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The Dispersed States of Medicinal Molecules in Ground Mixtures with α- or β-Cyclodextrin[1)]

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Aspirin, benzoic acid and *p*-hydroxybenzoic acid, all of which form intermolecularly hydrogen-bonded dimer structures in the crystalline form, were ground with α- or β-cyclodextrin. Inclusion compounds were also prepared by the coprecipitation method, except in the case of the aspirin–α-cyclodextrin system. The dispersed state of the medicinal molecules were investigated by analysis of the infrared spectra in the carbonyl stretching regions. It was suggested that the dispersed state of medicinals in the α-cyclodextrin system was different from that in the β-cyclodextrin system. It was assumed that, by grinding, aspirin molecules were included in the cyclodextrin cavity in the β-cyclodextrin system, but were dispersed monomolecularly in the hydrogen-bonded network structure of cyclodextrin in the α-cyclodextrin system. Interaction between medicinals and α- or β-cyclodextrin in aqueous solution was investigated by means of nuclear magnetic resonance studies.

The sublimation of *p*-hydroxybenzoic acid from the ground mixtures or inclusion compounds with α- or β-cyclodextrin systems was determined by thermogravimetry. The effects of cyclodextrin on the hydrolysis of aspirin under acidic conditions were investigated as well.

Keywords——cyclodextrin; dispersion; grinding; IR; inclusion; sublimation; hydrolysis; NMR

In the previous paper, the authors reported that the amorphous states of medicinals can be obtained by the grinding of medicinals with microcrystalline cellulose.[2)] The dispersed states of medicinals in the microcrystalline cellulose ground mixtures were discussed using β-cyclodextrin, which is well known to form inclusion compounds with various organic molecules,[3)] as a model compound. Recently, the application of cyclodextrins to pharmaceutical technology has become of more interest for various reasons, and the crystal structures of several inclusion compounds have been determined.[4)]

In the present paper, in order to investigate the mechanism of the conversion of crystalline medicinals into the amorphous state by grinding with cyclodextrins or microcrystalline cellulose, medicinals were ground with cyclodextrins of different cavity sizes. The molecular behavior of the medicinals in the ground mixtures is discussed on the basis of the infrared (IR) spectra.

Experimental

Materials——α-[5)] and β-cyclodextrin[6)] were kept in a desiccator containing P_2O_5 at room temperature after being heated at 110 °C for 3 h *in vacuo*. *p*-Hydroxybenzoic acid[5)] was of special reagent grade. Aspirin[7)] and benzoic acid[5)] were of JPX grade.

Preparation of Ground Mixtures——Equimolar mixtures of a medicinal and cyclodextrin were made using a mortar and pestle. The ground mixtures were prepared by grinding the physical mixtures in a vibrational mill (Heiko Seisakusho, model TI-200). The total weight of each specimen was 2.0 g.

Preparation of Inclusion Compounds——For systems that showed Bs-type phase solubility diagrams,[8)] inclusion

compounds were made as precipitates from the aqueous solution. The required amounts of medicinal and cyclodextrin were calculated from the descending curvature of the phase solubility diagram obtained beforehand.

Powder X-Ray Diffraction——Powder X-ray diffraction patterns were measured as described previously.[3]

IR Absorption Spectroscopy——A Hitachi 295 IR spectrophotometer was used. The measurements were made by the KBr disk method. Wave numbers were corrected on the basis of standard absorptions of polystyrene film.

Measurement of Sublimation Rates of *p*-Hydroxybenzoic Acid——A Shimadzu TG-20 thermogravimetry apparatus was used. Ten mg of sample was raised to the desired temperature (180 or 210 °C) at a heating rate of 20 °C/min and then kept at constant temperature. The operating conditions were as follows: range 50 μV, full scale 2 mg. Sublimation rates were determined from the weight loss observed after keeping the samples at the desired temperature for 30 min.

Nuclear Magnetic Resonance (NMR) Measurement——The 100 MHz ^{1}H NMR spectra were observed using a JEOL JNH-MH-100 spectrophotometer. The 200 MHz ^{1}H and ^{13}C NMR spectra were observed using a Varian XL-200 spectrometer. Tetramethylsilane was used as an external reference for D_2O solutions and no correction was made for susceptibility of the capillary.

Kinetic Study of Aspirin Hydrolysis——Kinetic studies were carried out at 15, 25, 35, 45 and 55 °C. Aspirin (6.0×10^{-4} M) and cyclodextrin (3.0×10^{-3} M) were added to pH 1.0 buffered solution (0.20 M KCl–0.20 M HCl). Aliquots were withdrawn periodically from the vials and analyzed spectrophotometrically at 276 and 304 nm in a Hitachi model 124 spectrophotometer. The amount of aspirin remaining was determined by using two-component analysis.[9] The hydrolysis rate constants were calculated from the slopes of the first-order curves.

Results and Discussion

IR Spectra of the Ground Mixtures with Cyclodextrins

(a) *p*-Hydroxybenzoic Acid System——It was recognized from powder X-ray diffraction studies that the grinding of medicinals with α-cyclodextrin converted both components into the amorphous state readily, as was the case with β-cyclodextrin. Figure 1 shows the carbonyl stretching band in the IR spectra of the *p*-hydroxybenzoic acid and α-cyclodextrin system. Curve C shows the IR spectrum of the inclusion compound, in which the carbonyl band is shifted to a higher frequency compared with the *p*-hydroxybenzoic acid crystals, and appears at 1701 cm^{-1}. The crystal structure of α-cyclodextrin and *p*-hydroxybenzoic acid inclusion compound has been determined by an X-ray method.[10] It was demonstrated that the α-cyclodextrin molecule forms a 1 : 1 complex with *p*-hydroxybenzoic acid and that hydrogen bonding occurs between hydroxyl of α-cyclodextrin and carbonyl of *p*-hydroxybenzoic acid. Therefore, the observed higher frequency shift is a result of the destruction of structure in the *p*-hydroxybenzoic acid crystal, and the formation of hydrogen bonding of monomeric *p*-

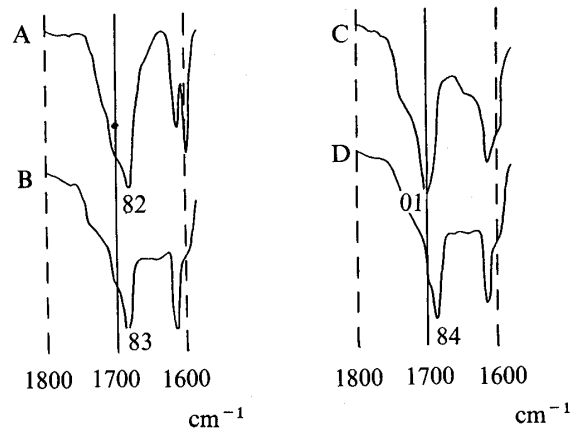

Fig. 1. IR Spectra of *p*-Hydroxybenzoic Acid in the Presence of α-Cyclodextrin

A, *p*-hydroxybenzoic acid (*p*-HBA) crystal; B, *p*-HBA–α-cyclodextrin ground mixture (ground for 5 min); C, *p*-HBA–α-cyclodextrin inclusion compound; D, ground sample of *p*-HBA–α-cyclodextrin inclusion compound (ground for 15 min).

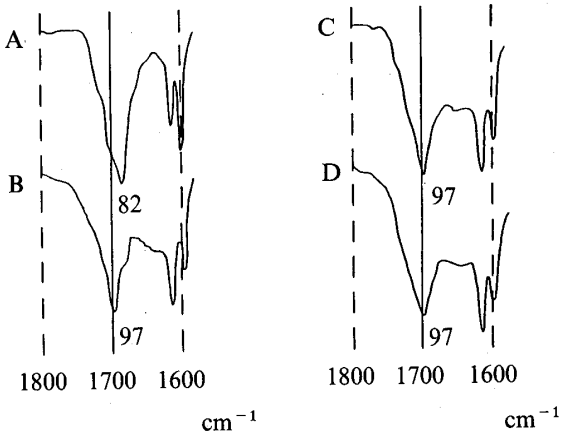

Fig. 2. IR Spectra of *p*-Hydroxybenzoic Acid in the Presence of β-Cyclodextrin

A, *p*-HBA crystal; B, *p*-HBA–β-cyclodextrin ground mixture (ground for 5 min); C, *p*-HBA–β-cyclodextrin inclusion compound; D, ground sample of *p*-HBA–β-cyclodextrin inclusion compound (ground for 15 min).

hydroxybenzoic acid with α-cyclodextrin. Curves B and D show the ground mixture and the ground sample of the inclusion compound, respectively. In these samples, however, the carbonyl bands were observed at 1683 and 1684 cm^{-1}, which are almost the same positions as in the crystal. This phenomenon suggests that the bond strength of carbonyl groups of both ground samples is nearly the same as that of the crystal, in which a cyclic dimer structure is formed.[11)]

The relative intensity of the doublet bands due to skeletal vibration of the bnezene ring, observed at 1594 and 1607 cm^{-1} in the crystal, was remarkably changed in curves B, C and D, that is, the ground mixture, the inclusion compound and the ground inclusion compound, respectively. This suggests that the environment of the benzene ring of the *p*-hydroxybenzoic acid molecule is significantly changed in these samples. It seems unlikely that the grinding of the inclusion compound causes a change in the included state of *p*-hydroxybenzoic acid.

These results for the ground samples leave the following possibilities: (1) two *p*-hydroxybenzoic acid molecules form a dimeric structure and both of the phenyl moieties are included in the cyclodextrin cavity, or (2) *p*-hydroxybenzoic acid molecules are dispersed monomolecularly and strong hydrogen bondings are formed between carbonyl groups of *p*-hydroxybenzoic acid and hydroxyl groups of cyclodextrin.

Figure 2 shows the IR spectra of the *p*-hydroxybenzoic acid and β-cyclodextrin system. In the ground mixture, the inclusion compound and the ground sample of inclusion compound, the carbonyl bands were all similarly shifted 15 cm^{-1} to higher frequency and appeared at 1697 cm^{-1}. This indicates that *p*-hydroxybenzoic acid molecules in the ground mixture are dispersed monomolecularly in the cyclodextrin cavity in the same manner as in the inclusion compound, and hydrogen bondings are formed between carbonyl of *p*-hydroxybenzoic acid and hydroxyl of β-cyclodextrin. These bondings are weaker than those in the *p*-hydroxybenzoic acid crystal.

To clarify the dispersed state of *p*-hydroxybenzoic acid molecules in the ground mixtures in the α- and β-cyclodextrin systems, sublimation rates were measured with a thermogravimetry apparatus to keep the samples at constant temperature. Table I shows the sublimation rates of *p*-hydroxybenzoic acid in the cyclodextrin systems at 180 and 210 °C. The sublimation was considerably inhibited for both inclusion compounds. In the α-cyclodextrin system, some sublimation was observed in the ground mixture and the ground sample of inclusion compound at 180 °C. If *p*-hydroxybenzoic acid molecules form strong hydrogen bonds with the hydroxyl group of α-cyclodextrin molecules, sublimation must be inhibited significantly. Hence it is reasonable to consider that dimer structure of *p*-hydroxybenzoic acid is present in the α-cyclodextrin ground systems, that is, possibility (1) mentioned above.

TABLE I. Sublimation Rates of *p*-Hydroxybenzoic Acid from Various States

	α-Cyclodextrin		β-Cyclodextrin	
	180 °C	210 °C	180 °C	210 °C
Physical mixture	1.04	1.01	1.07	1.03
Ground mixture	0.13	0.59	0.04	0.24
Inclusion compound	0.00	0.00	0.01	0.10
Ground sample of inclusion compound	0.23	0.63	0.07	0.42

Apparent rate constants were calculated by equation. $(dW/W \cdot 30) \times 100$; dW=amount of sublimed *p*-hydroxybenzoic acid; W=initial amount of *p*-hydroxybenzoic acid; measurement time 30 min.

In the β-cyclodextrin system, the ground mixture and the ground sample of inclusion compound exhibited appreciable inhibition of sublimation, though this effect was weaker than that in the case of the inclusion compound. These results are consistent with the IR data. Hence, the ground mixture and the ground inclusion compound are in the same state as the inclusion compound.

The inclusion compound with α-cyclodextrin markedly inhibited the sublimation, presumably because of greater restraint due to the tighter fit in the cavity.

(b) Aspirin System——X-Ray diffraction peaks disappeared after a 5-min grinding of the equimolar mixture of α-cyclodextrin and aspirin, indicating the production of the amorphous state. Figure 3 shows the IR spectra of the aspirin and α-cyclodextrin, β-cyclodextrin or microcrystalline cellulose systems. The latter two have already been discussed.[2,3] Aspirin crystal shows two carbonyl absorption bands; the one at 1757 cm^{-1} is assigned to acetoxyl carbonyl stretching and the other at 1695 cm^{-1} to carboxyl carbonyl stretching. In the ground mixture with β-cyclodextrin, three absorption bands were observed around 1771, 1749 and 1718 cm^{-1}. In the α-cyclodextrin system, however, only a weak shoulder is observed at the higher frequency. In Fig. 3, it is clear that spectrum B is similar to the spectrum D rather than to C. From these spectral shifts, the state of aspirin molecules in the ground mixture with α-cyclodextrin is concluded to be similar to that in the ground mixture with microcrystalline cellulose.

Figure 4 shows the solubility phase diagram of aspirin with the addition of cyclodextrins. It is known that the relationship between the apparent solubility of a medicinal and the concentration of additive is influenced by the stability constant and the solubility of the inclusion compound.[12] In the β-cyclodextrin system, a Bs-type phase diagram (indicating that an insoluble complex is formed[8]) was obtained. In the α-cyclodextrin system, however, a gently sloping A_L-type diagram was obtained, indicating a weak interaction between the host and the guest molecules. These results suggest that the molecular size of aspirin is too large for inclusion in the α-cyclodextrin cavity. Thus, aspirin and α-cyclodextrin can not form the inclusion compound during grinding. In the α-cyclodextrin ground mixture, the aspirin molecules appear to be dispersed monomolecularly in an intermolecular hydrogen bonding network by grinding. This mode of dispersion is similar to that of the ground mixture with microcrystalline cellulose. IR spectral studies are also in accord with this view.

NMR Studies——NMR spectra were measured to examine the interaction between

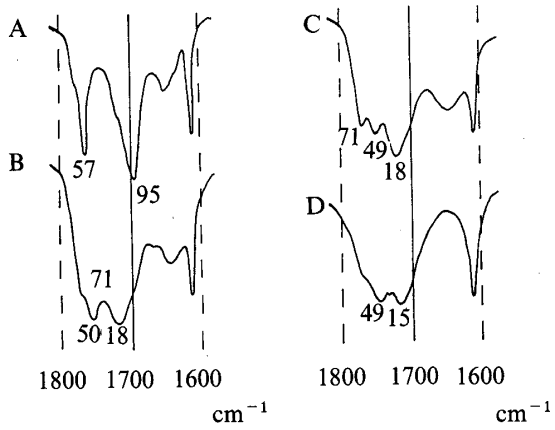

Fig. 3. IR Spectra of Aspirin Ground Mixtures

A, aspirin crystal; B, aspirin–α-cyclodextrin ground mixture (ground for 5 min); C, aspirin–β-cyclodextrin ground mixture (ground for 5 min); D, 10% aspirin–microcrystalline cellulose ground mixture (ground for 30 min).

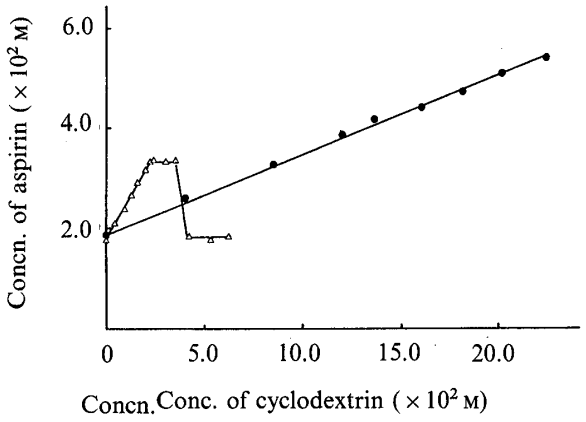

Fig. 4. Phase Solubility Diagram of Aspirin–Cyclodextrin Systems in Water at 20 °C

●, aspirin–α-cyclodextrin system; △, aspirin–β-cyclodextrin system.

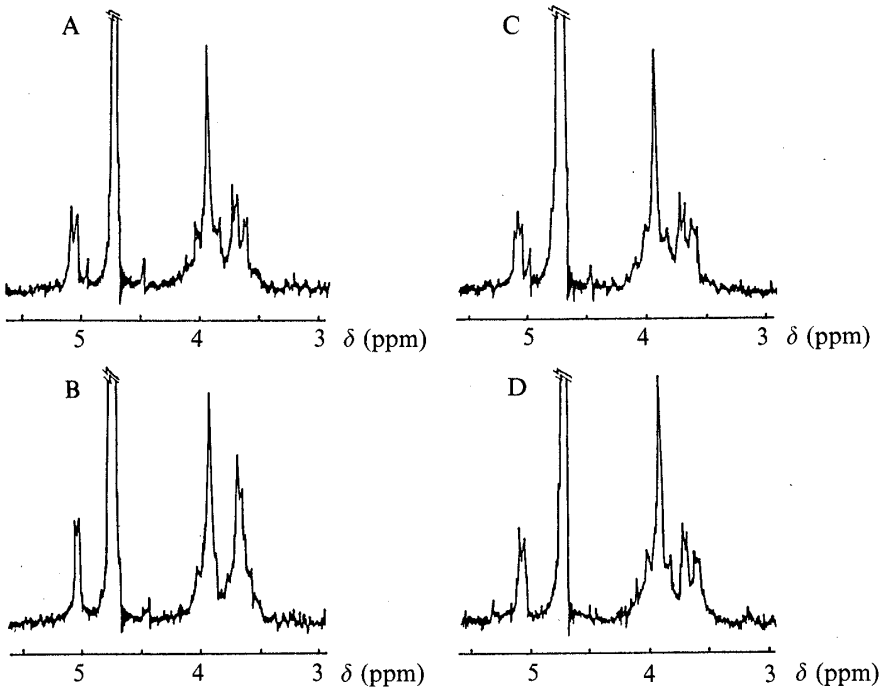

Fig. 5. The 100 MHz ^{1}H NMR Spectra of α-Cyclodextrin and Drug Mixtures in D_2O

A, α-cyclodextrin; B, α-cyclodextrin and *p*-hydroxybenzoic acid mixture; C, α-cyclodextrin and aspirin mixture; D, α-cyclodextrin and benzoic acid mixture.

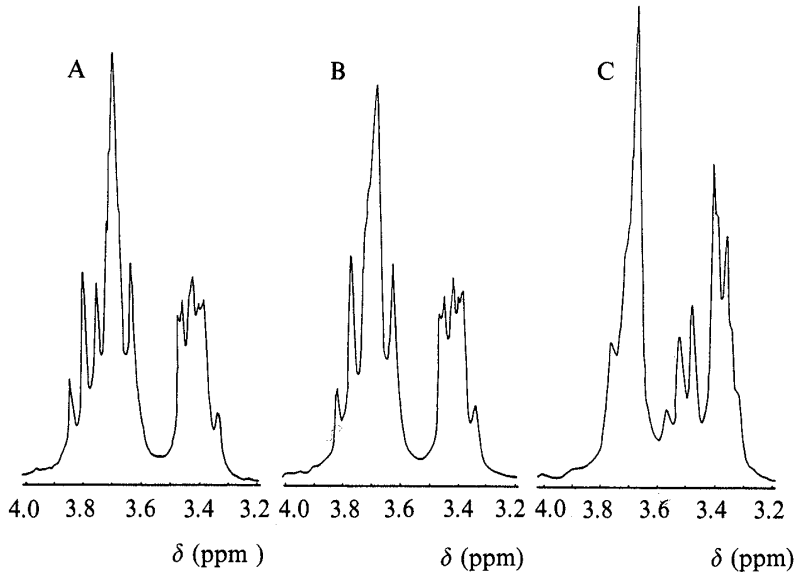

Fig. 6. The 200 MHz ^{1}H NMR Spectra of α-Cyclodextrin and Drug Mixtures in D_2O

A, α-cyclodextrin; B, α-cyclodextrin and aspirin mixture; C, α-cyclodextrin and benzoic acid mixture.

cyclodextrins and medicinals.[13] Figure 5 shows the 100 MHz ^{1}H NMR spectra of α-cyclodextrin (1.5×10^{-2} M) and equimolar mixtures of medicinals with α-cyclodextrin in D_2O. Proton signals assigned to cyclodextrin are observed in the range of $\delta = 3.0 - 5.5$. The addition of *p*-hydroxybenzoic acid caused a high-field shift of cyclodextrin proton signals. Demarco and his coworkers reported that if cyclodextrin forms an inclusion complex with aromatic substrates, protons located within or near the cavity should be strongly shielded.[13] In the case of the α-cyclodextrin and *p*-hydroxybenzoic acid system, it is considered that the medicinals

are included in the cyclodextrin cavity. However, appreciable changes were not observed on the addition of aspirin or benzoic acid to α-cyclodextrin D_2O solutions. The 200 MHz ^1H NMR spectra were also measured and are shown in Fig. 6. Appreciable high-field shifts and spectral changes were observed upon addition of benzoic acid, but only a change of the triplet bands at $\delta = 3.78$ was observed upon addition of aspirin. The ^{13}C NMR spectra of aspirin or benzoic acid with α-cyclodextrin were also examined. The mean values of chemical shifts of α-cyclodextrin were determined as $\Delta\delta = (\sum |\delta_{free} - \delta_{add}|)/n$, where δ_{free} and δ_{add} mean the chemical shifts observed without and with medicinals, respectively, and n is the number of chemical shifts used in the calculation (in this case $n = 6$). Upon addition of aspirin or benzoic acid, the values were calculated to be 0.09 and 0.24, respectively. The data show that the magnetic environmental changes around carbons of cyclodextrin are significant when benzoic acid is added.

It is concluded from the NMR spectra that *p*-hydroxybenzoic acid and benzoic acid are included in the cavity of α-cyclodextrin in aqueous solution.

The 100 MHz ^1H NMR spectra were also measured for β-cyclodextrin systems. High-field shifts and marked signal variations of β-cyclodextrin were observed upon addition of medicinals. In the case of the β-cyclodextrin system, aspirin, benzoic acid and *p*-hydroxybenzoic acid are all included in the cavity of β-cyclodextrin in aqueous solution.

Effect of Cyclodextrin on the Hydrolysis of Aspirin

Cyclodextrins have been studied extensively as a model for enzymes because of their catalytic activity resulting from the formation of inclusion compounds, causing microscopic changes in the surroundings of the guest molecules.[14] Lach *et al.* have studied the cyclodextrin-catalyzed hydrolysis of aspirin in alkaline solution.[15] They demonstrated that α- and β-cyclodextrins accelerated the hydrolysis of aspirin, and this acceleration effect was due primary to the partial inclusion of aspirin in the cyclodextrin cavity followed by interaction of the ester with the hydroxyl group of cyclodextrin present as an alkoxide ion.

In pH 1.0 acidic solution, in which cyclodextrin is in the molecular form, the hydrolysis of aspirin in the presence of α-, β-, or γ-cyclodextrin was found to follow first-order kinetics. The rate constants are given in Table II. While the rate constants showed no significant variations in the presence of α-cyclodextrin, a deceleration was observed in the presence of β-cyclodextrin at all temperatures investigated. Addition of γ-cyclodextrin resulted in lower rate constants, but not as low as in the case of β-cyclodextrin. It is known that hydrolysis of esters under acidic conditions is accelerated by the addition of a proton to the oxygen of the carbonyl group, and that the nucleophilic attack of a water molecule at the carbon of a

TABLE II. First-Order Rate Constants of Aspirin Decomposition at pH 1.0

	Rate constants (d^{-1}) at					E_a (kJ/mol)
	15 °C	25 °C	35 °C	45 °C	55 °C	
Control (without cyclodextrin)	0.0760	0.200	0.536	1.17	2.86	71.1
With α-cyclodextrin	0.0759 (−0.13)	0.206 (+2.94)	0.528 (−1.62)	1.16 (−1.28)	2.83 (−1.26)	70.2
With β-cyclodextrin	0.0493 (−35.2)	0.150 (−25.4)	0.450 (−16.0)	1.09 (−7.17)	2.74 (−4.26)	78.6
With γ-cyclodextrin	0.0703 (−7.55)	0.190 (−5.39)	0.524 (−2.20)	1.16 (−1.11)	2.88 (+0.59)	72.7

The numbers in parentheses show the difference (%) from the control value. E_a is the activation energy of decomposition calculated from Arrhenius plots.

carbonyl group is possible, but the direct action of water on the hydrolysis is negligible.[9] Because the p*K*a value of β-cyclodextrin is about 12,[16] β-cyclodextrin is wholly in the molecular form at pH 1.0. From the NMR analysis, aspirin molecules are assumed to be included in the cavity of β-cyclodextrin, so it is difficult for a proton or water molecule to approach the acetoxyl carbonyl group of included aspirin molecules. This is not in conflict with the fact that the stability constant of the complex formation increased as the temperature was decreased, and that the activation energy of the hydrolysis of aspirin is large only in the β-cyclodextrin system.

On the other hand, addition of α-cyclodextrin did not affect the hydrolysis of aspirin. This is consistent with the NMR data that aspirin molecules are not included in the α-cyclodextrin cavity in aqueous solution. γ-Cyclodextrin has little inhibiting effect on the hydrolysis. This may be attributed to the large void space, into which proton or water molecules are able to penetrate.

References and Notes

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