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Effectiveness of mechanochemical treatment with cyclodextrins on increasing solubility of glimepiride

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We investigated the enhancement of the solubility of glimepiride (GLM), a poorly water soluble antidiabetes drug, by cogrinding it with various cyclodextrins (CDs) using a ball mill. The phase solubility profiles of GLM with β-CD and its derivatives were classified as A_L-type, indicating the formation of a 1 : 1 stoichiometric water-soluble complex. When GLM crystals were coground with β-CD using a ball mill for 48 h, the aqueous solubility of GLM increased to approximately 250 μg/mL. The powder X-ray diffraction pattern showed that the peak intensity of crystalline GLM decreased after cogrinding. Endothermic peaks of around 208 °C, which were assigned to the fusion of GLM crystals, disappeared in the DSC measurement of the ground mixture. After cogrinding, two sharp peaks assigned to sulfonylurea and benzoyl carbonyl stretching bands varied to broaden the peak to around 1700 cm^{-1} in the C=O stretching region. These results suggested the formation of a complex between GLM and β-CD during cogrinding.

1. Introduction

Most of the active pharmaceutical ingredients (APIs) developed in recent years are poorly water soluble (Timpe 2007). APIs often show low bioavailability when administered orally, because the dissolution rate of the drugs in the gastrointestinal tract is usually a rate-limiting step (Yamashita et al. 2003; Blagden et al. 2007). Various techniques have been attempted to improve the dissolution behavior of insoluble APIs, including the use of solubilizing excipients (Strickley 2004; Avdeef et al. 2007), the micronization of drug particles (Itoh et al. 2003; Kecka and Müller 2006), the preparation of solid dispersions (Hasegawa et al. 2007), and the formation of molecular complexes (Bhatt et al. 2005; Umeda et al. 2007).

Cyclodextrins (CDs) have been extensively studied as solubilizing agents, and are used to enhance the bioavailability of APIs in pharmaceutical preparations (Uekama et al. 2006; Loftsson and Duchêne 2007). The micronization of drug substances by milling is also a popular technique for enhancing the drug dissolution rate. Wongmekiat et al. (2002) reported a noticeable improvement in the formation of drug nanoparticles by cogrinding with CDs. Complexation with CDs as well as particle size reduction contributes to modification of the dissolution property of some drugs (Wongmekiat et al. 2006).

Glimepiride (GLM), a third-generation sulphonylurea drug, has been widely used for the control of type 2 diabetes mellitus (Massi-Benedetti 2003; Inukai et al. 2005). GLM exhibits very poor solubility at 37 °C (< 0.004 mg/mL) in acidic and neutral media and relatively high permeability (30.4×10^{-6} cm/s) through CaCo-2 cell monolayers (Frick et al. 1998). Thus, GLM is categorized as a Class 2 drug by the Biopharmaceutics Classification System (Amidon et al. 1995). Solubilization of GLM would, therefore, be an effective way to ensure absorption of the drug and to achieve a reproducible clinical response.

Complexation of GLM with CDs and water-soluble polymers was demonstrated using the kneading method to maintain the concentration of dissolved GLM, which was incorporated into the CD cavity in aqueous solvent (Ammar et al. 2006b). The polymers may work as inhibitors for the dissociation of a GLM-CD inclusion complex, or may cause crystallization of GLM molecules by increasing the viscosity of the solution, or both. In this study, our approach focused on further enhancing GLM solubility. We previously reported an improvement in the dissolution behavior of some compounds by cogrinding them with several cyclic glucans in the solid state (Fukami et al. 2004, 2006). Thus, the application of this technique was expected to enhance the reactivity between GLM and CDs. In addition, the intermolecular interaction was examined by using phase-solubility techniques, powder X-ray diffractometry (XRD), differential scanning calorimetry (DSC), and infrared (IR) spectroscopy.

2. Investigations, results and discussion

2.1. Solubilization of glimepiride

2.1.1. Phase solubility diagram

In order to study the compatibility of different kinds of CDs with GLM, a phase solubility technique was used to

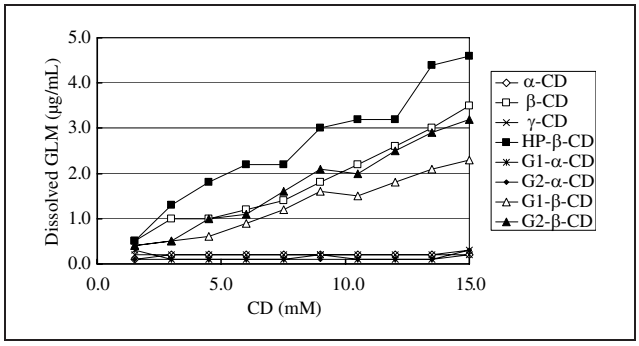

Fig. 1: Phase-solubility diagram of the GLM-CDs system

examine the effects of CDs on the solubility of GLM in aqueous solution (Fig. 1). The phase solubility profiles of GLM with β-CD and its derivatives were classified as A_L-type, indicating the formation of a 1 : 1 stoichiometric water-soluble molecular complex. Since the solubility of GLM increased linearly up to approximately 2.3 (G1-β-CD) or 4.6 (HP-β-CD) μg/mL, water-soluble complexes would be formed within the range of CD concentrations examined. No solubilizing effect was observed for α- and γ-CDs, which have 6 and 8 glucopyranose units, respectively. It was suggested that the cavity of the β-ring, which consists of 7 glucopyranose units, was suitable for accommodating part of a GLM molecule.

2.1.2. Cogrinding of glimepiride with cyclodextrins in solid state

In an attempt to further improve GLM solubility, the cogrinding technique was performed on GLM and CDs in solid state. Based on a previous study, GLM and the CDs were weighed and mixed at a 1 : 5 weight ratio, as opposed to molar ratio, since excess amounts of dextrin are required for solid state cogrinding (Fukami et al. 2004, 2006). The concentration of each CD was estimated to be approximately 2.5 mmol/L in this experiment. Figure 2 shows the GLM concentrations in distilled water after using the cogrinding treatment with various CDs. Cogrinding with β-CD and its derivatives improved the solubility of GLM with a propensity similar to the phase solubility diagram, with the exception of hydroxypropyl (HP) β-CD. In this case, GLM concentrations were 5 to 10 times greater than those observed in the phase solubility study. In general, powder properties play an important role in the cogrinding process, thus the combination of GLM and HP-β-CD, the ratio, or both may not be suitable for the mechanochemical reaction. Further studies of this phenomenon are currently under investigation in our laboratory.

The drug concentrations decreased to ca. 80% of the initial level 4 h after the powder mixture was suspended into distilled water, and were stable for at least 24 h. This suggests that some GLM molecules initially existed in a supersaturated state. That is, mechanical shear stress forced GLM molecules to disperse into β-CD and its branched derivatives during the cogrinding process. This preparation method in solid state would be effective for the solubilization of poorly water-soluble APIs.

2.1.3. Cogrinding of glimepiride with cyclodextrins using glass balls

Since mechanical energy could activate the reactivity between solid samples, light-weight glass balls were used instead of alumina balls to increase the collision frequency. Interestingly, the concentration of GLM increased drastically up to ca. 250 μg/mL with β-CD (Fig. 3). Other β-CD derivatives also enhanced GLM solubility by approximately 10-fold compared with that observed after the phase solubility technique. Hence, the material of the milling balls may affect the mechanochemical reaction between GLM and CDs due to physical and chemical factors, such as mobility, hardness and the surface activity of the milling balls.

Redenti et al. (2001) reviewed the application of simultaneous CD complexation and salt formation for improving the pharmaceutical performance of APIs, reporting that complexation between CDs and salts of acidic drugs increased the total solubility of the drugs (free ionized drug + free un-ionized drug + ionized drug complex + un-ionized drug complex). This method has been widely applied to poorly water-soluble nonsteroidal anti-inflammatory drugs, bile acids, and hypoglycemic agents. Glibenclamide (GBC), a second generation sulphonylurea agent for diabetes mellitus, was also reported to form a 1 : 1 : 3 GBC : Na : CD complex, which was freely soluble in water and exhibited an improved *in vitro* dissolution rate, even at acidic pH (Chiesei et al. 1998). Thus, we compared the pH values of solutions prepared from the coground mixtures of CDs and GLM using alumina and glass balls, which yielded results around 5.5 and 7.0, respectively. These results indicate that a slight amount of the alkaline components in the soda-lime glass ball may have been incorporated into the ground mixture during the cogrinding process. However, a change in pH may not be the

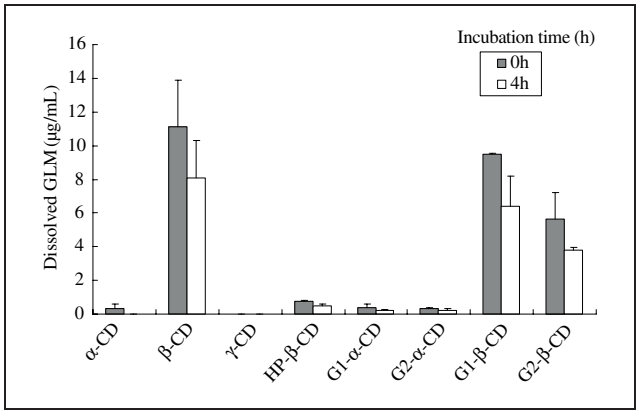

Fig. 2: Solubility of GLM improved by alumina-ball milling with CDs

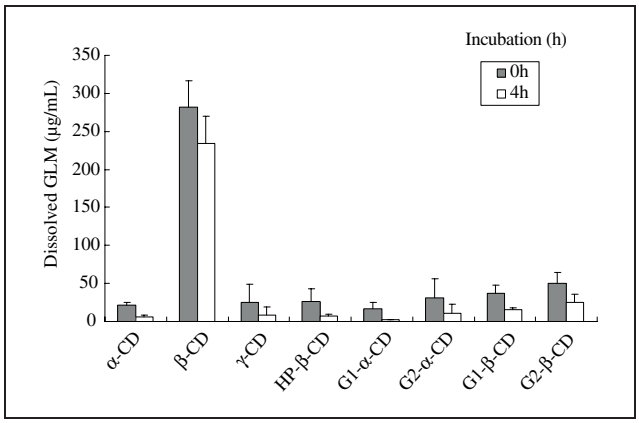

Fig. 3: Solubility of GLM improved by glass-ball milling with CDs

only factor contributing to the enhancement of solubility in this system since the solubility of GLM was reported to be 1.20 (μg/mL) at pH 7.0 (Endo et al. 2003). The activity coefficient of neutral organic compounds has been shown to increase with increasing ionic strength of the dissolution medium, thereby favoring the transfer of the compounds from the hydrophilic medium to the hydrophobic cavity of the CD (Ferreira et al. 2001). Considering these aspects, the ionic components of glass balls may belong to the factors contributing to the enhancement of complexation between GLM and CDs in aqueous solution.

2.2. Characterization of glimepiride in the binary mixture

A molecular complex appeared to have been formed by cogrinding GLM with particularly β-CD in the dissolution study. Thus, the intermolecular interaction in the solid state was evaluated by several analytical techniques, including powder XRD, DSC, and infrared spectroscopy (FTIR).

2.2.1. Powder X-ray diffractometry

In order to study the molecular states of GLM in the solid state, powder XRD was employed for the mixture of GLM and β-CD. As shown in Fig. 4, characteristic diffraction peaks were observed at 13.5, 18.2 and 21.1° (2θ), corresponding to the crystalline form I of GLM (Endo et al. 2003). The diffraction pattern of the physical mixture was a superposition of those of the raw materials.

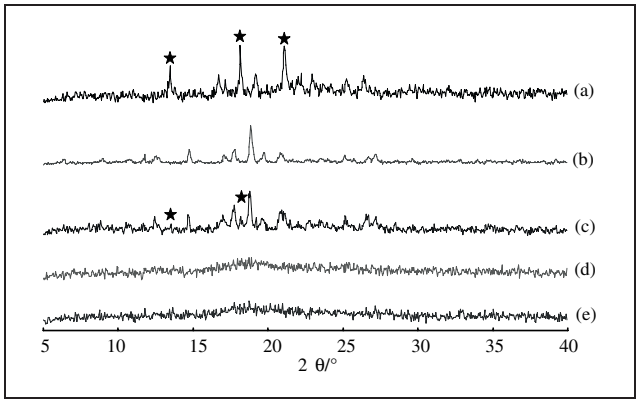

Fig. 4: Changes in powder XRD patterns of GLM during cogrinding: (a) GLM, (b) β-CD, (c) PM, (d) GM with alumina-ball milling, (e) GM with glass-ball milling

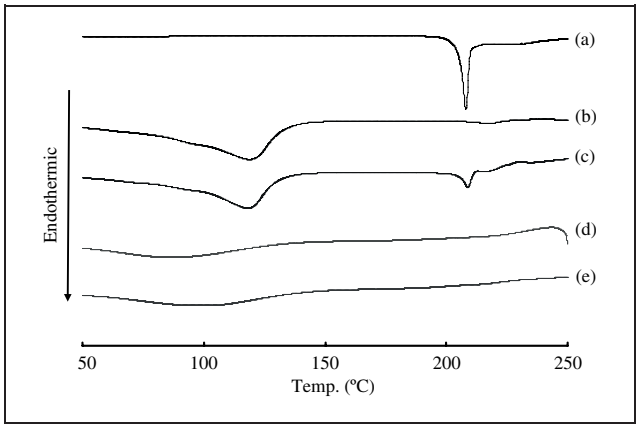

Fig. 5: DSC curves of GLM, β-CD and their mixtures: (a) GLM, (b) β-CD, (c) PM, (d) GM with alumina-ball milling, (e) GM with glass-ball milling

Although the peak intensities of crystalline GLM decreased after cogrinding regardless of milling ball materials, new diffraction peaks indicating the formation of a crystalline complex did not appear. This suggested that the crystalline structures of β-CD as well as GLM were disrupted and that GLM molecules were substantially dispersed into amorphous β-CD in the solid state.

2.2.2. Differential scanning calorimetry

DSC measurements were carried out to clarify the thermal behavior of GLM, β-CD, and their binary mixtures (Fig. 5). The physical mixture showed endothermic peaks around 110 °C and at 208 °C, which were assigned to water vaporization from β-CD and the fusion of GLM crystals, respectively. On the contrary, the sharp endothermic peak corresponding to GLM melting disappeared in the ground mixture. Since the thermal reaction due to water loss from β-CD also decreased, this indicated that GLM and the crystalline waters of β-CD were molecularly dispersed in the amorphous binary mixtures. This result agreed well with that of powder XRD, suggesting that a mechanochemical effect induced the interaction between GLM and β-CD molecules during the cogrinding process.

2.2.3. IR measurement

IR spectroscopy was used to obtain a structural characterization of the complex. Characteristic bands were observed at 1707 and 1673 cm^{-1} in the IR spectra for both GLM alone and for the physical mixture, which corresponded to the carbonic stretching band of GLM (Fig. 6a, c). These absorption bands were assigned to the ureidic and amidic C=O, respectively. Two peaks were also observed at 1347 and 1156 cm^{-1}, which were assigned to the sulphonyl amide vibration of GLM (Moyano et al. 2003). After cogrinding, the carbonyl stretching bands united into a broad peak at around 1700 cm^{-1} in the C=O stretching region. In addition, a general reduction in the intensity of the bands and a loss of spectral resolution were observed. Hence, it was suggested that each intact hydrogen bond network had collapsed and some intermolecular interactions between GLM and β-CD had formed because carbonyl absorptions of GLM showed a band shift when interacting with the CD molecule. Although the spectral change was visually unclear in previous reports, it was explained that sulphonylurea and amide groups of GLM interacted with β-CD (Moyano et al. 2003; Ammar et al. 2006a). Consequently, molecular complexes of GLM and β-CD would be formed during cogrinding, which would be responsible for the enhancement of GLM solubility.

In conclusion, almost insoluble GLM was significantly solubilized by forming water-soluble molecular complexes with CDs in aqueous solution. A remarkable effect was observed on the solubilization of GLM coground with β-CD and its branched derivatives, particularly when glass balls were used. The dominant dissolution enhancing factors were estimated as follows: (i) complex formation between GLM and CDs, (ii) pH and/or ionic strength changes appropriate for complexation in solution, and (iii) micronization of GLM and CD particles. Further studies are expected to elucidate the interactive manner between a targeted sample and cyclodextrins, which would assure application of this method to various insoluble compounds, including drug substances. A new technique has been added to the solubilization technologies available for poorly water-soluble drugs.

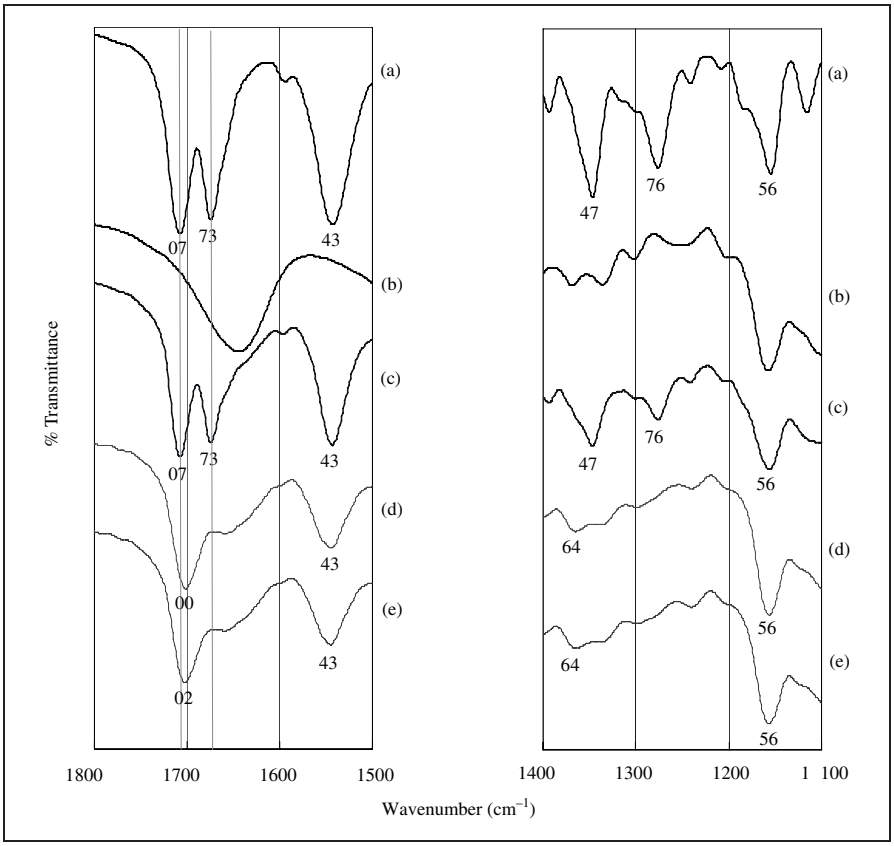

Fig. 6:
FT-IR spectra for GLM-CDs systems: (a) GLM, (b) β-CD, (c) PM, (d) GM with alumina-ball milling, (e) GM with glass-ball milling

3. Experimental

3.1. Chemical and reagents

Four kinds of CDs, α-, β-, and γ-CD and HP-β-CD were provided by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Branched CD derivatives, including G1-α-CD, G2-α-CD, G1-β-CD, and G2-β-CD, were received as gifts from Ensuiko Sugar Refining Co., Ltd. (Tokyo, Japan). GLM was extracted from commercially available tablets, Amaryl® (Sanofi-Aventis K.K., Tokyo, Japan), and purified according to previous reports (Iwata et al. 1997, Endo et al. 2003). All other chemicals were of reagent grade, purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and used without further purification.

3.2. Phase solubility diagrams

Solubility experiments were performed as described by Higuchi and Connors (1965). Approximately 10 mg of GLM were added to various concentrations of each CD in aqueous solution (1.5–15 mmol/L, 10 mL). The suspensions were shaken in a water bath at 37 °C for 72 h. An aliquot was filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and assayed by HPLC (LC-2000, Jasco, Tokyo, Japan) to obtain a phase solubility diagram. The HPLC conditions were as follows: mobile phase, acetonitrile-50 mM phosphate buffer (13 : 7); column, Inertsil ODS-3 (5 μm, 4.6 mm i.d. × 150 mm, GL Sciences, Tokyo, Japan); flow rate, 1.0 mL/min; column temperature, room temperature; UV detection, 233 nm.

3.3. Solubilization of glimepiride by cogrinding

Physical mixtures of GLM and CDs were prepared at a weight ratio of 1 : 5 using a pestle and mortar. For the preparation of a ground mixture, 15 mg of physical mixture was loaded into a 5 mL shade glass vial with alumina or glass balls (diameter 5 mm) and coground using a Desktop Ball Mill (V-1M, Irie, Tokyo, Japan) at 150 rpm at room temperature for 48 h. After cogrinding, 9 mg of ground mixture were dispersed in 3 mL distilled water, and incubated under gentle shaking at 25 °C. The suspensions were filtered through a 0.45 μm membrane. The filtrate was subjected to HPLC analysis under the same conditions described above.

3.4. Powder X-ray diffractometry (XRD)

Powder XRD was carried out using a Geigerflex Rad-II with a Cu-Kα radiation source (Rigaku Corporation, Tokyo, Japan). Data were collected at a scan rate of 6° min^{-1} over a 2θ range of 5° to 40°. The accelerating voltage was 35 kV and the current was 25 mA.

3.5. Differential scanning calorimetry

DSC curves were obtained using DSC 8230 (Rigaku, Tokyo, Japan). Approximately 3 mg of powdered sample were placed into an aluminum crimped pan and measured at a scanning speed of 5 °C min^{-1} under a flow of nitrogen gas.

3.6. Infrared spectroscopy

A model 230 FT-IR spectrometer (JASCO Corporation, Tokyo, Japan) was used. The measurements were carried out using the KBr method. Spectra (64 scans at 4 cm^{-1} resolution) were collected in the 4000 to 400 cm^{-1} range.

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