Amorphization and Dissolution Studies of Acetaminophen-β-Cyclodextrin Inclusion Complexes

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ABSTRACT

In order to improve the solubility and the dissolution rate of acetaminophen, the equimolar inclusion complex of acetaminophen and β-cyclodextrin was prepared by freeze drying, grinding and sealed heating methods. The physical mixture of acetaminophen and β-cyclodextrin was also prepared by means of mechanical mixing. The molecular behaviors of the drug in all samples were characterized by X-ray powder diffraction, fourier transformed infrared spectroscopy and differential scanning calorimetry. The dissolution and the stability studies of the samples were performed. The results showed that the amorphous inclusion complex of acetaminophen in β-cyclodextrin was successfully obtained by freeze drying and grinding methods. These inclusion complexes showed a substantially higher dissolution rate than acetaminophen and physical mixture. The characterization techniques provided useful information to support the amorphousness of the samples as well as the successful inclusion of the drug molecule into the cyclodextrin cavity. Upon storage under certain conditions at a specific time, molecular behaviors of acetaminophen in freeze-dried mixture were partially transformed into the crystalline state, whereas the sealed heating mixture and physical mixture showed little change in their molecular behaviors.

On the basis of these results, the improvement of acetaminophen bioavailability is expected by inclusion complex formation with cyclodextrin. However, from the stability point of view, the retardation of phase transition in order to maintain the amorphous state of the drug should be taken into consideration.

Key words: Acetaminophen, Amorphization, β-Cyclodextrin, Inclusion complexes

INTRODUCTION

The bioavailability of most drugs depends primarily on their dissolution properties. Fast dissolution rate and complete dissolution usually enables better absorption. It is recognized that the internal structure of a compound exerts a profound effect on its dissolution characteristics, i.e., the ease of escaping to the dissolution medium of each molecule depends on the nature of the internal structure. Accordingly, crystalline and amorphous forms of a compound usually show different dissolution behaviors. As the amorphous form of a drug dissolves much more rapidly and completely than its crystalline

form, the amorphization technique was previously shown as a useful manipulation to achieve better bioavailability of certain drugs (Hancock and Zografi, 1997). Unfortunately, the amorphous form is quite unstable due to its higher thermodynamic activity and tends to change to the more stable form, a crystalline form, which is less soluble.

In addition to the attempt of preparing and stabilizing the amorphous drugs, the formulation of an inclusion complex was evidenced as a promising means for increasing the solubility and the dissolution rate which were attributed to the enhancement of the bioavailability of poorly soluble drugs (Corrigan and Stanley, 1982; Ma et al., 1999; Ozkan et al., 2000; Veiga et al., 2000; Wong and Yuen, 2001). β-cyclodextrin has been extensively used as a host for this type of complex formation (Loftsson and Brewster, 1996).The molecular configuration of ?ñcyclodextrin provides the hydrophobic cavity of specific size in which the molecules of certain drugs can be enclosed. It was of interest that by appropriate method of preparing, an amorphous inclusion complex could be possibly obtained. This complex is generally more thermodynamically stable in contrast to the amorphous drug.

In the present study, attempts were made in order to obtain the amorphous form of acetaminophen, a widely-used analgesic and antipyretic drug by complex formation with β-cyclodextrin. The molecular behaviors of the samples were then characterized by powder X-ray diffractometry, differential scanning calorimetry (DSC) and FTIR spectroscopy. The measurements of the particulate dissolution rate of all samples were performed. The storage-stability test was also conducted to verify whether the amorphousness of the samples existed for a period of time.

MATERIALS AND METHODS

Materials

Acetaminophen, purchased from Iwaki, Seiyuku Co.

β-Cyclodextrin

Both were dried separately before use by vacuum sample apparatus Model VSD-95, then kept in a desiccator over phosphorous pentoxide. The moisture content of β-cyclodextrin was triplicately determined by Karl-Fisher, Aquacounter AQ-6, Hiranuma.

Other materials and solvents used were analytical reagent grade.

Methods

1. Preparation of mixtures

1.1 Preparation of physical mixture

The physical mixture of acetaminophen (intact drug, ID) in β-cyclodextrin of 1:1 molar ratio was prepared by simple blending, using a vortex mixer.

1.2 Preparation of ground mixture

The intact drug and the physical mixture were separately ground for 10 minutes, using a Vibrational mill (Model TI-200, Heiko, Seisakusho) made of tungsten carbide. The ground drug and ground mixture were subsequently obtained.

1.3 Preparation of freeze-dried mixtures

The freeze-dried drug and freeze-dried mixture were prepared by dissolving each intact drug or physical mixture in a suitable medium. The solution was primarily frozen by immersing in liquid nitrogen and then freeze dried for 48 hours, using Freeze dryer (Model Neocool DS 55-8, Yamoto)

1.4 Preparation of sealed heating mixture

The sealed heating mixture was obtained by heating the physical mixture in a sealed glass ampoule at 90˚C for 2 hours.

After preparing, the ground mixture, the freeze-dried mixture and the sealed heating mixture were washed successively with ethyl ether to remove any excess of free drug. After being dried in a vacuum overnight, they were analyzed for the amount of drug included in β-cyclodextrin, using UV-Visible Recording Spectrophotometer (UV-160) at 244 namometers.

2. Characterization of the molecular behaviors of mixtures

2.1 Powder X-ray diffractometry

The powder X-ray diffractograms of all mixtures were obtained, using X-ray diffractometer (Model Rigaku Geigorflex 2027). The measurement conditions were as follow: target, Cu ; filter, Ni; voltage, 30 KV ; current, 5mA ; time constant, 0.5 sec. ; scanning speed, 4˚C/min.

2.2 Differential scanning calorimetry

The thermal behaviors of the mixtures were determined, using differential scanning calorimeter DuPont 9900 under nitrogen gas stream at flow rate of 20 mL/min. Each mixture, weighing about 2.0-3.0 milligrams, was placed in a liquid pan which was hermetically sealed. The heating range was from 50˚C to 250˚C with the heating rate of 10˚C/min.

2.3 Infrared absorption spectroscopy

The IR spectra were recorded by Nujol mull method, using Infrared spectrophotometer (Model Hitachi 295).

3. Dissolution studies

The experiments were conducted according to the following procedure: 900 milliliters of distilled water, maintained at 37±0.5˚C was used as a dissolution medium. The stirring speed of the paddle was at 50 rev./min. After the required amount of each sample had been placed into the dissolution medium, an aliquot portion of the solution was withdrawn at appropriate time intervals to be analyzed by spectrophotometer for the amount of the dissolved drug. Each point on the dissolution profiles represented the average of three determinations.

4. Storage-stability studies

The mixtures were stored in the desiccator over the saturated aqueous solution of ammonium chloride to provide 75% of relative humidity. The desiccator was kept in the thermostated cabinet at 40˚C. After the end of storage time, the mixtures were characterized for their molecular behaviors by powder X-ray diffractometry, DSC and FTIR spectroscopy.

RESULTS AND DISCUSSION

1. Acetaminophen (intact drug), freeze-dried drug and ground drug

The X-ray diffractograms of freeze-dried drug and ground drug are illustrated in Figure 1. They were very similar to that of the intact drug. It might be suggested that the crystallinity of the drug still remained, even though it was subjected to the freeze drying and grinding processes. This suggestion was also supported by the DSC thermograms (Figure 2) that the sharp endothermic peaks exhibited by freeze-dried drug and ground drug existed at nearly the same melting temperature as the intact drug. Moreover, the identical IR spectra of the intact drug, freeze-dried drug and ground drug (Figure 3)provided additional evidence to support this suggestion.

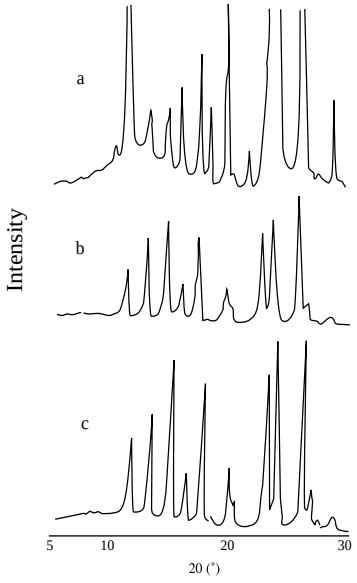

Figure 1. X-ray diffractograms of acetaminophen: (a) intact drug; (b) freeze-dried drug; (c) ground drug

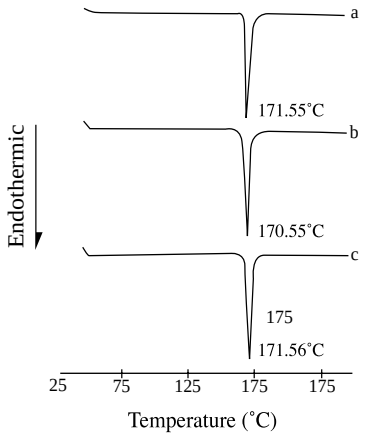

Figure 2. DSC thermograms of acetaminophen: (a) intact drug; (b) freeze-dried drug; (c) ground drug

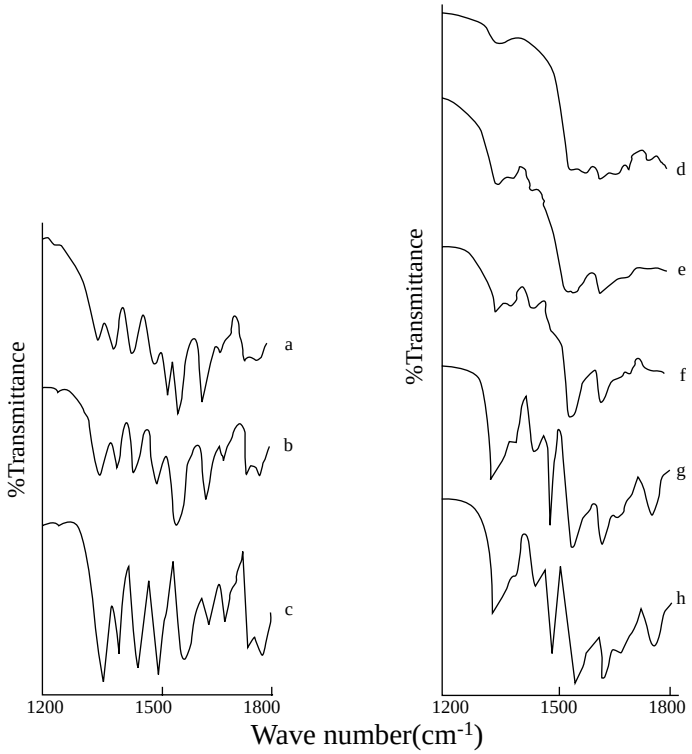

Figure 3. IR spectra of acetaminophen; β-cyclodextrin and equimolar mixtures of acetaminophen and β-cyclodextrin: (a) intact drug, (b) freeze-dried drug, (c) ground drug, (d) β-cyclodextrin, (e) physical mixture, (f) sealed heating mixture, (g) freeze-dried mixture, (h) ground mixture

2. Acetaminophen in β-cyclodextrin mixtures

2.1 Physical mixture and sealed heating mixture

The X-ray diffractograms of physical mixture and sealed heating mixture were quite similar as shown in Figure 4(A). Although these X-ray diffractograms were somewhat different from that of the intact drug, they still indicated that the physical mixture and sealed heating mixture were in crystalline form. Their DSC thermograms, illustrated in Figure 4(B), also suggested that the crystalline structure of the drug in β-cyclodextrin mixtures was not affected by the simple blending and sealed heating technique. However, it was of interest that the melting endothermic peaks exhibited by physical mixture and sealed heating mixture existed at much lower temperatures than the melting point of the intact drug, i.e., 159.1˚C and 164.0˚C compared to 171.5˚C, respectively. The most likely explanation for the lowering of the melting point of the drug in the presence of β-cyclodextrin may be firstly, due to the drug and β-cyclodextrin interaction and secondly, the melting point of the mixtures might be affected by the liberation of water molecules from β-cyclodextrin when heated in the hermetically-sealed pan. From IR spectra of physical mixture and sealed heating mixture shown in Figure 3, the shifts of the stretching vibration of phenolic group at 1226 cm^{-1} to 1245 cm^{-1} and 1241 cm^{-1} were observed. It was previously reported (Nakai et al., 1980) that this shift was responsible for the dissociation of intermolecular hydrogen bonding of acetaminophen through the inclusion complex formation with β-cyclodextrin. This finding,

therefore, suggested the possibility of interaction between the drug and β-cyclodextrin as mentioned before. In spite of the interaction, the crystalline complexes were obtained. The other interesting absorption peak was at 1612 cm^{-1} which was responsible for C=C in benzene ring of acetaminophen. In physical mixture and sealed heating mixture, this peak became smaller compared to that exhibited by the intact drug. This was caused by the dilution of the drug by β-cyclodextrin.

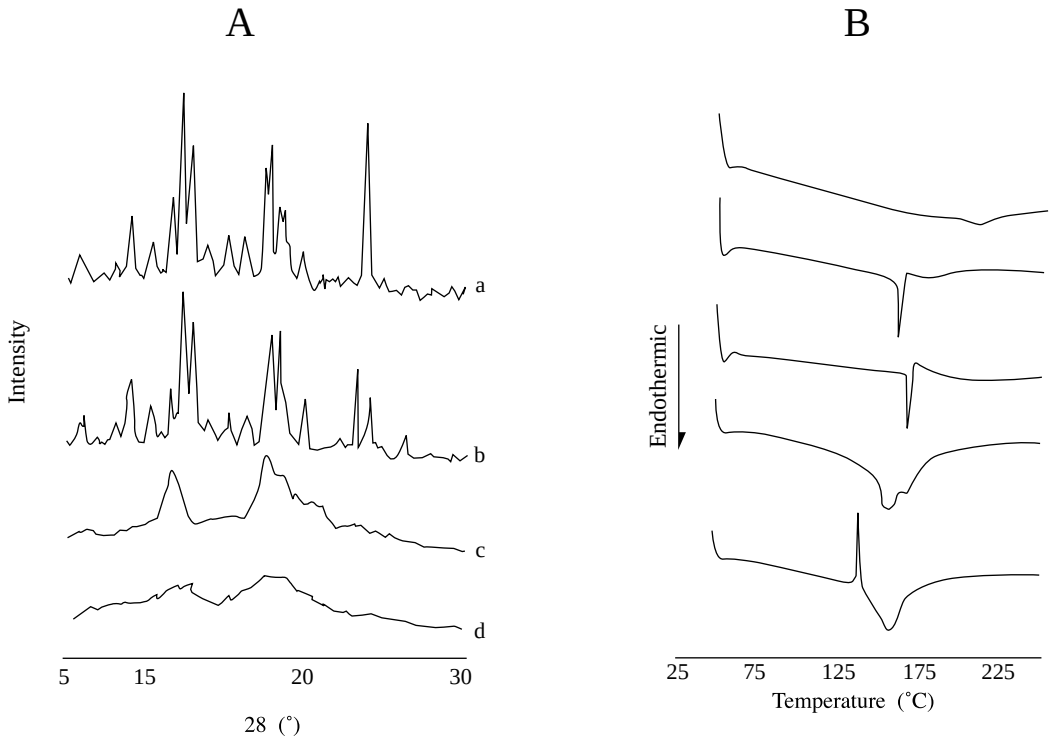

Figure 4. X-ray diffractograms (A) and DSC thermograms (B) of the equimolar mixtures of acetaminophen and β-cyclodextrin. (a) physical mixture; (b) sealed heating mixture; (c) freeze-dried mixture; (d) ground mixture.

2.2 Freeze-dried mixtures and ground mixture

The freeze-dried mixture and ground mixture of acetaminophen in β-cyclodextrin were completely converted to the amorphous state after being subjected to freeze drying and grinding processes. All characterizing techniques used in this study provided full-supported information and this result was consistent with the others. It was previously reported that grinding was a useful method for obtaining the amorphous molecular dispersion systems (Lin et al., 1988). The freeze drying technique was also proved to be superior to the coprecipitation method, obtaining the amorphous inclusion complexes (Kurozumi et al., 1975). The absence of crystallinity of freeze-dried mixture and ground mixture was clearly evident by the halo pattern of X-ray diffractograms shown in Figure 4(A). From DSC thermograms, illustrated in Figure 4(B), the endothermic curves shown by freeze-dried mixture was very broad. It might be ascribed to non-crystalline structure of the mixture which generally

exhibited non-sharp and non-definite melting point curve. In the case of ground mixture, the sharp exothermic curve appeared at 135.7˚C which was attributed to the crystallization of the amorphous mixture. The resulting crystalline mixture then melted at 156.8˚C. Even though the crystallization was evident, the crystalline mixture obtained was not well-ordered crystal lattice pattern because a wide melting point curve was exhibited. The IR spectra shown in Figure 3 also provided useful evidence for supporting the complex formation between the drug and β-cyclodextrin. The shift of stretching band at 1226 cm^{-1} and 1243 cm^{-1} was observed on IR spectra shown by freeze-dried mixture and ground mixture. The significance of this shift has already been mentioned. Moreover, the shifts of the stretching bands at 1563 cm^{-1} which belonged to the amide group of the drug, to 1555 cm^{-1} for freeze-dried mixture and to 1553 cm^{-1} for ground mixture, were also observed. These observations signified the intermolecular hydrogen bonding between the amido group of acetaminophen and the hydroxyl group of β-cyclodextrin (Lin et al., 1988; Lin, 1990).

3. Dissolution studies

The dissolution profiles of the intact drug and its mixtures in β-cyclodextrin are illustrated in Figure 5. It was shown that the drug in the mixtures dissolved faster than the intact drug. This significant difference was observed within the first fifteen minutes of dissolution profiles but became quite comparable thereafter. The higher dissolution rate of the drug in the physical mixtures than that of the intact drug was due to the surfactant-like property of β-cyclodextrin. Among those mixtures, the differences in their dissolution rates were noted. Because the mixtures were subjected to different conditions upon preparing, the degree of successive particle size reduction as well as the extent of the amorphousness of the mixtures thus differed. Accordingly, the dissolution rates of ground mixture and freeze-dried mixture were faster than those of physical mixture and sealed heating mixture which were of crystalline form.

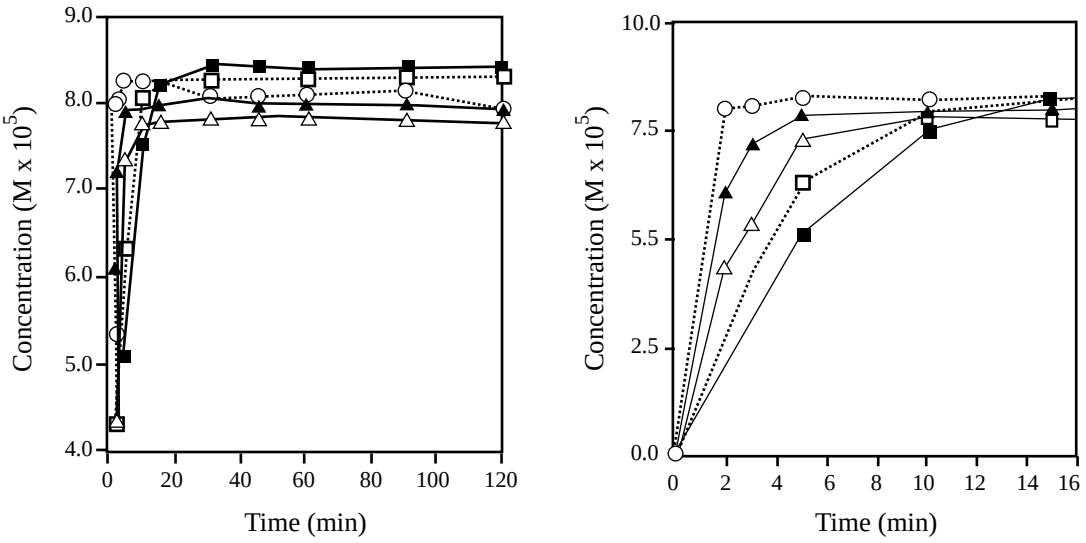

Figure 5. Dissolution profiles of acetaminophen and the equimolar mixtures of acetaminophen and β-cyclodextrin in distilled water at 30±0.5˚C. ■ intact drug; □ sealed heating mixture; ○ freeze-dried mixture; ▲ ground mixture; △ physical mixture

4. Storage-stability studies

4.1 Acetaminophen, freeze-dried drug and ground drug

After storage, the freeze-dried drug and the ground drug as well as the intact drug exhibited same molecular conformations compared to those before storage. This was evident by the X-ray diffractograms, the DSC thermograms and IR spectra which were very similar to those before storage (the figures not shown). It might be suggested that these samples were in very stable crystalline state so that the phase transition no longer took place during storage.

4.2 Acetaminophen in β-cyclodextrin mixtures

4.2.1 Physical mixture and sealed heating mixture

The X-ray diffractograms of physical mixture and sealed heating mixture after storage are shown in Figure 6(A). They were almost identical to each other, but obviously different from those recorded before storage. A number of intense peaks existed, implying that the mixtures were in crystalline form. Their DSC thermograms, shown in Figure 6(B), were also quite similar. Unfortunately, no more meaningful information could be drawn from these overlapping peaks. It could only be expected that the systems consisted of mixed crystals that each melted at different temperature. Under storage condition of high humidity and high temperature, the drug dissolved in the adsorbed water and then recrystallized to the new crystals. Since, the transition process occurred only in a limited time which was insufficient for complete recrystallization, the mixed crystals were presumably yielded. However, further investigation is required for a clear explanation of these thermal behaviors. The IR spectra of physical mixture and sealed heating mixture (Figure not shown) also indicated the interaction between the drug and β-cyclodextrin through hydrogen bond formation. Interestingly, the disappearance of the absorption peak at 1612 cm^{-1}, which was responsible for the C=C in the benzene ring of the drug, was noted in their IR spectra. This finding suggested the possibility of insertion of the drug molecule into β-cyclodextrin cavity. The space of the phenyl structure of the drug molecule (7 x 7 x 3.4˚A) was comparable to the β-cyclodextrin cavity which is approximately 8.0˚A. It is also interesting that the characterizing parameters of physical mixture and sealed heating mixture were very similar at the beginning of storage. During storage, these parameters changed but in the same manner so that the similarity remained at the end of storage time. It might be suggested that the physical mixture and sealed heating mixture were virtually the same. In other words, the sealed heating technique used in this study had negligible effect on the molecular conformation of the drug.

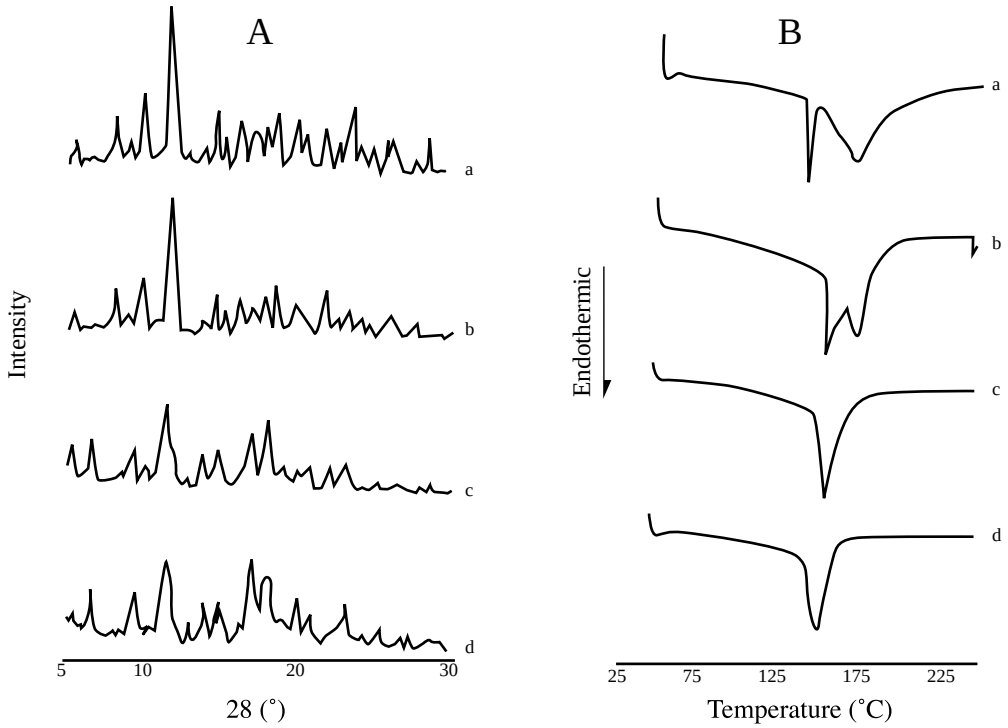

Figure 6. X-ray diffractograms (A) and DSC thermograms (B) of the equimolar mixtures of acetaminophen and β-cyclodextrin after stored at 40˚C; 75% R.H. for 21 days.
(a) physical mixture; **(b)** sealed heating mixture; **(c)** freeze-dried mixture; **(d)** ground mixture.

4.2.2 Freeze-dried mixture and ground mixture

After storage, the freeze-dried mixture and ground mixture are changed from the amorphous state to crystalline form. This was evident by a number of pronounced X-ray diffraction peaks shown in Figure 6(A). These X-ray diffractograms had changed from the halo pattern as previously illustrated in Figure 4(A). The similarity of the diffraction peaks of the freeze-dried mixture and ground mixture was also observed. The DSC thermograms of the freeze-dried mixture and ground mixture after storage were shown in Figure 6(B). They exhibited somewhat broader endothermic peaks at 153.5˚C and 150.1˚C respectively. This finding supported the above suggestion that both the freeze-dried mixture and ground mixture were in crystalline form. Since they were in an amorphous state at the beginning of storage, they could readily dissolve in the adsorbed water and further crystallized to the crystalline form. The new crystalline mixtures obtained were, however, distinctly different from the crystalline drug and the crystalline physical mixture. These results disagreed with the data reported by Lin (1990). According to his report, the acetaminophen and β-cyclodextrin ground mixture could retain its amorphousness for 60 days under the same storage condition. However, the grinding technique used was different. The IR spectra of the freeze-dried mixture and ground mixture recorded after storage still denoted the interaction between the drug and β-cyclodextrin.

CONCLUSIONS

The amorphous inclusion complexes of acetaminophen and β-cyclodextrin were successfully obtained by freeze drying and grinding methods. This conclusion was supported by X-ray diffraction, DSC and IR spectroscopy. Among these characterizing techniques, the X-ray diffraction seemed to be the most useful method, providing the meaningful information to support the conclusion. These amorphous inclusion complexes showed markedly higher dissolution rates than the intact drug, the physical mixture and the sealed heating mixture, which were in crystalline state. However, the amorphous inclusion complexes readily converted to crystalline form upon storage at 40˚C, 75% R.H. for three weeks.

Based on the results of this study, the preparation of the amorphous inclusion complexes with β-cyclodextrin seems to be a promising means to enhance the dissolution property of acetaminophen, a poorly soluble drug. Indeed, it should be realized that the improved properties might not be long lasting due to the instability of the amorphous state. Accordingly, it is necessary to make an attempt to retard the phase transition, in order to maintain the amorphous state of the drug as long as possible, at least through the shelf-life of the products.

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