a) CA crystals, (b) MPB crystals, (c) equimolar physical mixture of CA–MPB and (d) sample (c) after grinding for 15 min.

ecules should be monomolecularly included in the channel of complex. It was also noted that C=C benzene ring stretching of MPB crystals was shifted from 1589 to 1593 cm^{-1} . The van der Waals interaction between CA molecules and accommodated MPB molecules might be associated with the IR shifts as previously observed in other bile acid-guest complex.²¹⁾

Figure 4 illustrates the DSC curves of CA-MPB system. Cholic acid and MPB crystals showed endothermic peaks due to melting at 204 and 127 °C, respectively. The physical mixture demonstrated the fusion peak due to MPB crystals whereas the ground mixture did not show the fusion peak of MPB crystals. The disappearance of the fusion peak of MPB after grinding suggested MPB molecules should be monomolecularly dispersed in the channel of complex. The endothermic peak at *ca.* 170 °C was assumed to be due to the fusion of newly formed crystalline complex between CA and MPB. The result was agreed well with that of IR spectroscopy, confirming the complex formation between CA and MPB.

In order to determine the stoichiometric ratio of the complex, ground mixtures of CA and MPB were prepared at various mixing ratios. DSC measurements were carried out as shown in Fig. 5. The endothermic peak due to melting of MPB crystals was not observed in the 1:1 CA-MPB ground mixture (curve c) whereas the 1:3 and 1:2 CA-MPB ground mixtures (curves a, b) showed clear melting peaks of MPB crystals, indicating the existence of excess amount of MPB crystals in these systems. In contrast, the ground mixtures of CA-MPB at mixing ratio 2:1 and 3:1 showed the endothermic peaks due to melting of excess amount of CA in the 2:1 and 3:1 systems. It is also noted that exothermic peaks at 94 °C due to crystallization of excess CA, which existed in amorphous state in the ground mixtures, were also observed. As a result, it could be reasonable to conclude that

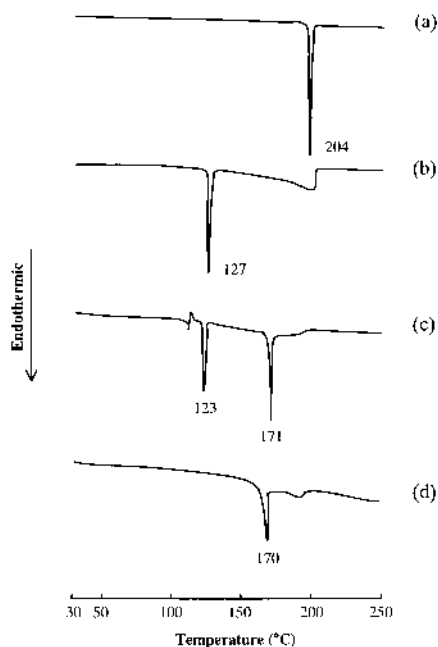


Fig. 4. DSC Curves of CA-MPB Systems

(a) CA crystals, (b) MPB crystals, (c) equimolar physical mixture of CA-MPB and (d) sample (c) after grinding for 15 min.

the stoichiometry of the CA-MPB complex was 1:1.

Co-grinding of CA with Ibuprofen In order to study the effect of guest species on the complex formation, we employed IBP that had larger *p*-substituted groups than MPB as a model compound. Figure 6 shows the change of PXRD of the equimolar physical mixture of CA-IBP after grinding. The new diffraction peaks, which were not observed in PXRD patterns of CA and IBP crystals, were observed after grinding for 15 min. An increase in the new peak intensities through grinding was accompanied by a decrease in peak intensities of CA and IBP crystals. After grinding for 60 min, the diffraction peaks of CA crystal were disappeared while a

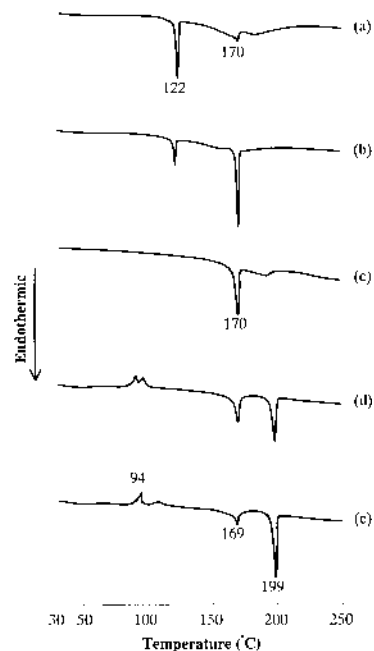


Fig. 5. DSC Curves of CA-MPB Ground Mixtures at Different Molar Ratios

(a) 1:3, (b) 1:2, (c) 1:1, (d) 2:1 and (e) 3:1.

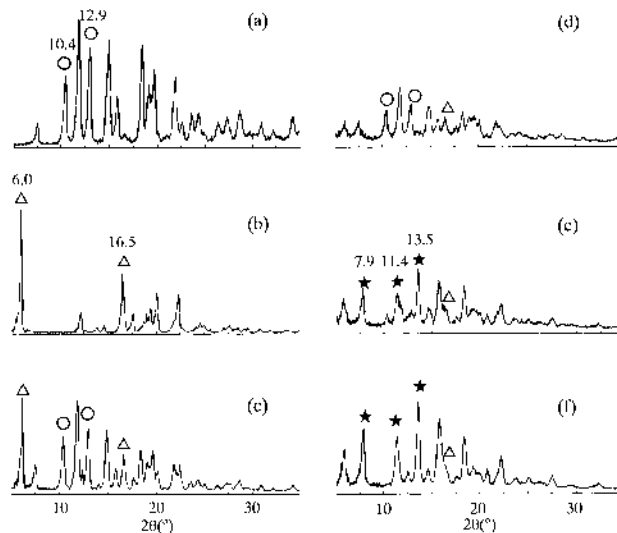


Fig. 6. Change of PXRD Patterns of Equimolar Physical Mixture of CA-IBP after Grinding

(a) CA crystals, (b) IBP crystals, (c) equimolar physical mixture of CA-IBP, (d) sample (c) after grinding for 5 min, (e) after grinding for 15 min, and (f) after grinding for 60 min.

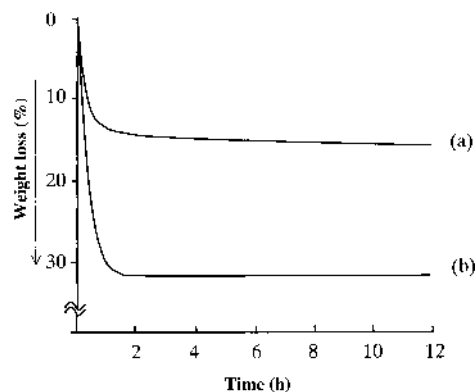


Fig. 7. Sublimation Curves of 1 : 1 CA-IBP Systems (Isothermal at 60 °C, Under Vacuum)

(a) 60 min ground mixture, and (b) physical mixture.

small diffraction peak due to IBP (as indicated by triangle) was still observed. The results indicated that the complex was formed between CA and IBP, however, the stoichiometry of CA-IBP complex seemed to be different from 1 : 1. To determine the exact stoichiometry and study molecular interaction of IBP in the complex, the sublimation experiment was performed. Figure 7 shows sublimation curves of CA-IBP system at 60 °C in a vacuum. Under this condition, IBP crystals were completely sublimated within 1 h. The 1 : 1 CA-IBP physical mixture showed the weight loss of 31% (w/w) after 12 h sublimation (initial IBP content was 33% (w/w)) while the 1 : 1 CA-IBP ground mixture showed weight loss of 15% (w/w), which was almost half of the initial IBP contents. The result suggested that the other half of IBP contents in the ground mixture was suppressed from sublimation because of strong interaction between IBP and CA molecules in the channel of the complex. After sublimation, the content of IBP in the ground sample was assayed as 19.5% (w/w) by UV-spectroscopic method that was in good agreement with the calculated value of 2 : 1 CA-IBP stoichiometry (20.1% (w/w)). Notably, the higher stoichiometry of CA-IBP complex suggested that the channel of complex could be adjusted to accommodate larger guest molecules during the grinding process.

In addition, we attempted to compare the channel dimension of CA-guest complex with other bile acids-guest complexes by using phenanthrene which was reported to form complex with DCA²³) or UDCA.²⁴) However, co-grinding of CA with the phenanthrene could not induce the formation of complex.

Application of Co-precipitation and Sealed-Heating Methods for Complex Formation We also attempted to prepare CA-guest complexes by co-precipitation and sealed-heating methods and compared them with the complexes formed by the grinding method. For the co-precipitation method, we used alcohols that had been empirically used as solvents for preparing DCA-guest complexes. The complex formation between CA and either MPB or IBP, however, was not observed. The solvents preferentially included into the channel over the guest used and the CA-solvent complexes were formed. Miyata and co-workers⁷) also reported the formation of stable complexes with these solvents when using benzene as a guest compound. We also tried another solvents

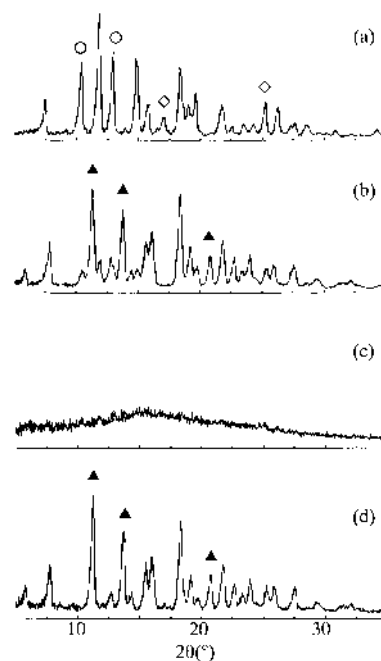


Fig. 8. Effect of Sealed-Heating and Grinding at Low Temperature on the Complex Formation between CA and MPB

(a) 1 : 1 CA-MPB physical mixture; (b) sample (a) after sealed-heating at 115 °C for 60 min; (c) sample (a) after grinding at low temperature for 60 min; and (d) sample (c) after sealed-heating at 60 °C for 60 min.

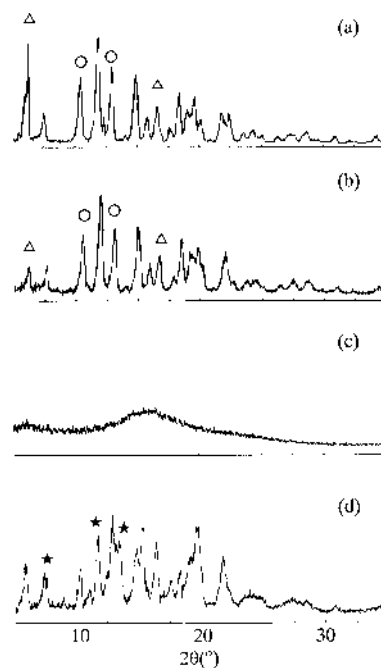


Fig. 9. Effect of Sealed-Heating and Grinding at Low Temperature on the Complex Formation between CA and IBP

(a) 1 : 1 CA-IBP physical mixture; (b) sample (a) after sealed-heating at 100 °C for 60 min; (c) sample (a) after grinding at low temperature for 60 min; and (d) sample (c) after sealed-heating at 100 °C for 60 min.

and found that only CA-IBP complex could be partially formed by co-precipitation from acetone.

In case of sealed-heating method, the complex was prepared by heating the physical mixture of CA-guest compounds at the temperature around the melting point of guest compounds and the results are illustrated in Figs. 8 and 9.

For CA–MPB system, the PXRD pattern of sealed-heated sample (Fig. 8b) was changed to the same pattern as observed in the ground mixture (Fig. 2f), indicating the complex formation by the sealed-heating method. However, the complex formation was not observed in the sealed-heated sample of CA–IBP (Fig. 9b), even after the heating temperature was increased to higher than the melting point of IBP crystals (75 °C). The results suggested that not only the heating temperature but also the mechanical force was required for the complex formation of CA–IBP system. In order to clarify the mechanism, low temperature grinding was applied for the physical mixture of CA–guest compounds. Powder X-ray diffractometry indicated that all the ground samples (Figs. 8c, 9c) were obtained in amorphous state. After the heat treatment to the ground samples, the characteristic X-ray diffraction peaks that observed in corresponding complexes were found (Figs. 8d, 9d). From the results, we could assume that there were two factors concerning to mechanochemical complexation. One was mechanical force required to destroy the crystal structure of CA and guest compounds into unstable state. The other was thermal activation to promote structure relaxation resulting in the crystallization of CA–guest complexes. In the case of CA–IBP system, which has higher CA–guest stoichiometry, it might need more mechanical energy when comparing with CA–MPB system. The results were considered to correlate well with the grinding time for complex formation as illustrated in Fig. 2 and Fig. 6. The complex formation was observed after grinding for 5 and 15 min for CA–MPB and CA–IBP systems, respectively.

Conclusion

The solid-state complex formation between CA and guest compounds was observed during the grinding process. The guest compounds were assumed to be included into the channel that could adjust depending on the size of guest molecules. The higher CA–guest stoichiometry was achieved when using larger guest molecules. Complexation between CA and a guest by co-grinding was considered to consist of two processes, mechanochemical amorphization and molecular arrangement for the inclusion crystal. The latter process was significantly influenced by grinding temperature.

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