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Effects of Grinding on Physical and Chemical Properties of Crystalline Medicinals with Microcrystalline Cellulose. III. Infrared Spectra of Medicinals in Ground Mixtures

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Several drugs, such as benzoic acid, aspirin, salicylic acid, and methyl *p*-hydroxy benzoate, all of which had intermolecular hydrogen bondings, were ground with microcrystalline cellulose respectively. Each drug was ground with β-cyclodextrin too. The inclusion compounds of each drug with β-cyclodextrin were prepared from aqueous solution. When the drugs were ground with β-cyclodextrin, rapid disappearance of X-ray diffraction peaks and that of heat of fusion of drugs, compared with microcrystalline cellulose, were observed. About the inclusion compounds, it was concluded from X-ray diffraction, thermal analysis, and ultraviolet measurements that β-cyclodextrin formed 1:1 adduct with each drug and the drug molecules were preffered oriented.

The conditions of the drug molecules in the ground mixtures and in the inclusion compounds were investigated using infrared spectroscopy, especially by monitoring the carbonyl stretching bands near 1700 cm^{-1}. When the dispersion of drugs into matrices were completed, the distinct frequency shift of the carbonyl stretching bands was observed in the infrared spectra of these sample.

From these results, it would be reasonable to consider that in the ground mixture of microcrystalline cellulose, the drug molecules were present monomolecularly, presumably interacting with cellulose molecules by hydrogen bondings.

Keywords——microcrystalline cellulose; cyclodextrin; hydrogen bonding; grinding; aspirin; amorphous; mechanochemistry; IR; X-ray diffraction

In the previous studies,[2] amorphous state of organic medicinal was obtained by grinding of the medicinal with microcrystalline cellulose. It was also reported that the ground mixture had various peculiar properties, such as rapid dissolution, disappearance of melting endotherm on the thermograms, inhibition of sublimation of volatile medicinals.[3] These phenomena were explained by the concept that the ground mixture was "entropy frozen solution", that is, dissolving in cellulose without the ability of molecular transition movement.

The aim of this investigation is to study the dispersed state of medicinals in the ground mixture using infrared (IR) spectral analysis. Similar to microcrystalline cellulose, β-cyclo dextrin consists of glucopyranose units and is known to form inclusion compounds easily with various medicinals,[4] so it is interesting to compare the behavior of the ground mixture with microcrystalline cellulose with that of β-cyclodextrin inclusion compound and its ground mixture. Physical properties of the inclusion compound and the ground mixture with β-cyclodextrin were investigated by X-ray diffraction, thermal analysis, and IR spectrum. The dispersion state of the ground mixture with microcrystalline cellulose was also discussed.

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Experimental

(1) **Materials**——Microcrystalline cellulose[5] and β-cyclodextrin[6] were kept in a desiccator containing P_2O_5 at room temperature after heating at 110° for 3 hr *in vacuo*. Aspirin,[7] benzoic acid[8] and salicylic acid[9] were of JP IX grade. Methyl *p*-hydroxy benzoate was of special reagent grade.

(2) **Preparation of Ground Mixtures**——Vibrational mill of Heiko Seisakusho Model TI-200, which was made of tungsten carbide, was used. Volume of the mill was 140 cm^3, and height of the rod 55 mm. Total weight of specimen was 2.0 g.

(3) **Preparation of β-Cyclodextrin Inclusion Compounds**——β-Cyclodextrin inclusion compounds were prepared according to the coprecipitation method.[10] 2.5×10^{-3} mol of β-cyclodextrin was dissolved in 50 ml of water, and added 1.25×10^{-2} mol of drug. The solution was agitated vigorously for 5 hr at 60°. The solution was filtrated and stored for 2 days at room temperature. The inclusion compound was obtained as the coprecipitate which was separated by filtration. It was dried for 24 hr at room temperature and washed with ethyl ether, then dried for 24 hr at room temperature. The amount of the drug in the inclusion compound was determined spectrophotometrically using a Hitachi 124 spectrophotometer after the product was dissolved in 65% aqueous ethanol solution of 0.1 N hydrochloric acid.

(4) **X-Ray Diffraction (Powder Method)**——Rigakudenki 2204 Diffractometer was used. The measurement conditions were the same as those reported in the previous paper.[11]

(5) **Differential Scanning Calorimetry**——Perkin-Elmer Model DSC-1B Differential Scanning Calorimeter was used. The measurement was done using the sample pan for the solid sample at the scanning speed of 8°/min from 360 to 450 K. Sample weight was about 4 mg.

(6) **IR Absorption Spectroscopy**——Hitachi EPI-G3 infrared spectrophotometer was used. The measurements were according to the KBr disk method.

Results

(1) IR Spectra of the Ground Mixtures with Microcrystalline Cellulose

Figure 1 shows the IR spectra of microcrystalline cellulose (M.C.C.), aspirin, and the ground mixtures of aspirin with M.C.C. The spectrum of M.C.C. showed strong absorption bands in the region of 3600—3200 cm^{-1} assigned as hydrogen bonded hydroxyl stretching and at 1640 cm^{-1} due to adsorbed water.[12] M.C.C. has no absorption bands in the region of carbonyl stretching vibration (1780—1650 cm^{-1}). So, the discussion will be focussed on the region of carbonyl stretching bands near 1700 cm^{-1}. Curve (B) shows the IR pattern of aspirin crystals, which appeared two absorption bands, the one at 1757 cm^{-1} is due to acetoxyl carbonyl stretching and the other at 1695 cm^{-1} is due to carboxyl carbonyl stretching.[13] These two bands were not affected by the grinding of aspirin alone. Curves (C) and (D) show the IR patterns of the ground mixtures. The broad absorption bands were observed around 1748 cm^{-1} and 1715 cm^{-1} in these IR curves.

Figure 2 shows the IR spectra of the ground mixtures of benzoic acid with M.C.C. The carbonyl stretching band of benzoic acid crystals appeared at 1691 cm^{-1} as shown in curve (B). Curve (C) shows the IR pattern of the ground mixture of 10% benzoic acid and 90% M.C.C. The carbonyl stretching band was shifted to a higher frequency and appeared at 1703 cm^{-1}. Curve (D) shows the IR pattern of the ground mixture of 20% benzoic acid which showed the diffraction peaks due to crystalline benzoic acid on the X-ray diffractogram.

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The carbonyl stretching band was shifted slightly to a higher frequency and appeared at 1694 cm^{-1}. As reported previously,[2] there exists a critical amount over which a crystalline portion remained in the ground mixture. The IR absorption band seemed to consist of the sum of the overlapping spectral lines of crystalline and amorphous benzoic acid.

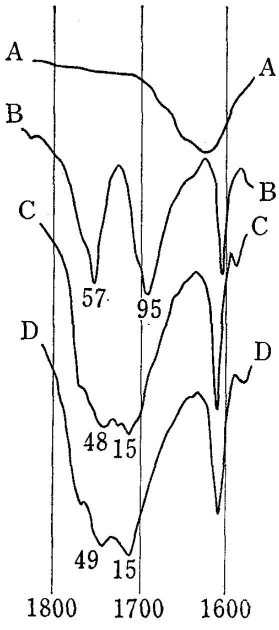

Fig. 1. IR Spectra of the Ground Mixtures of Aspirin with M.C.C.

A: M.C.C.,
B: aspirin crystals,
C: ground mixture (10% aspirin, ground for 30 min),
D: ground mixture (20% aspirin, ground for 240 min).

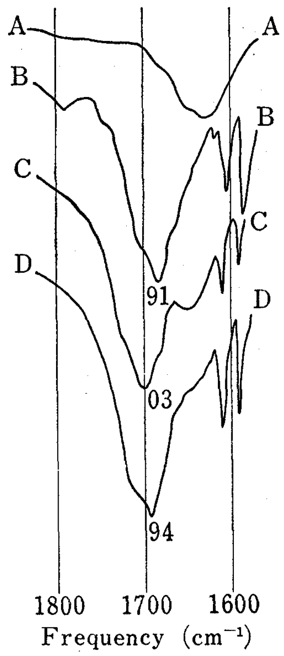

Fig. 2. IR Spectra of the Ground Mixtures of Benzoic Acid with M.C.C.

A: M.C.C.,
B: benzoic acid crystals,
C: ground mixture (10% benzoic acid, ground for 120 min),
D: ground mixture (20% benzoic acid, ground for 240 min).

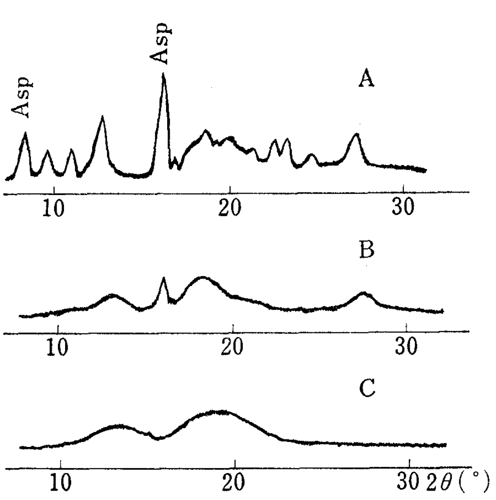

Fig. 3. X-Ray Diffraction Patterns of the Mixture of Aspirin with β-Cyclodextrin (Molar Ratio=1:1)

A: physical mixture,
B: ground mixture (ground for 2 min),
C: ground mixture (ground for 3 min).

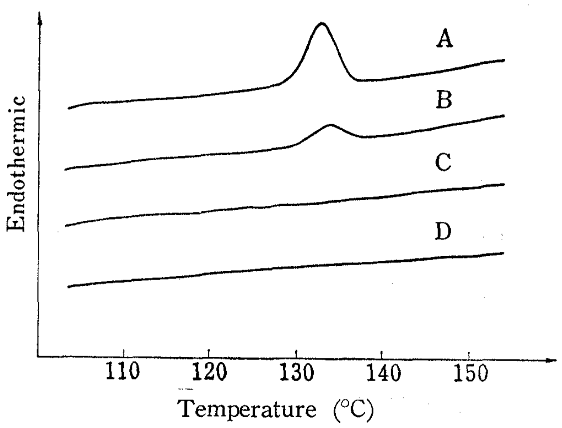

Fig. 4. DSC Curves of the Mixture and Inclusion Compound of Aspirin with β-Cyclodextrin (Molar Ratio 1:1)

A: physical mixture,
B: ground mixture (ground for 2 min),
C: ground mixture (ground for 3 min),
D: inclusion compound.

For methyl *p*-hydroxy benzoate crystals, the carbonyl stretching band appeared at 1684 cm^{-1}. In the ground mixture of 10% methyl *p*-hydroxy benzoate with 90% M.C.C. which showed halo pattern on the X-ray diffractogram, carbonyl stretching band was shifted 17 cm^{-1} to a higher-frequency and appeared at 1701 cm^{-1}. In the ground mixture of 10% salicylic acid with 90% M.C.C., the carbonyl stretching band appeared at 1671 cm^{-1}, while the IR spectrum of salicylic acid crystals showed carbonyl stretching band at 1664 cm^{-1}.

(2) The Ground Mixture of Medicinals with β-Cyclodextrin

As the shifting of carbonyl stretching bands of the medicinals was observed upon grinding with M.C.C., crystalline medicinals were also ground with β-cyclodextrin, which contained seven D(+)-glucopyranose units attached by α-(1,4) linkages, in order to examine the state of the medicinals in the ground mixture. X-ray diffraction patterns of the equimolar mixture of aspirin and β-cyclodextrin (containing 12% of aspirin) are shown in Fig. 3. The mixture of 2 min grinding, the crystalline peaks were still remained, but the peak intensities of each characteristic line decreased considerably, then only halo pattern was observed after 3 min grinding. The thermal characteristics of the ground mixture were also measured by DSC as shown in Fig. 4. Endothermic heat due to the fusion of aspirin was observed in the mixture of 2 min grinding at about 130°, but the mixture of 3 min grinding did not show the heat of fusion of aspirin on the thermogram. It agreed very closely with the phenomena that obtained from the ground mixture with M.C.C. The grinding of the medicinals with

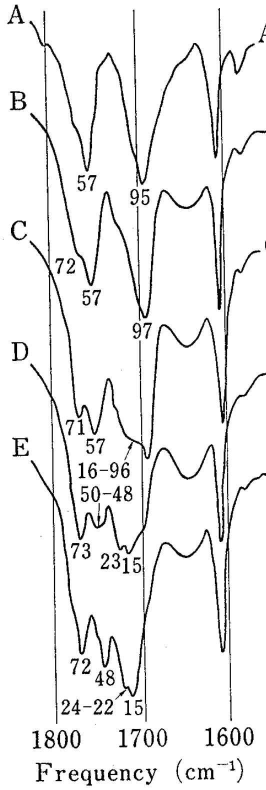

Fig. 5. IR Spectral Change of Ground Mixture of Aspirin with β-Cyclodextrin (Molar Ratio=1:1)

A: aspirin crystals,
B: ground for 2 min,
C: ground for 3 min,
D: ground for 4 min,
E: ground for 15 min.

Table I. Combining Ratio of Drug to β-Cyclodextrin in Inclusion Compounds

Drugs	mol/mol of β-CD
Aspirin	0.97
Benzoic acid	1.00
Methyl *p*-hydroxy benzoate	0.98
Salicylic acid	0.86

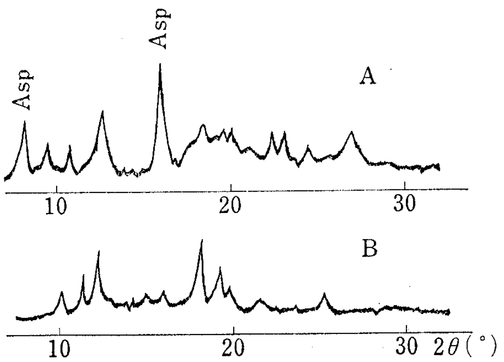

Fig. 6. X-Ray Diffraction Patterns of Inclusion Compound and Physical Mixture of Aspirin with β-Cyclodextrin

A: physical mixture.
B: inclusion compound.

β-cyclodextrin converted the medicinals into amorphous state more rapidly than the grinding with M.C.C. β-Cyclodextrin is macrocyclic polymer of glucose while M.C.C. is rigid linear polymer. It seems likely that the efficiency of grinding is affected by the difference of the structure between these compounds.[14] Fig. 5 shows the time course of IR spectra of the ground mixture of aspirin with β-cyclodextrin. From these spectra, it can be seen that the carbonyl stretching absorption frequencies are changed with increasing grinding time. Curve (B) shows the IR spectrum of the mixture of 2 min grinding. A new shoulder appeared at 1772 cm^{-1} which was higher frequency than acetoxyl carbonyl stretching band and eventually became a well resolved peak, whereas acetoxyl carbonyl stretching band observed at 1757 cm^{-1} diminished with increasing grinding time. Curve (C) shows the IR spectrum of the mixture of 3 min grinding, which showed halo pattern on the X-ray diffractogram. The carboxyl carbonyl stretching band of aspirin was shifted to a higher frequency compared to aspirin crystal and a broad band was observed from 1716 to 1696 cm^{-1}. Curve (D) shows the IR spectrum of the mixture of 4 min grinding. The 1757 cm^{-1} band due to aspirin crystals disappeared and new band appeared from 1748 to 1750 cm^{-1}. Continued grinding leads to the disappearance of the 1757 cm^{-1} and 1695 cm^{-1} bands, and the appearance of new bands at 1772 cm^{-1}, 1748 cm^{-1}, and 1715 cm^{-1}, as shown in curve (E) which was 15 min ground mixture. These three peaks did not change their frequencies after longer time grinding. From the time course of the IR spectra of the ground mixture, it can be probably said that the band at 1772 cm^{-1} is assigned to acetoxyl carbonyl stretching, the band at 1715 cm^{-1} is assigned to carboxyl carbonyl stretching. The assignment of the band at 1748 cm^{-1}, however, is not determined on the basis of above result.

(3) Comparison of the Inclusion Compound and the Ground Mixture of Medicinals with β-Cyclodextrin

Aspirin, benzoic acid, salicylic acid, and methyl *p*-hydroxy benzoate formed the inclusion compounds with β-cyclodextrin, the ratio of medicinal to β-cyclodextrin in the inclusion compound is shown in Table I. In all medicinals, the combining ratio of the medicinal to β-cyclodextrin was determined as about 1:1 by chemical analysis. This indicates that one molecule of the medicinal interacts with one molecule of β-cyclodextrin in the crystal.

The thermal characteristics of the inclusion compounds were measured by DSC. The endothermic peak due to the fusion of aspirin was observed in the physical mixture of aspirin with β-cyclodextrin, but the endothermic peak was disappeared in the inclusion compound (Fig. 4). Fig. 6 shows the X-ray diffraction patterns of the inclusion compound and the physical mixture of aspirin with β-cyclodextrin. The diffraction pattern of the physical mixture (A) consists of the superposition of the patterns of aspirin and β-cyclodextrin, but the diffraction pattern of inclusion compound (B) is different from that of the physical mixture. Other medicinals used in this experiment also showed similar behavior in X-ray diffractogram observed in the case of aspirin.

The crystal structures of β-cyclodextrin inclusion compounds are not very known at present,[15] while many X-ray crystallographic studies are reported about α-cyclodextrin inclusion compounds.[16] Preliminary results from X-ray analyses of cyclodextrin complexes suggest that the guest molecules locate inside the void of the cyclodextrin molecules.[17]

From the obtained results of X-ray diffraction and DSC thermal analyses, the medicinal molecules might be included by β-cyclodextrin molecules, ensuring stable positions within the crystalline inclusion compounds.

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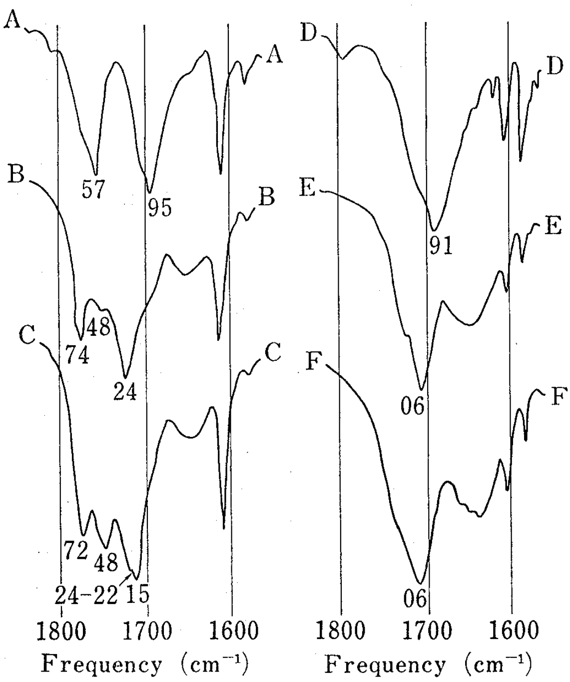

Fig. 7. IR Spectra of Inclusion Compounds and Ground Mixtures of Aspirin and Benzoic Acid with β-Cyclodextrin

β-Cyclodextrin–aspirin (left hand),
A: aspirin crystals,
B: inclusion compound,
C: ground mixture.
β-Cyclodextrin–benzoic acid (right hand),
D: benzoic acid crystals,
E: inclusion compound,
F: ground mixture.

The IR spectra of inclusion compounds and the ground mixtures of aspirin and benzoic acid with β-cyclodextrin are shown in Fig. 7. In the IR spectra of the inclusion compound of aspirin, two sharp peaks are observed at 1774 cm^{-1}, and 1724 cm^{-1} as shown in curve (B). These two peaks are well consistent with the bands in the ground mixture of aspirin with β-cyclodextrin assigned to acetoxyl carbonyl stretching band, and carboxyl carbonyl stretching band. Furthermore, a shoulder was observed at about 1748 cm^{-1} in the inclusion compound and is consistent with the unassigned peak in the ground mixture.

In the inclusion compound of benzoic acid, carbonyl stretching band was appeared at higher frequency than benzoic acid crystal. The carbonyl stretching band appeared at 1706 cm^{-1} and was consistent with the ground mixture. It is known that dissociation of the intermolecular hydrogen bonding causes shifting to a higher frequency.

Discussion

It is well known that the formation of hydrogen bonding causes lower frequency shift of carbonyl stretching bands. In the crystals of most simple carboxylic acids, the molecules are strongly linked to their neighbours by hydrogen bonding between oxygen atoms and hydroxy groups. Allen and his coworkers studied the association of benzoic acid in dilute solution of non-polar solvents by IR spectra, and reported that the carbonyl stretching band which was assigned to "free" carbonyl group was observed at 47 cm^{-1} higher frequency than the carbonyl stretching band which was assigned to "hydrogen bonded" carbonyl group.[18] In the IR spectra of the inclusion compounds and the ground mixtures of the medicinals with β-cyclodextrin, the carbonyl stretching bands shifted to a higher frequency. This seemed to have a strong relation with the dissociation of hydrogen bonding in the medicinal crystals.[19] The crystal lattice of medicinals was assumed to be destroyed by the grinding force and to convert into paracrystal lattice or monomolecular state.

The medicinal molecules interact with β-cyclodextrin stoichiometrically in the inclusion compounds. This supports that medicinal molecules in the inclusion compounds exist in the β-cyclodextrin matrix monomolecularly rather than micro-assembly of molecules. The IR spectra of the ground mixtures are almost the same as those of the inclusion compounds. So, it assumed that the medicinal molecules in the ground mixtures exist in monomolecular dispersion as same as in the inclusion compounds.

It has been suggested that cyclodextrin forms inclusion complexes with organic substrates in aqueous solution.[20] Moreover, it has been reported that even molecules, which

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were too large to be included within the void of cyclodextrin, interact with the cyclodextrin by means of intermolecular hydrogen bonding from outside of the cavity.[21]

In the IR spectra of the ground mixture of medicinals with M.C.C., the carbonyl stretching band is also observed at higher frequency than that of medicinal crystals. This also indicates that the medicinal molecules exist in the matrix as a monomolecular dispersion in a similar manner with the ground mixture of medicinal with β-cyclodextrin. Dissociation of the dimer in the medicinal crystals means the rupture of two hydrogen bondings. When the carbonyl group becomes "free", the carbonyl stretching bands move to 40—50 cm^{-1} higher frequency. The shift in frequency is a valuable measure of the strength of the hydrogen bonding.[19] In this study, observed band shifts were in less than 20 cm^{-1}, this suggests that the intermolecular hydrogen bonds were destroyed imperfectly or intermolecular hydrogen bondings other than those between medicinal molecules were formed by the grinding.

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