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GRINDING EFFECT ON SOME PHARMACEUTICAL PROPERTIES OF DRUGS BY ADDING β-CYCLODEXTRIN*

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ABSTRACT

The effect of grinding on the physicochemical properties of ground mixtures of crystalline drugs (acetaminophen, warfarin, indomethacin, diazepam and hydrocortisone acetate) with β-cyclodextrin was studied by IR analysis, x-ray diffraction method and thermal analysis. The crystallinities of drugs decreased with increasing grinding time and became amorphous or nearly amorphous, which depended on drug moiety and cavity size of β-cyclodextrin. The result indicates that acetaminophen became amorphous and only formed an inclusion complex in the ground mixture with β-

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cyclodextrin, although all five drugs interacted with β-cyclodextrin in water. The dissolution rate of drugs from the ground mixtures was shown to be higher than that of the ground drug, crystalline drug or physical mixture, while the dissolution rate of the inclusion complex was the very highest. Physicochemical stability of the ground mixtures stored under 40°C and 75% RH condition was measured by differential scanning calorimetry. In the case of diazepam, indomethacin, warfarin or hydrocortisone acetate and β-cyclodextrin ground mixture, drug was crystallized and the crystallinities increased with the increase of storage time, which reached an equilibrium state after 15 days storage. Whereas, acetaminophen-β-cyclodextrin ground mixture was still amorphous during 60 days storage.

INTRODUCTION

Recently, cyclodextrins have considerable raised interest and attention for their specific chemical modifications and ability to form inclusion complexes (1-3). In many studies, complexation was often found in an aqueous solution (4-8).

Grinding was widely performed as a means to reduce the particle size of powders (9-10). It has been found that grinding not only causes changes in the

molecular behavior of the ground drugs by the addition of additives but also improves the molecular interaction between drugs and additives in the ground mixtures (11-12).

In this study, the effect of grinding with β-cyclodextrin on the properties of the drugs is investigated by infrared spectroscopy, differential scanning calorimetry and X-ray diffractometry. The dissolution behavior of the ground mixtures in water is also determined. Physicochemical stability of ground mixtures in 40°C and 75% RH condition will be examined. In addition, the interaction of β-cyclodextrin with individual drugs in aqueous solution is also studied.

MATERIALS AND METHODS

Materials

Acetaminophen[1] warfarin[2], indomethacin[3], diazepam[2] and hydrocortisone acetate[4] were pharmaceutical grade. β-cyclodextrin[5] was used as an additive. All the other materials were of analytical reagent grade.

Preparation of ground mixtures

The ground mixtures of drugs with β-cyclodextrin in the 1:1 molar ratio were respectively prepared by grinding in a ceramic ball mill for 24 hours. During grinding, the sample was withdrawn at prescribed intervals for further examination.

Preparation of solid complexes

The solid complexes were prepared by dissolving an appropriate amount of the β-cyclodextrin and drugs (acetaminophen, warfarin, indomethacin) in water or ammonia water, molecular ratio=1:1, afterwards these samples were freeze-dried.

Solubility studies

Solubility measurements were carried out at 37±0.5°C according to the method of Higuchi and Connors (13). An apparent stability constant was calculated from the initial straight line portion of the phase solubility diagrams (13).

Membrane permeation studies

The membrane permeation apparatus and method have been described previously (4,14). The permeation of the drug has been expressed according to the following equation (15-16).

$$\ln \left(\frac{Cd - Cr}{Co} \right) = -Pt \quad \cdots\cdots\cdots (1)$$

$$P = \frac{A \; D}{V \; \ell} \quad \cdots\cdots\cdots (2)$$

where Cd and Cr are the concentrations of drug in the donor and receptor cell, respectively, at the time t. Co is the initial concentration of drug in donor cell and P is the apparent permeability constant. The apparent diffusion constant (D) can be obtained from

Eq.(2), where A is the effective surface area of membrane, V is the volume of solution in each cell, and ℓ is the thickness of membrane.

Powder x-ray diffraction study

Powder x-ray patterns of ground mixtures were carried out using a x-ray diffractometer[6] with Ni-filtered Cu-K_α radiation.

Differential scanning calorimetry (DSC)

The DSC patterns of ground mixtures were carried out with DSC calorimeter[7] at a scanning rate 10°C/min under N_2 steam.

Infrared absorption spectroscopy

Studies of the IR spectra of ground mixtures were carried out with IR spectrophotometer[8] using KBr disc method.

Dissolution rate from directly compressed tablets

The physical mixtures and ground mixtures were compressed into a flat-faced tablet, 10 mm diameter and 2 mm thickness, using a hydraulic press for KBr pellets for infrared spectroscopy under 300kg/cm^2 for 10 minutes. The release of drug from flat-faced tablet was carried out using a rotating disc method at 100 rpm and 37±0.5°C in distilled water.

Physicochemical stability of ground mixtures in storage condition

The designed amount of a ground mixture was placed in a vessel and stored over saturated NaCl solution placed in desiccators. The desiccators were kept in a thermostated cabinet. The stroage condition was controlled at 40°C and 75% RH. The stored sample was withdrawn from each vessel at various time intervals for thermal analysis. After the measurement, the sample was returned to the vessel for further storage.

RESULTS AND DISCUSSION

Interaction of drug with β-cyclodextrin in water

The interaction of five separate drugs with β-cyclodextrin in water was respectively studied by solubility determinations. The solubility diagrams obtained for five drugs with β-cyclodextrin in water at $37 \pm 0.5^{\circ}C$ are shown in Fig. 1. The solubility of five drugs obviously increased with the increase of the concentrations of β-cyclodextrin. All the solubility diagrams showed a typical A_L type phase diagram. In addition, no system yielded any solid complex. The 1:1 apparent stability constant (K) was estimated by using Eq. (3), on the basis of assumption that 1:1 complex is initially formed.

$$K = \frac{\text{slope}}{\text{intercept } (1\text{-slope})} \quad \ldots\ldots\ (3)$$

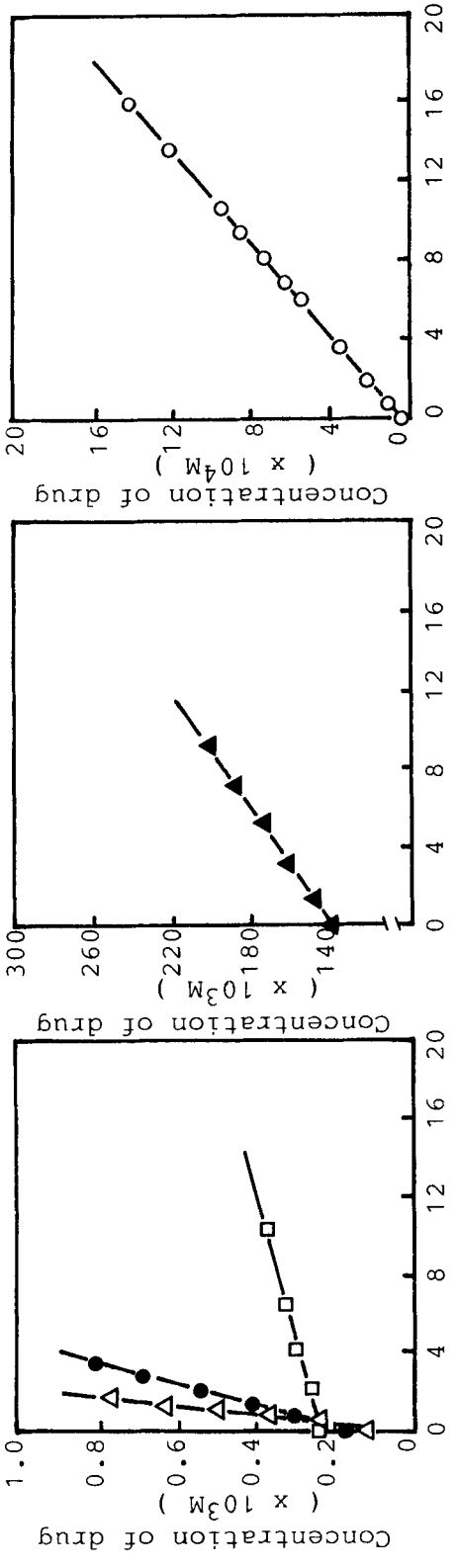


FIGURE 1
Solubility Diagrams of Five Separate Drug-β-Cyclodextrin in Water at 37°C
Key:
△; Diazepam □; Indomethacin ●; Warfarin
▲; Acetaminophen ○; Hydrocortisone acetate

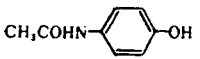
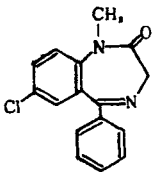
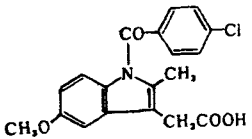
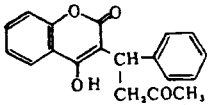
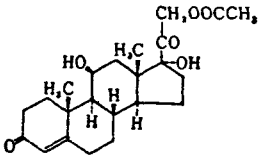
The magnitude of apparent stability constant for five drugs with β-cyclodextrin in water is listed in Table I. Different values of apparent stability constant for five drugs might be due to their linear or cyclic moiety in molecular structure which is capable of penetrating into the cavity of β-cyclodextrin (17). Table II shows the apparent permeability constant and diffusion constant of five drugs effected by β-cyclodextrin. It is obvious that in the presence of β-cyclodextrin, drug penetration was slower compared to the permeation of drug alone. This might be because the inclusion complexation was achieved in the donor cell before permeation, resulting in a lower concentration of drug in the receptor cell. When drug and β-cyclodextrin were in a separated cell compartment, drug permeation was faster since the complex formation has a sucking effect (18). From the above data, inclusion complexation of five separate drugs with β-cyclodextrin in water was obviously achieved.

Physicochemical properties of drug- β-cyclodextrin ground mixture

In order to characterize the ground mixtures and the inclusion complexes of drugs and β-cyclodextrin, examinations by DSC, x-ray diffractometry and IR spectroscopy were carried out and the results were compared with the corresponding physical mixtures in the same molar ratio. Fig. 2 shows DSC curves of drugs,

TABLE I

The Structure and Stability Constant of Five Separate Drugs

Drugs	Acetaminophen	Diazepam	Indomethacin	Warfarin	Hydrocortisone acetate
Structure					
Stability * constant (M^{-1})	44.24	402.37	50.47	148.88	2691.13

* in water at 37°C

TABLE II

Effect of β-Cyclodextrin on the Apparent Permeability Constant and Apparent Diffusion Constant of Separate Drugs Through Membrane with Various Methods at 37°C

	Apparent permeability constant (hr^{-1})			Apparent diffusion constant ($x10^4$ cm^2/hr)			Medium
Permeation method	I	II	III	I	II	III	
Acetaminophen	0.0488	0.0581	0.0450	0.894	1.071	0.826	H_2O
Warfarin	0.0198	0.0250	0.0058	3.644	4.593	1.060	pH 7.0 McIlvaine buffer solution
Diazepam	0.0366	0.0402	0.0330	6.719	7.384	6.058	2% ethanolic-aqueous solution
Indomethacin	0.0342	0.0367	0.0321	6.279	6.738	5.893	pH 7.0 McIlvaine buffer solution
Hydrocortisone acetate	0.0119	0.0122	0.0110	2.198	2.242	2.019	30% ethanolic-aqueous solution

Note: Method I: Donor cell; drug/ Receptor cell; blank medium
Method II: Donor cell; drug/ Receptor cell; β-cyclodextrin
Method III: Donor cell; drug + β-cyclodextrin/ Receptor cell; blank medium

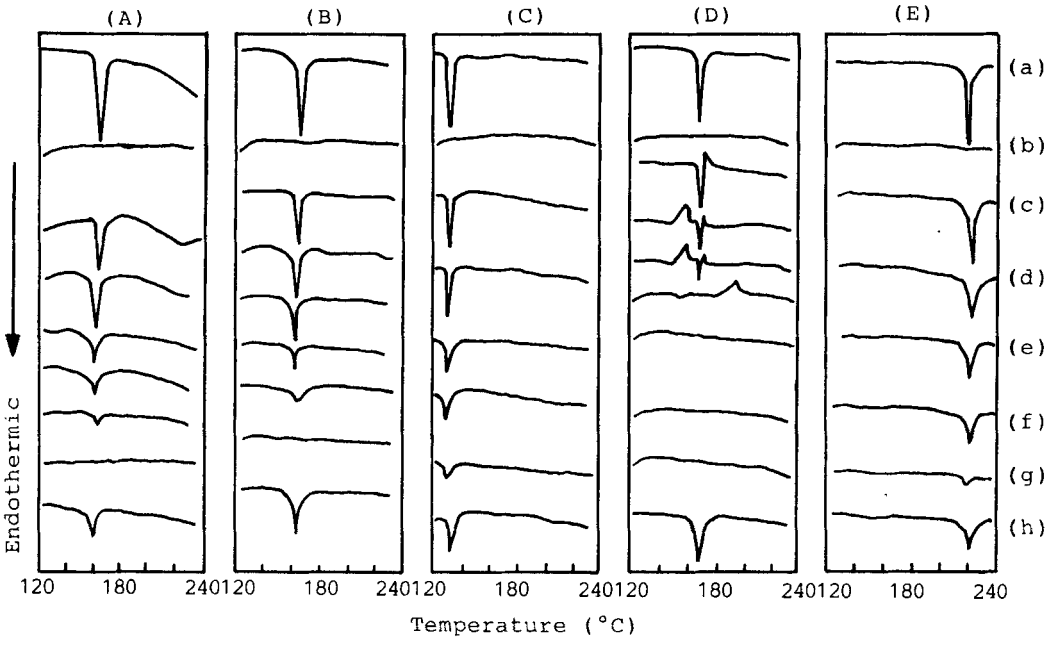


FIGURE 2
DSC Thermograms of Ground Mixtures of Separate Drug with β-Cyclodextrin (Molar Ratio=1:1)
Key:
(A) Warfarin (B) Indomethacin (C) Diazepam
(D) Acetaminophen (E) Hydrocortisone acetate
(a) crystalline drug (b) β-cyclodextrin (c) physical mixture (d) ground mixture (ground for 0.5 hr) (e) ground mixture (ground for 3 hr) (f) ground mixture (ground for 7 hr) (g) ground mixture (ground for 24 hr) (h) ground drug alone (ground for 24 hr)

β-cyclodextrin and ground mixtures. It is evident that the endothermic peak of each drug (mp. peak) decreased with the increasing grinding time. In addition, we also found that the endothermic peak around 168°C for acetaminophen disappeared, as it did with freeze dried mixtures. This disappearance of the endothermic peak might be due to the inclusion complex formation. This indicates that there is a possibilty of

inclusion complex formation by the grinding of acetaminophen-β-cyclodextrin mixture. However, the endothermic peak of another four drugs still exhibited a smaller peak in the DSC curves. This smaller peak indicates that there is a less crystalline nature of ground mixtrure as a result of grinding, but which do not cause inclusion complex formation.

The x-ray diffraction patterns in Fig. 3 show the crystallinities of all the ground mixtrues decreased with the increase of grinding time and became amorphous (acetaminophen) or nearly became amorphous (warfarin, indomethacin, diazepam, hydrocortison acetate). However, in the absence of β-cyclodextrin the crystallinity of each ground drug was still stronger than the ground mixtures. It is remarkable that the grinding efficiency was improved by the addition of β-cyclodextrin.

Fig. 4 shows the IR spectra of drug-β-cyclodextrin system. To acetaminophen-β-cyclodextrin system, it can be seen that the amide absorption frequency (1568 cm^{-1}) was shifted to a lower frequency (1555 cm^{-1}) with the increase of grinding time. Moreover, C=C absorption frequency (1612 cm^{-1}) completely disappeared with increasing grinding time. This also shows the IR spectrum of the 24 hr ground mixture of acetaminophen and β-cyclodextrin was the same as the freeze dried (acetaminophen-β-cyclodextrin). This suggests that

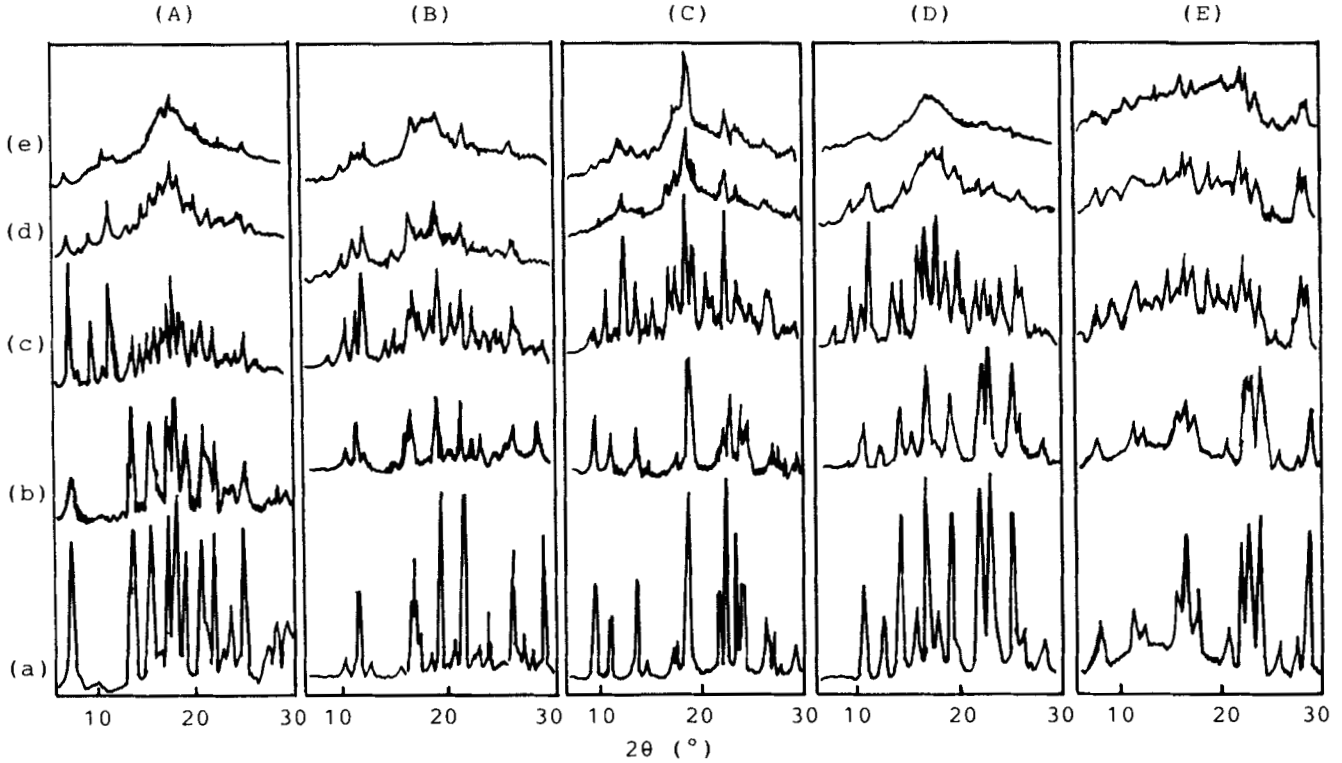


FIGURE 3

X-ray Diffraction Patterns of Ground Mixtures of Separate Drugs with β-Cyclodextrin (Molar Ratio=1:1)

Key:

see FIGURE 2

(a) crystalline drug (b) ground drug alone (ground for 24 hr) (c) physical mixture (d) ground mixture (ground for 3 hr) (e) ground mixture (ground for 24 hr)

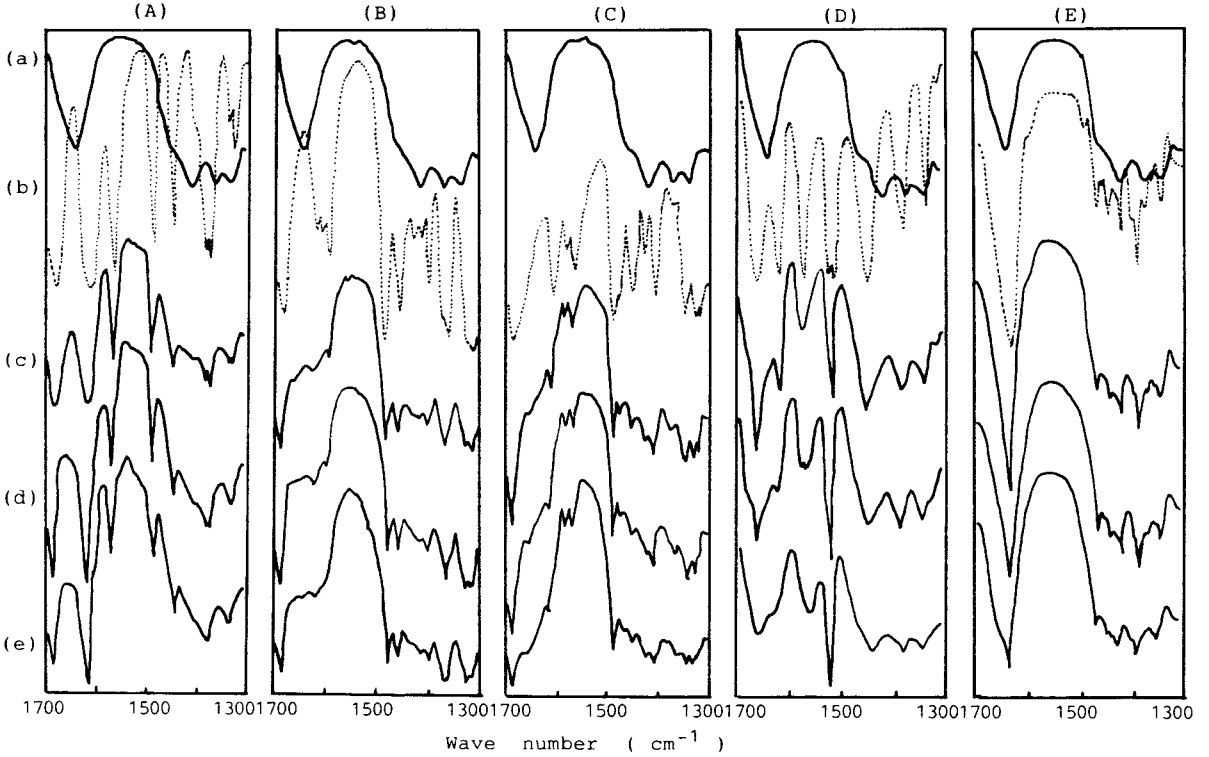


FIGURE 4
IR Spectra of Ground Mixtures of Separate Drugs with β-Cyclodextrin (Molar Ratio=1:1)
Key:
see FIGURE 2
(a) β-cyclodextrin (b) crystalline drug (c) physical mixture (d) ground mixture (ground for 3 hr) (e) ground mixture (ground for 24 hr)

benzene ring of acetaminophen was included into the cavity of β-cyclodextrin, leading to a inclusion complex formation. However, in the other drug-β-cyclodextrin system the IR spectrum of the 24 hr ground mixture was similar to the IR spectrum of the raw material, this indicates that warfarin, indomethacin, diazepam or hydrocortison acetate did not form an inclusion complex with β-cyclodextrin when ground.

Dissolution behavior of drug-β-cyclodextrin systems

Fig.5 shows dissolution profiles of drug from disc with constant surface area in water at 37° C. It is evident that the ground mixtures of drug with β-cyclodextrin dissolved faster than the ground drug, physical mixture and crystalline drug. This might be because the ground mixture was more nearly in a amorphous state than other samples. Moreover, β-cyclodextrin has a surfactant-like property which can reduce the interfacial tension between a water-insoluble drug and a dissolution medium, leading to a higher dissolution rate (4,19). Thus the enhanced dissolution rate might be due to an decrease in crystallinity and an increase in solubility of the drug in the ground mixtures. Since the freeze-dried mixture formed an inclusion complex, the higher release rate of drug from freeze-dried inclusion complexes is reasonable.

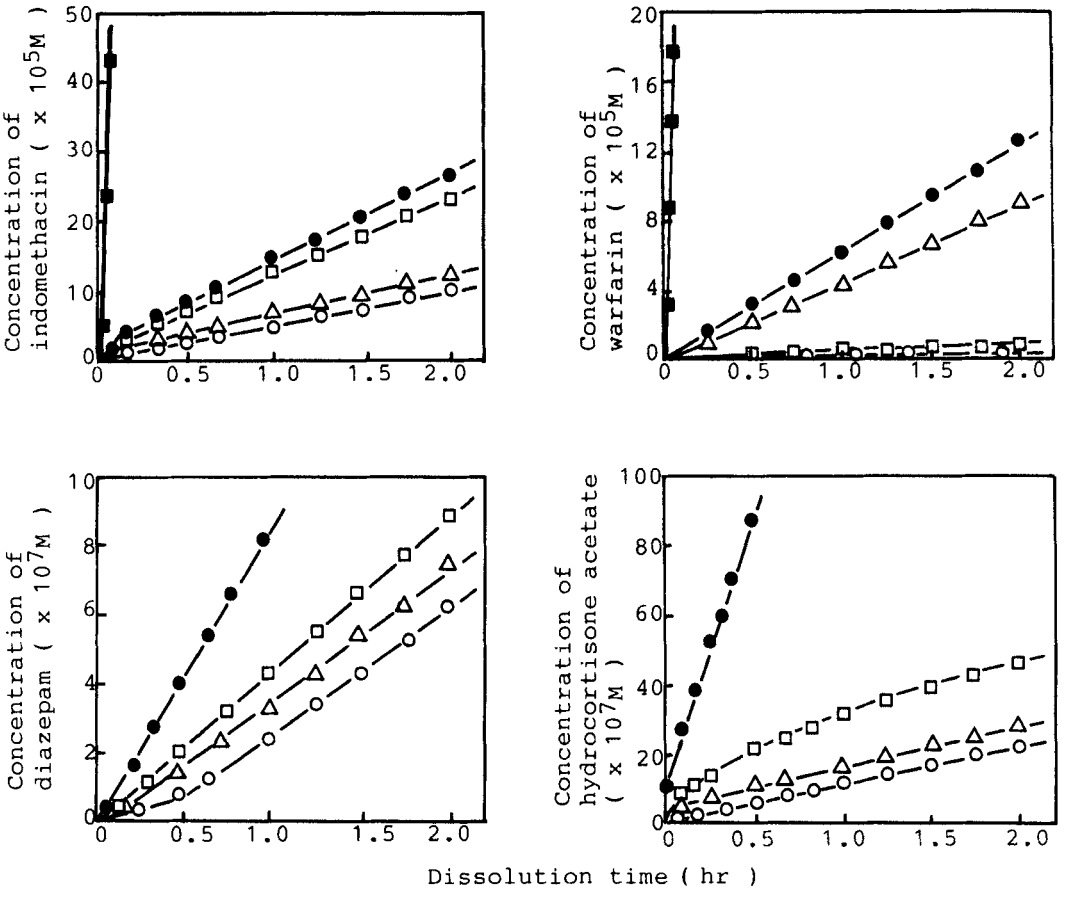


FIGURE 5
Dissolution Profile of Drugs and Ground Mixtures in Water at 37°C
Key:
■; freeze-dried inclusion complex ●; ground mixture (ground for 24 hr) □; physical mixture
△; ground drug (ground for 24 hr)
○; crystalline drug

Physicochemical stability of ground mixtures in 40 °C and 75% RH storage condition

Fig. 6 shows the change of heat of fusion of endothermic peak in DSC curves of the ground mixtures stored at 40 °C and 75% RH. It is remarkable that heat of fusion of crystalline drug was significantly larger than that of the ground mixtures in the presence

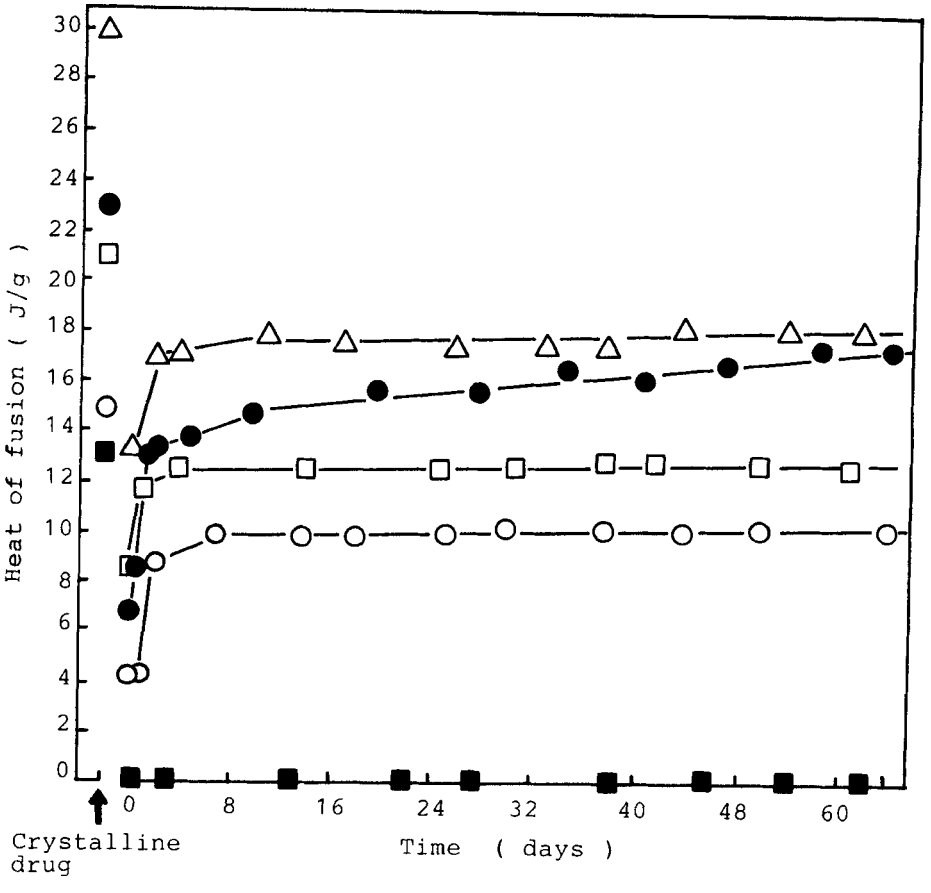


FIGURE 6
Change in Heat of Fusion of Ground Mixtures During Storage at 40 °C and 75% RH Condition
Key:
■; Acetaminophen □; Indomethacin ●; Warfarin
△; Hydrocortisone acetate ○; Diazepam

of β-cyclodextrin. This indicates that grinding caused a loss of the heat of fusion. An increase of heat of fusion of the endothermic peak for each drug markedly progressed when the ground mixtures stored at 40°C and 75% RH condition. This might be due to the crystallization of the drug in the ground mixtures

(20). The equilibrium state obtained after 15 days was maintained during prolonged storage (60 days), however, the heat of fusion of the warfarin-β-cyclodextrin system seemed to increase somewhat during storage. The heat of fusion of each ground mixture at equilibrium state was remarkably smaller than that of each crystalline drug. This indicates that when the drug was crystallized within β-cyclodextrin there was a smaller size of crystal, resulting a lower value of heat of fusion. In addition, it was found that acetaminophen-β-cyclodextrin ground mixture did not exhibit any endothermic peak under 60 days storage condition. This might be attributed to the inclusion complex formation by grinding. It also reveals that the inclusion complex was markedly stable under storage conditions.

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Foot notes

1. Seven Star Pharm. Co. Ltd., Taipei, R.O.C.
2. Sigma Chem. Co., St. Louis, U.S.A.
3. Sumitomo Chem. Co. Ltd., Osaka, Japan
4. Roussel-UCLAF S.A., Paris, France
5. Nihon Shokuhin Kako Co. Ltd., Tokyo, Japan
6. Rigaku Geigerflex D/Max-IIIA, Japan
7. Dupont DSC-1090, U.S.A.
8. Perkin-Elmer IR-580, U.S.A.

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