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EFFECT OF HUMIDITY ON THE FORMATION AND STABILITY OF ACETAMINOPHEN-β-CYCLODEXTRIN INCLUSION COMPLEXES

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ABSTRACT

The effect of humidities (75% RH and 100% RH) on the inclusion complex formation and crystallizing behavior of physical mixture and ground mixture of acetaminophen with α-cyclodextrin, β-cyclodextrin or microcrystalline cellulose at 30 ℃ were studied by DSC thermal analysis, X-ray diffractometer, IR spectrophotometer. The change of water content in the physical mixture and ground mixture when stored at 30 ℃, 75% RH or 100% RH was determined. The results indicate that the inclusion complex formation and crystallizing behavior of these mixtures were dependent on the relative humidity and types of cyclodextrins. The physical mixture of acetaminophen and β-cyclodextrin stored at 30 ℃ and 100% RH was transformed to crystalline inclusion complex, but

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stored at 75% RH still exhibited a behavior of physical mixture. The ground mixture of acetaminophen and β-cyclodextrin was converted amorphous inclusion complex to crystalline inclusion complex whether stored at 75% RH or 100% RH. However, physical mixture and ground mixture of acetaminophen and α-cyclodextrin or microcrysatlline cellulose when stored at 75% RH or 100% RH belonged to a crystalline physical mixture.

INTRODUCTION

Cyclodextrins have attracted growing interest in the pharmaceutical sciences due to the ability of their central cavity to form inclusion complexes with many drugs [1-4]. The inclusion complexes may give desirable properties to drug molecules, such as increasing the aqueous solubility and stability of drugs, improving bioavailability of poorly absorbable drugs and masking unpleasant tastes or odour of compounds [5-8].

Cyclodextrins can not only improve the dissolution rate of warfarin (> 1000 fold) but also control the release rate of warfarin from the inclusion complex resulting in the prolongation of the prothrombin time [9-10]. It is found that water-insoluble drugs ground with β-cyclodextrin may increase their dissolution rate and prevent recrystallization from an amorphous state [11-13].

The experiment that acetaminophen molecules can be included step by step into the cavity of β-cyclodextrin molecules by mechanical grinding process and form an amorphous inclusion complexs has been investigated by the authors [14]. In the present study, the effect of humidity on the stability of ground mixtures of acetaminophen with α-cyclodextrin, β-cyclodextrin or microcrystalline cellulose as well as on the stability of the physical mixtures of acetaminophen and previous excipients was determined.

Materials and Methods

Materials

Acetaminophen was chosen as a model drug and purchased from Seven Star Pharm. Co. Ltd., Taiwan, R.O.C. α and β-cyclodextrins were obtained from Nihon Shokuhin Kako Co. Ltd., Tokyo, Japan, and used without any treatment. Microcrystalline cellulose (Avicel 101) was bought from Asahi Kasei KoGyo Co., Tokyo, Japan. All other materials and reagents were of analytical reagent grade.

Preparation of Ground Mixtures

The ground mixture of acetaminophen with α or β-cyclodextrin in a 1:1 molar ratio was prepared separately by 24 hours grinding in a ceramic ball mill. A ground mixture of acetaminophen with microcrystalline cellulose (weight ratio=1:1) was also prepared. The

decomposition of acetaminophen was not observed during the grinding process according to HPLC determination.

Preparation of Freeze-dried Samples

Freeze-dried samples were prepared via dissolving α or β-cyclodextrin and acetaminophen (molar ratio = 1:1) in water. These dissolved samples were freeze-dried for 2 days and stored in desiccator.

Effect of Humidity on Ground Mixtures and Physical Mixtures

Each ground mixture or physical mixture was weighed in two groups of bottles that were kept in two different desiccators. One desiccator had relative humidity (RH) controlled at 75% in a 30 °C thermostatic cabinet by means of a saturated NaCl solution. The other one maintained at 100% RH in a 30°C thermostatic cabinet by distilled water.

Examination of All the Samples

Physical mixtures, freeze-dried samples, ground mixtures and stored samples were identified by X-ray power diffractometry (Geigerflex D/Max-IIIA, Rigaku Denki, Japan; Ni filter, Cu K_α radiation, 25 KV, 15 mA, scanning speed, 2θ/min), differential scanning calorimetry (DSC-1090, Dupont, USA; Open pan system in N_2 gas flow, heating rate: 10 °C/min) and infrared spectrophotometry with KBr disc (IR-700, Jasco, Japan). Water content assays (n=5) were performed according to the

Karl-Fischer method (MKS-3p, Kyoto Electronics, Japan). The stability of acetaminophen in the stored samples was also determined by HPLC at varying time intervals [15-16]. All HPLC analysis was performed with a high-pressure pump (Familic-300 S, Jasco, Japan) and a variable wavelength UV detector (UVIDEC-100-VI, Jasco, Japan) set at 254 nm. The chromatograph was equipped with a 4.6 mm X 25 cm reversed-phase high efficiency C_{18} column (Finepak Sil C_{18}, Jasco, Japan). The mobile phase was methanol-water (75:25) which was filtered and degassed before use. The flow rate was 1.0 ml/min. The detector was set at a sensitivity of 0.0025 AUFS. The sample size was 10 μl.

RESULTS AND DISCUSSION

Fig. 1 shows the DSC thermograms and IR spectra of physical mixture of acetaminophen and β-cyclodextrin when stored at 30 °C and 100% RH. A sharp endothermic peak at 168 °C was obviously observed due to the fusion of acetaminophen. After melting, a small exothermic peak was also exhibited, this exothermic peck might be responsible for the complex formation by heating [17]. The endothermic peak for acetaminophen at 168 °C decreased with the increase of storage time. It disappeared completely after 48 hours of storage, suggesting the complex formation. We also found that a small exothermic peak could be transformed if we

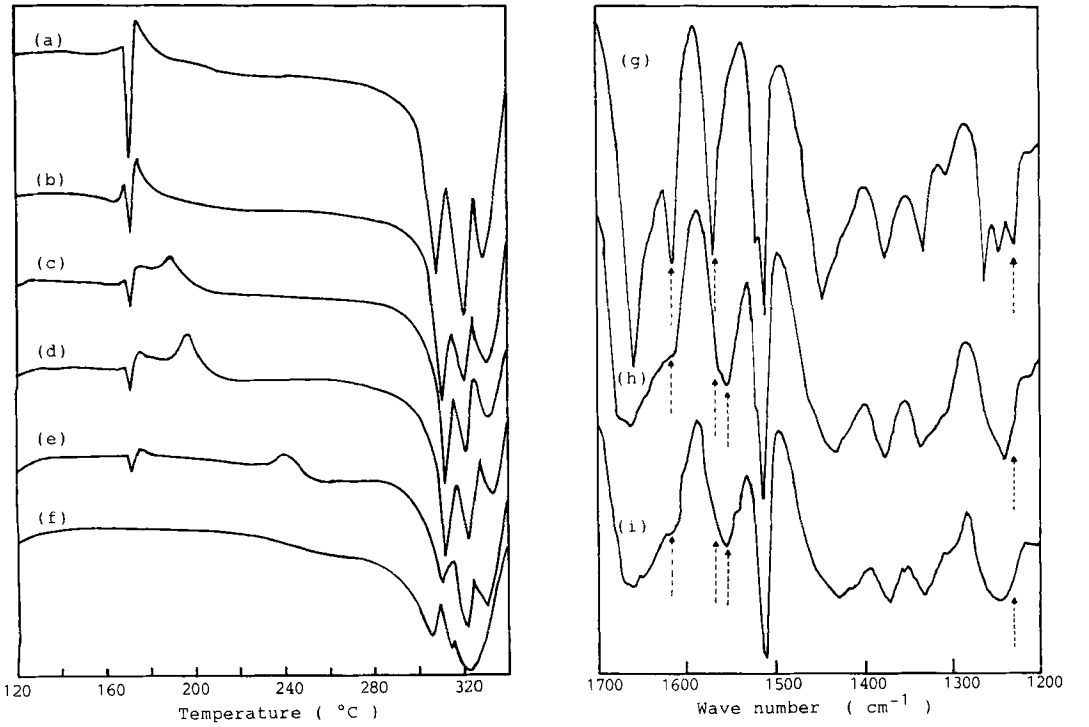

FIG. 1
DSC curves and IR spectra of physical mixture of acetaminophen-β-cyclodextrin stored at 30 °C and 100% RH
Key:
Grinding time: (a,g), 0 hr; (b), 3 hr; (c,h), 7 hr; (d), 9 hr; (e), 16 hr; (f,i), 48 hr

increased the storage time. These DSC results were consistent with those of IR spectra. The 1226 cm^{-1} belonging to the phenolic group of acetaminophen in IR spectra shifted to 1245 cm^{-1}, indicating the dissociation of the intermolecular hydrogen bonds of acetaminophen through the inclusion complex formation.

Moreover, the IR spectra of 1612 cm^{-1} belonging to the c=c of the benzene ring of acetaminophen disappeared due to the formation of acetaminophen-β-cyclodextrin inclusion complex. The intermolecular hydrogen bonding also occurred between the amido group of acetaminophen and the hydroxyl group of cyclodextrin because the 1568 cm^{-1} of amido groups in acetaminophen molecule shifted to 1555 cm^{-1}[14, 17-18].

The physical mixture of acetaminophen and β-cyclodextrin stored at 30°C and 100% RH presented the same X-ray diffraction patterns with the freeze-dried sample, showing a crystalline inclusion complex (Fig. 2-f) [14]. This indicates that the physical mixture of acetaminophen and β-cyclodextrin might form a crystalline inclusion complex by storage in higher humidity condition. However, the physical mixture of acetaminophen and β-cyclodextrin stored at 30 °C and 75% RH did not form an inclusion complex until 48 days, since the endothermic peak of acetaminophen at 168 °C still existed and manifested the same X-ray diffraction pattern with patterns of acetaminophen and physical mixture of acetaminophen and β-cyclodextrin before storage (Fig. 2-a,b,d,e). On the other hand, the physical mixture of acetaminophen and β-cyclodextrin at 30 °C and 100% RH could still form a crystalline inclusion complex 48 hours after, the results which

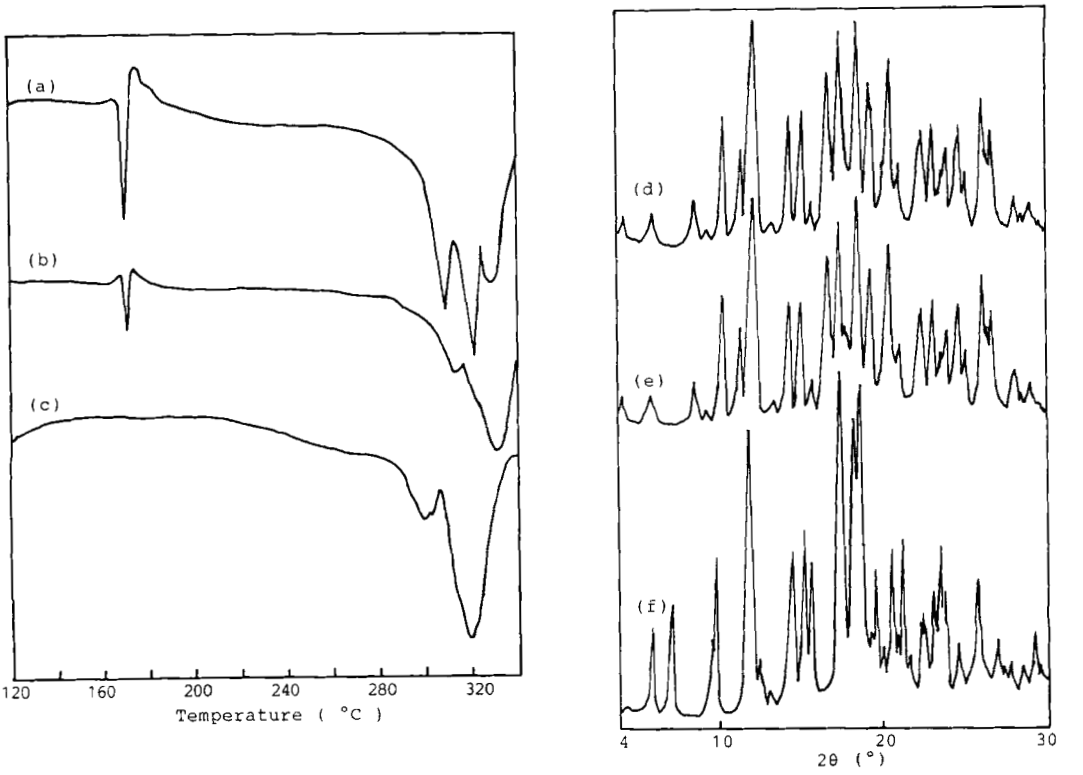

FIG. 2
DSC curves and X-ray diffraction patterns of physical mixture of acetaminophen-β-cyclodextrin
Key:
Storage time and condition:
(a,d), before storage
(b,e), stored at 30°C and 75% RH for 48 days
(c,f), stored at 30°C and 100% RH for 48 days

could be proved by DSC thermograms and X-ray diffractograms (Fig. 2-c,f).

Fig. 3 indicates the DSC curves and the X-ray diffractograms of the ground mixture of acetaminophen and β-cyclodextrin after being stored at 30°C and 75% RH or 100% RH. It has been shown that the ground mixture

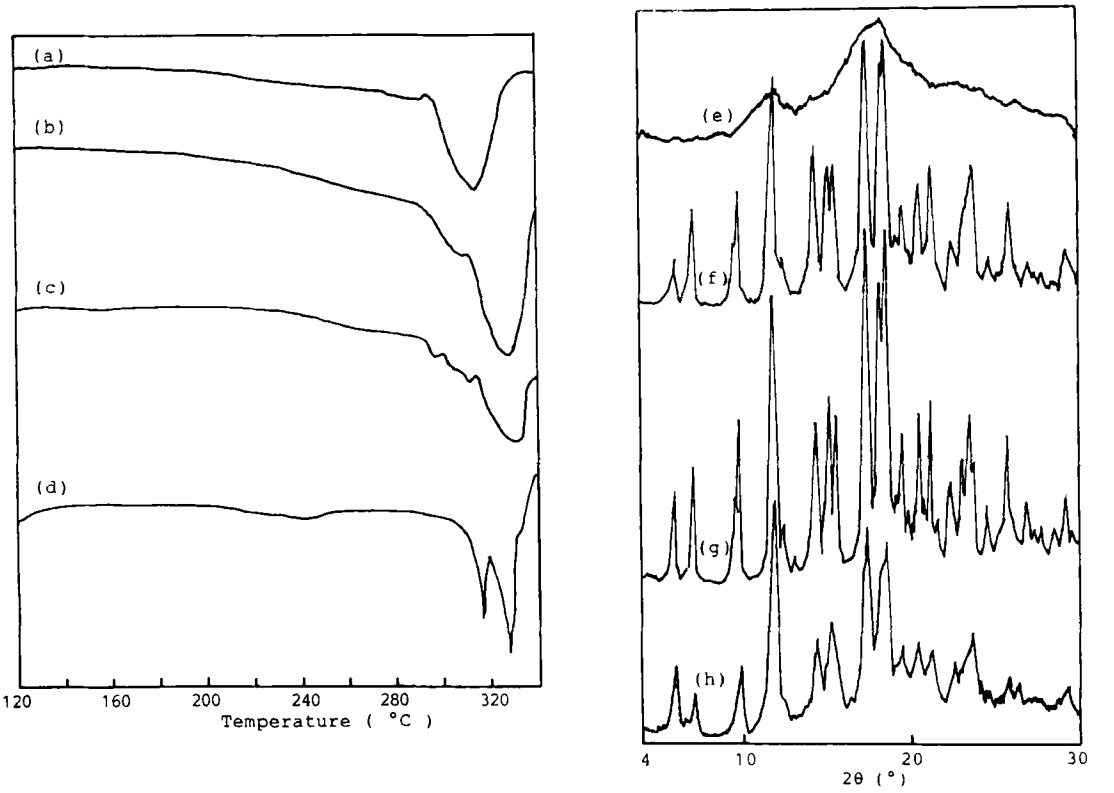

FIG. 3
DSC curves and X-ray diffraction patterns of ground mixture of acetaminophen-β-cyclodextrin
Key:
Storage time and condition:
(a,e), before storage
(b,f), stored at 30 °C and 75% RH for 48 days
(c,g), stored at 30 °C and 100% RH for 48 days
(d,h), freeze-dried sample stored at 30 °C 75% RH or 100% RH

after 24 hours grinding should be an amorphous inclusion complex (Fig 3-a,e) [14]. However, after the 48 day storage at 30°C, 75% RH or 100% RH, the amorphous state of inclusion complexes was transformed into crystalline state (Fig. 3-b,c,f,g), as was proved by IR specta and

X-ray diffractograms. The freeze-dried samples exhibited the same corresponding peaks after storage at 30 °C and 75% RH or 100% RH for 48 days, but a sharper X-ray diffraction patterns than the original freeze-dried samples (Fig. 3-d,h).

The physical mixture of acetaminophen and α-cyclodextrin indicated a different behavior from the physical mixture of acetaminophen and β-cyclodextrin (Fig. 4). Seeing from DSC thermograms and X-ray diffraction patterns, the physical mixture of acetaminophen and α-cyclodextrin after being stored at 30 °C and 75% RH for 48 days, showed the same corresponding peaks as original physical mixture did, although some intense peak appeared in the stored samples. We also found that the X-ray diffraction peaks of the stored samples at 100% RH were somewhat different from the patterns of the original physical mixture and the stored samples at 75% RH. But according to the DSC thermograms and IR spectra, it still should be the physical mixture of acetaminophen and α-cyclodextrin. The different X-ray patterns might be attributed to the crystallization of α-cyclodextrin in higher humidity condition [19], suggesting that the physical mixture of acetaminophen and α-cyclodextrin did not accept the humidity effect to form inclusion complex. This might be due to the central cavity of α-

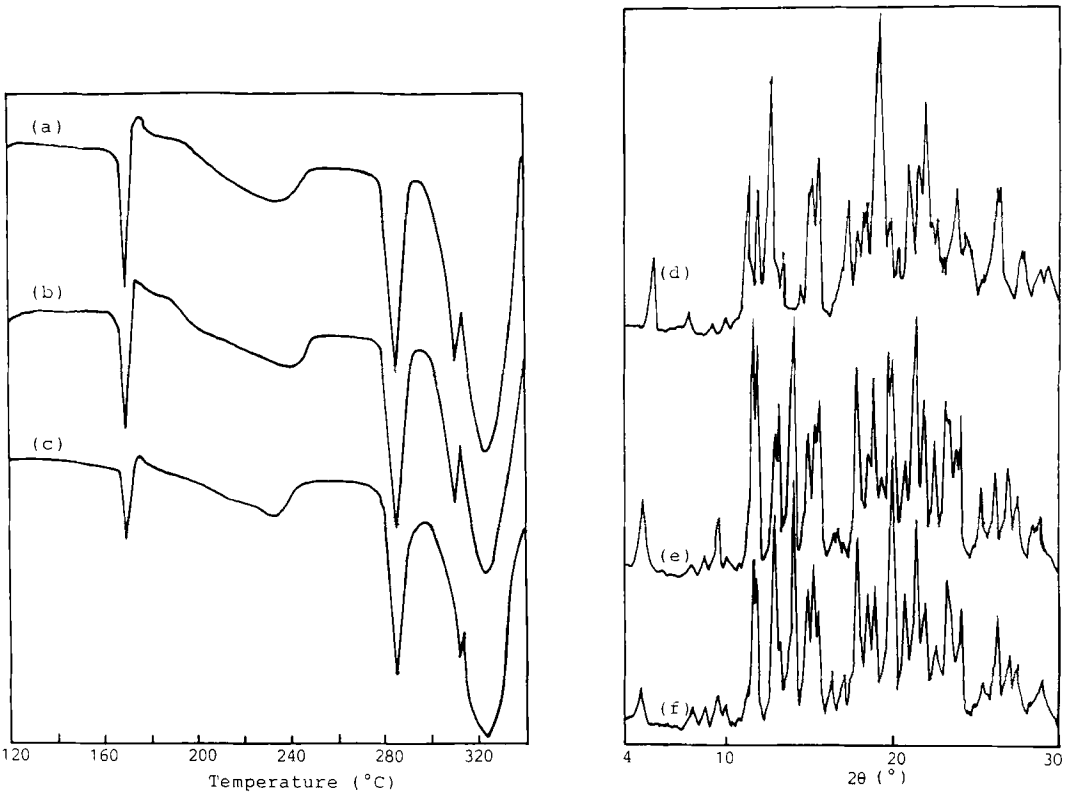

FIG. 4
DSC curves and X-ray diffraction patterns of physical mixture of acetaminophen-α-cyclodextrin
Key:
Storage time and condition:
(a,f), before storage
(b,e), stored at 30 °C and 75% RH for 48 days
(c,d), stored at 30 °C and 100% RH for 48 days

cyclodextrin was smaller than that of β-cyclodextrin [1-4].

Fig. 5 shows the DSC curves and X-ray diffraction patterns of the ground mixture and freeze-dried sample of acetaminophen and α-cyclodextrin stored at 30 °C and

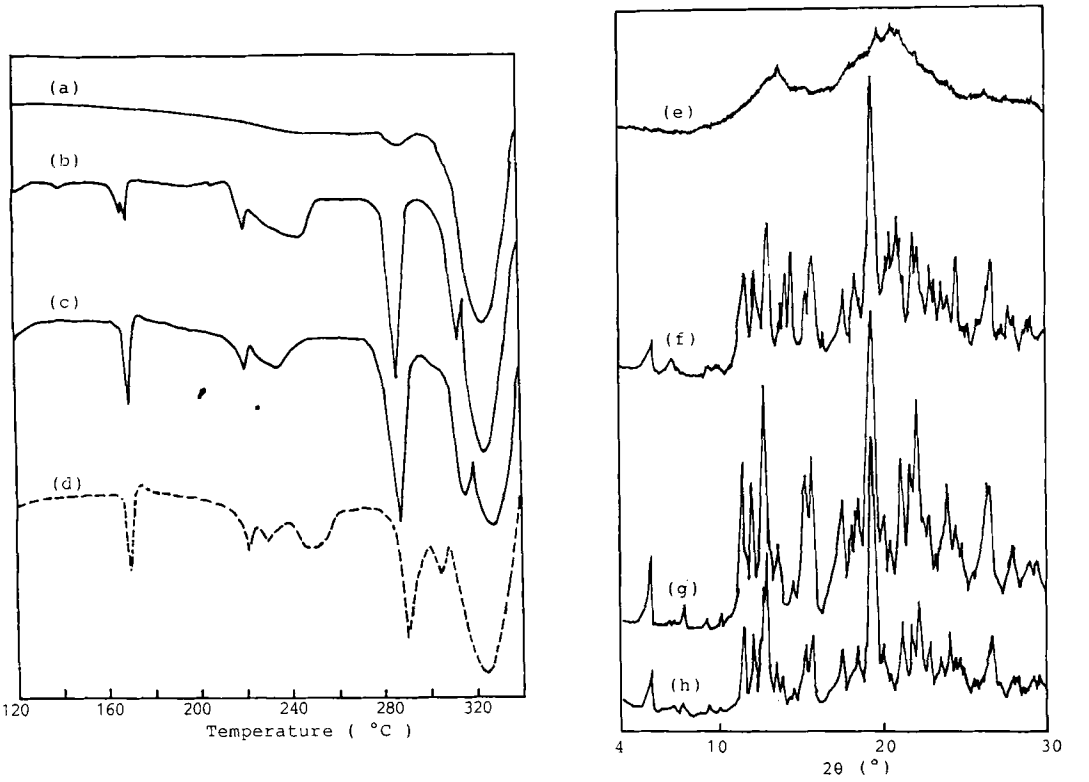

FIG. 5
DSC curves and X-ray diffraction patterns of ground mixture of acetaminophen-α-cyclodextrin
Key:
Storage time and condition:
(a,e), before storage
(b,f), stored at 30 °C and 75% RH for 48 days
(c,g), stored at 30 °C and 100% RH for 48 days
(d,h), Freeze-dried sample stored at 30 °C and 100% RH for 48 days

75% RH or 100% RH. The ground mixture of acetaminophen and α-cyclodextrin has been proved to be in an amorphous state of physical mixture (Fig. 5-a,e) [14]. However, the endothermic peak disappeared at 168°C for ground mixture in this study was reappeared at 168°C in the stored samples which were stored at 30°C, 75% RH and 100% RH for 48 days (Fig. 5-b,c). A smaller endothermic peak of ground mixture was found at 75% RH rather than at 100% RH. The marked increase of the endothermic peak at 168°C for ground mixture stored at 100% RH might be due to the higher humidity, that led to accelerating the recrystallization of amorphous state of acetaminophen. The X-ray diffractograms of the ground mixtures after 48 days of storage was transformed from amorphous state to crystalline state. The same phenomena happened to the original physical mixture and the freeze-dried samples of acetaminophen and α-cyclodextrin (Fig. 5-f,g,h). This indicated that the amorphous state of ground mixture (belonging to physical mixture) of acetaminophen and α-cyclodextrin was transformed to the crystalline state whether stored at 75% RH or 100% RH. It did not form an inclusion complex whereas the ground mixture of acetaminophen and β-cyclodextrin did. It was suggested that hydrogen bonds in the α-cyclodextrin were broken in process of the moisture adsorption and that acetaminophen molecules dispersing within the α-

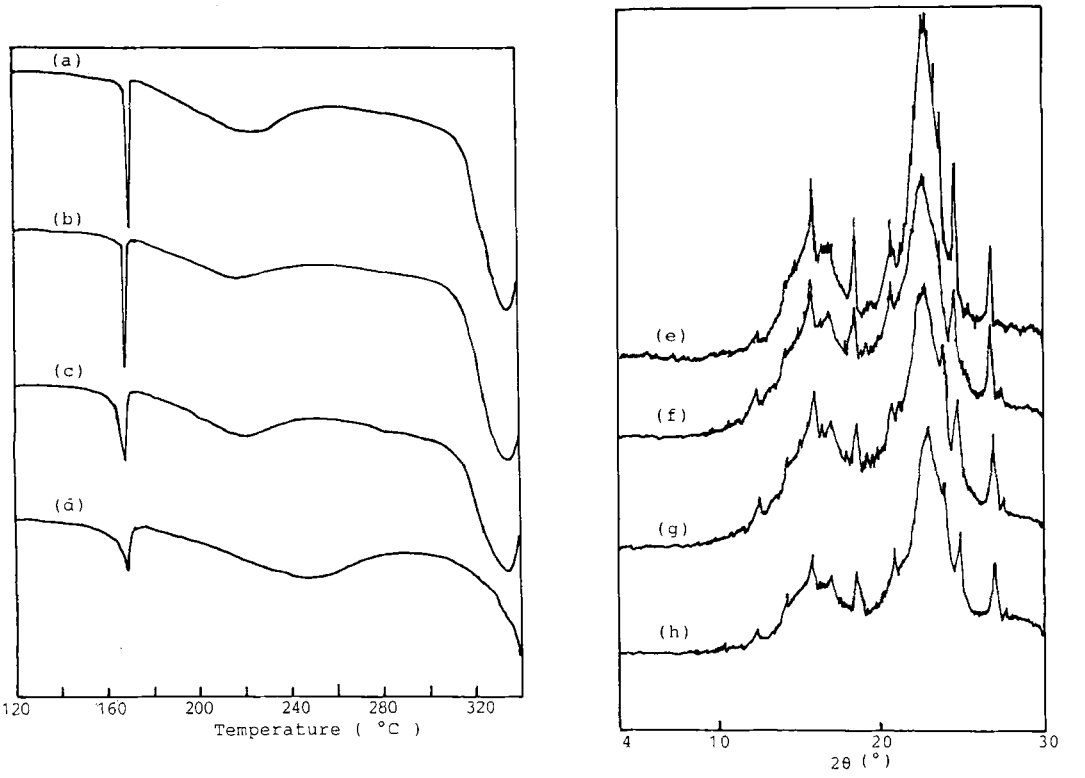

FIG. 6
DSC curves and X-ray diffraction patterns of the physical mixture and ground mixture of acetaminophen-microcrystalline cellulose stored at 30 ℃ and 100% RH
Key:
(a,e), physical mixture before storage
(b,f), physical mixture stored for 48 days
(c,g), ground mixture before storage
(d,h), ground mixture stored for 48 days

cyclodextrin obtained the ability to move and led to recrystallization [20].

DSC curves and X-ray diffraction patterns of the physical mixture and ground mixture of acetaminophen and microcrystalline cellulose stored at 30°C and 100% RH

are shown in Fig. 6. Apparently, the physical mixtures stored for 48 days presented the same crystallinity as the original physical mixture did (Fig. 6-a,b,e,f). However, the crystallinity of the ground mixtures stored at 100% RH condition for 48 days was less intense than that of the original ground mixture (Fig. 6-c, d, g, h). This might be attributed to the ground acetaminophen with higher water solubility which was dissolved and hydrated with adsorbed water or adsorbed water and microcrystalline cellulose, resulting in decreasing the intense of endothermic peak at 168°C [20].

Water contents of the physical mixtures and ground mixtures of acetaminophen and α-cyclodextrin, β-cyclodextrin or microcrystalline cellulose stored at 30°C and 75% RH or 100% RH are shown in Fig. 7. It is obvious that water contents of physical mixture and ground mixture of acetaminophen and α-cyclodextrin after one day storage reached to about 10.33 ± 0.5 % (n=5) whether stored at 75% RH or 100% RH and maintained on a constant level (Fig. 7-A). These contents agreed with the 10% of water contained in α-cyclodextrin 6 H_2O [21]. This also indicates that at higher relative humidity (> 75%), α-cyclodextrin in the amorphous state absorbed water and converted to the corresponding crystalline α-cyclodextrin hydrates [19,21]. The adsorbed water on the ground mixture of acetaminophen and microcrystalline

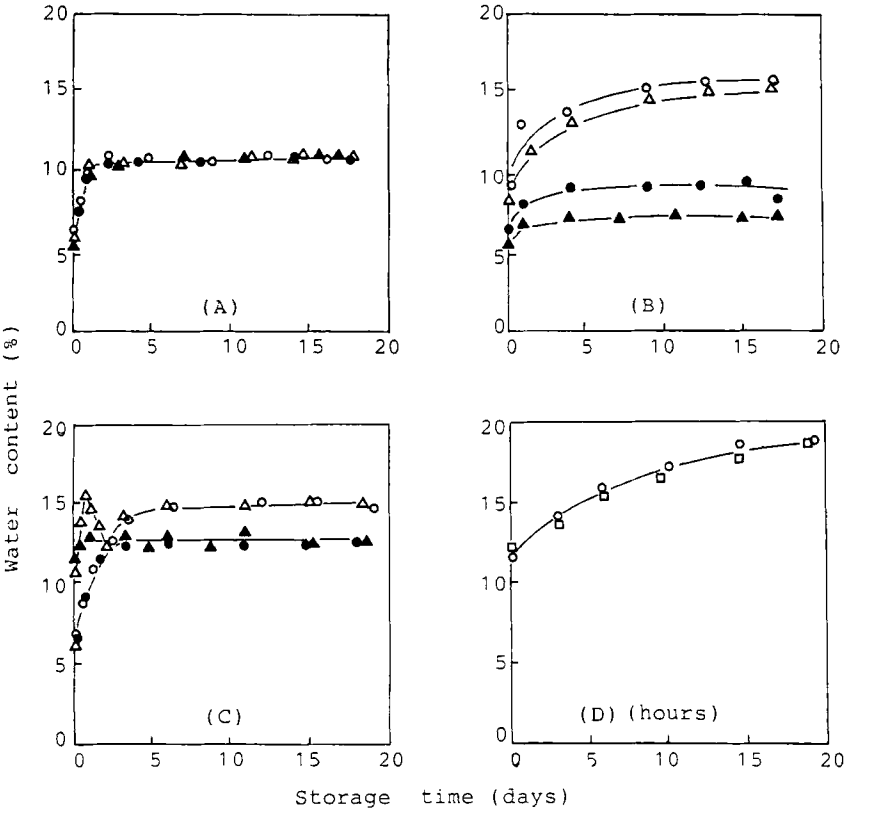

FIG. 7
Water content of physical mixture and ground mixture of acetaminophen-excipient determined by Karl Fischer
Key:
(A) α-cyclodextrin system
(B) microcrystalline cellulose system
(C,D) β-cyclodextrin system

▲, physical mixture stored at 75% RH
△, physical mixture stored at 100% RH
●, ground mixture stored at 75% RH
○, ground mixture stored at 100% RH
□, freeze-dried sample stored at 100% RH

cellulose stored at 30°C and 100% RH was larger in quantity than that of the corresponding samples stored at 30°C and 75% RH (Fig. 7-B). The samples stored at 100% RH obtained 15.6±1.8 % of water content, but when stored at 75% RH the samples exhibited 7.8 ± 0.9 % of water content. This data was consistent with the results of Kawano and Nakai [20]: they stored the amorphous cellulose at 30°C and 97% RH for 2 days and obtained 17.4 % of water content by TG analysis.

The water adsorption of the ground mixture of acetaminophen and β-cyclodextrin when stored at 30°C and 75% RH or 100% RH increased with the storage time (Fig. 7-C, D). This was similar to the behavior of the freeze-dried samples of acetaminophen and β-cyclodextrin (Fig. 7-D). At initial stage, adsorption isotherms rised rapidly, then the water content maintained constantly at 15.3±1.2% after 6 days of storage. Higher water content resulted in the crystallization of the amorphous ground mixture (amorphous inclusion complex) of acetaminophen-β-cyclodextrin, and converted to inclusion complex crystals (Fig. 3-g). We also found that the water content of ground mixture stored at 75% RH increased with storage time and reached to a constant level of 12.8±0.7%. The amorphous state of ground mixture of acetaminophen and β-cyclodextrin stored at 30 °C and 75% RH was also transformed to

(1) β-Cyclodextrin system

Physical mixture → 100% RH → Crystalline inclusion complex
Physical mixture → 75% RH → Crystalline physical mixture

Ground mixture → Amorphous inclusion complex
Amorphous inclusion complex → 75% RH → Crystalline inclusion complex
Amorphous inclusion complex → 100% RH → Crystalline inclusion complex

Freeze-dried sample → Crystalline inclusion complex
Crystalline inclusion complex → 75% RH → Crystalline inclusion complex
Crystalline inclusion complex → 100% RH → Crystalline inclusion complex

(2) α-Cyclodextrin system

Physical mixture → 100% RH → Crystalline physical mixture
Physical mixture → 75% RH → Crystalline physical mixture

Ground mixture → Amorphous physical mixture
Amorphous physical mixture → 75% RH → Crystalline physical mixture
Amorphous physical mixture → 100% RH → Crystalline physical mixture

Freeze-dried sample → Crystalline physical mixture
Crystalline physical mixture → 75% RH → Crystalline physical mixture
Crystalline physical mixture → 100% RH → Crystalline physical mixture

(3) Microcrystalline cellulose

Physical mixture → 100% RH → Crystalline physical mixture
Ground mixture → 100% RH → Crystalline physical mixture
Physical mixture → 75% RH → Crystalline physical mixture
Ground mixture → 75% RH → Crystalline physical mixture

Scheme I Humidity affecting the interaction between acetaminophen and excipients

crysatlline inclusion complex via adsorbing water vapor (Fig. 3-f).

Moreover, different behavior of water adsorption were observeded in the physical mixture of acetaminophen-β-cyclodextrin stored at 75% RH and 100% RH. At 100% RH, water adsorption was increased initially to a maximum level of 15.3±1.2% and then decreased with the storage time . The water increase at the initial stage might be due to the vapor adsorption aroused by β-cyclodextrin, and the following decrease might be attributed to the dissolved acetaminophen which was included into the central cavity of β-cyclodextrin, and led to repulsing the water molecule from central cavity [22-23]. When the acetaminophen molecule was stabilized in β-cyclodextrin cavity, the water moisture was restored around the exposed part of the acetaminophen and β-cyclodextrin molecules, then it turned into crystalline inclusion complex (Fig. 2-f) The crystalline inclusion complex gradually adsorbed water vapor to reach a constant level of 15.4±1.0%. However, the water content of phsical mixture of acetaminophen and β-cyclodextrin stored at 30°C and 75% RH was about 12.6 ± 0.8%, still being crystalline (Fig. 2-e).

The results of this study have shown that the formation and stability of inclusion complex between acetaminophen and cyclodextrin can be affected by the

humidity of the storage condition. These results are summaried in Scheme I. Therefore, it seems to be necessary to choose the optimal condition of storage when drugs are mechanically pretreated with other excipients.

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