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Preparation of amorphous indomethacin from aqueous 2,6-di-*O*-methyl-β-cyclodextrin solution

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Abstract

Indomethacin precipitated exclusively in an amorphous form from aqueous 2,6-di-*O*-methyl-β-cyclodextrin solutions, whereas it precipitated in Form V polymorph from the solutions of the drug alone, parent cyclodextrins and 2-hydroxypropyl-cyclodextrins. The polymorphic transition of the amorphous form to Form V crystals in aqueous solution was markedly inhibited by the addition of 2,6-di-*O*-methyl-β-cyclodextrin, keeping the amorphous state for at least 5 days at 4 °C, whereas it quickly transformed to Form V crystals in the absence of 2,6-di-*O*-methyl-β-cyclodextrin. 2,6-Di-*O*-methyl-β-cyclodextrin suppressed the solution-mediated polymorphic transition of amorphous form of indomethacin to Form V crystals in aqueous solution. The current results suggested that 2,6-di-*O*-methyl-β-cyclodextrin is useful for isolation of amorphous indomethacin that occurs at an early stage of crystallization according to "Ostwald's Rule of Stages".

Keywords: Indomethacin; Amorphous form; Solution-mediated polymorphic transition; 2,6-Di-*O*-methyl-β-cyclodextrin; Inclusion complex

1. Introduction

Different polymorphs exhibit different physicochemical properties such as solubility, dissolution rate, bioavailability, and chemical and physical stabilities (Byrn, 1982; Brittain, 1999; Bernstein, 2002). Therefore, it is of great importance to discover, produce and isolate polymorphs of a given solid drug and to control their polymorphic transformations for not only development of high-quality drugs but also protection of valuable intellectual property. Amorphous or metastable forms of poorly water-soluble drugs are purposely formulated in solid preparations to improve their low solubility, dissolution rate and oral bioavailability. However, they are physically or chemically labile and quickly transform to stable forms, which make difficult to prepare them with employing simple recrystallization techniques in solvents. In particular, amorphous forms are generally prepared with freeze-dried, spray-dried and chilled methods, *etc.* (Guillory, 1999). Polymorphic transformations proceed generally *via* the solid–solid or the solution-mediated mechanism (Rodriguez-Hornedo and Murphy, 1999; Bernstein, 2002). The solid–solid transformation is dependent on internal rearrangements or conformational changes of the molecules in crystals. On the other hand, the solution-mediated transformation is controlled by differences in solubility of stable and metastable forms, where a metastable form with higher solubility appears first from solution and it then dissolves, nucleates and transforms into a stable form with the lowest solubility, according to "Ostwald's Rule of Stages" (Ostwald, 1897). Therefore, we usually obtain only the final form that is most stable at the experimental conditions. On the other hand, it is difficult to isolate the intermediate metastable forms occurring during crystallization and transforming rapidly to the stable form.

Cyclodextrins (CyDs), cyclic oligosaccharides consisting of usually 6–8 D-glucose units, form inclusion complexes with various molecules in aqueous solution and in solid states, and are successfully utilized for improvement of pharmaceutical properties of drugs (Stella and Rajewski, 1997; Uekama et al., 1998; Uekama, 2004). In previous papers, we reported that 2,6-di-*O*-methyl-β-CyD (DM-β-CyD) markedly suppresses the solution-mediated polymorphic transformation of the Ostwald's

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metastable form of tolbutamide to its stable form, yielding exclusively the metastable form, which was ascribable to the inclusion complex formation of the drug with the host molecules (Sonoda et al., 2006a,b). Indomethacin, a nonsteroidal anti-inflammatory drug is known to show polymorphism and to transform to its stable form in solution (Kaneniwa et al., 1985). Therefore, we investigated the effects of CyD derivatives on crystallization of indomethacin from aqueous solution. Interestingly, we prepared amorphous indomethacin from aqueous DM-β-CyD solution and demonstrated that the host molecule significantly inhibits the solution-mediated polymorphic transition (Tse et al., 1994) of amorphous indomethacin to its other polymorph in water.

2. Materials and methods

2.1. Materials

Parent α-, β- and γ-CyDs, 2-hydroxypropyl-α-CyD (HP-α-CyD, degree of substitution (DS) of 2-hydroxypropyl groups is 5.2), HP-β-CyD (DS 4.8), and HP-γ-CyD (DS 5.4) were supplied by Japan Maize Co. (Tokyo, Japan). 2,6-Di-*O*-methyl-α-CyD (DM-α-CyD) and DM-β-CyD were purchased from Wako Pure Chemicals Co. (Kyoto, Japan). Indomethacin was obtained from Sumitomo Pharmaceutical Co. (Osaka, Japan). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

2.2. Crystallization

The crystallization of indomethacin in the absence and presence of CyDs in aqueous solution was conducted as follows: indomethacin (2.0 mM) was dissolved in the absence and presence of CyDs at various concentrations in pH 8.0 sodium phosphate buffer (25 mL, prepared with 0.1 M H_3PO_4/0.1 M NaOH, $I = 0.2$ with NaCl) in a 50 mL beaker at room temperature. The solution was slowly titrated with aqueous 0.5 M HCl solution (about 2 mL) to about pH 5.0 where the drug did not yet precipitate. The solution was paper-filtered and the filtrate was put in a refrigerator (4 °C) for 1 day unless otherwise stated. The precipitated indomethacin crystals were collected by filtration. In experiments of the competitive inclusion complexation, the competitors (benzoic acid derivatives) were dissolved at different concentrations in solutions with 2 mM indomethacin and 5 mM DM-β-CyD in pH 8.0 sodium phosphate buffer solutions and the pH was changed to 5.0 with 0.5 M HCl solution. The crystallization was conducted at the same conditions as described above. The contents of different polymorphs were determined by powder X-ray diffractometry (Rigaku RINT 2500) under the following conditions: Ni-filtered Cu Kα radiation (1.542 Å), 40 kV, 40 mA, divergent slit of 1.74 mm (1°), scanning slit of 0.94 mm (1°), receiving slit of 0.15 mm, and goniometer angular increment of 1°/min. Concentrations of indomethacin in the filtrates were determined by UV spectroscopic method at 265 nm.

2.3. Interaction of indomethacin with CyDs

The interaction of indomethacin with CyDs was studied by the solubility method and ^{1}H NMR spectroscopy. The solubility method was conducted according to the method of Higuchi and Connors (1965). An excess amount of indomethacin (about 5 mg) was added in a test tube containing CyD solutions at various concentrations in water (1 mL), and the mixture was shaken at 25 °C for about 3 days. After the equilibrium was attained, an aliquot (0.5 mL) was taken by a cotton-plugged pipette, diluted appropriately with water, and analyzed for indomethacin by UV spectroscopy at 265 nm. The interaction of DM-β-CyD with the competitors was also studied by the solubility method under the same conditions except for the added amounts (5–25 mg) and the analytical wavelengths (λ_{max} for each competitor). The stability constant (K) of CyD complexes was calculated by the equation of K = slope/[intercept (1 − slope)] (Higuchi and Connors, 1965) using slopes and intercepts of the initial straight line portion of the phase solubility diagrams. ^{1}H NMR spectra were taken at 30 °C on a Jeol JNM-α 500 spectrometer operating at 500 MHz, using a 5-mm sample tube. Indomethacin (50 mM) and CyDs (25–150 mM) were dissolved in 0.1 M borate buffer/D_2O solution (pH meter reading of 9.3). The continuous variation plots (Job, 1928) were made under a constant concentration (50 mM) of host and guest molecules. The 2D-ROESY spectra were taken at concentrations of guest (50 mM) and host (50 mM) compounds. ^{1}H NMR signals of DM-CyDs were assigned according to the report of Onda et al. (1998).

3. Results and discussion

As described in the section of Material and methods, indomethacin was dissolved in the absence and presence of CyDs in pH 8.0 sodium phosphate buffer and then the solution was slowly titrated with aqueous 0.5 M HCl solution to about pH 5.0. The solution was paper-filtered and the filtrate was put in a refrigerator (4 °C) for 1 day for crystallization of the drug. Fig. 1 shows powder X-ray diffraction patterns of solids precipitated

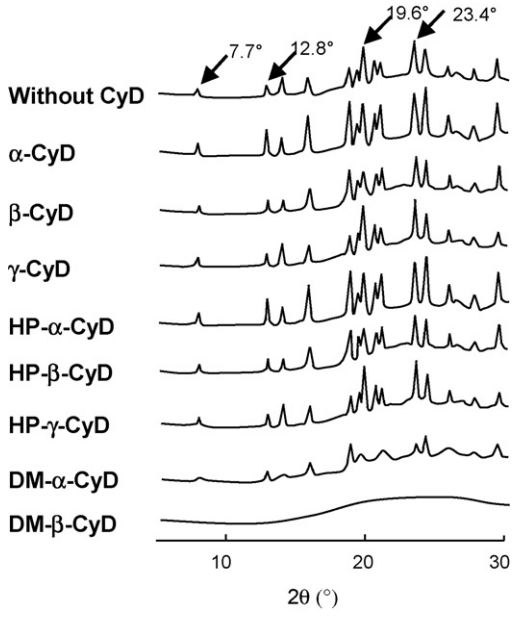


Fig. 1. Powder X-ray diffraction patterns of indomethacin precipitated in the absence and presence of CyDs (5 mM) in phosphate buffer (pH 5.0), stored at 4 °C for 1 day.

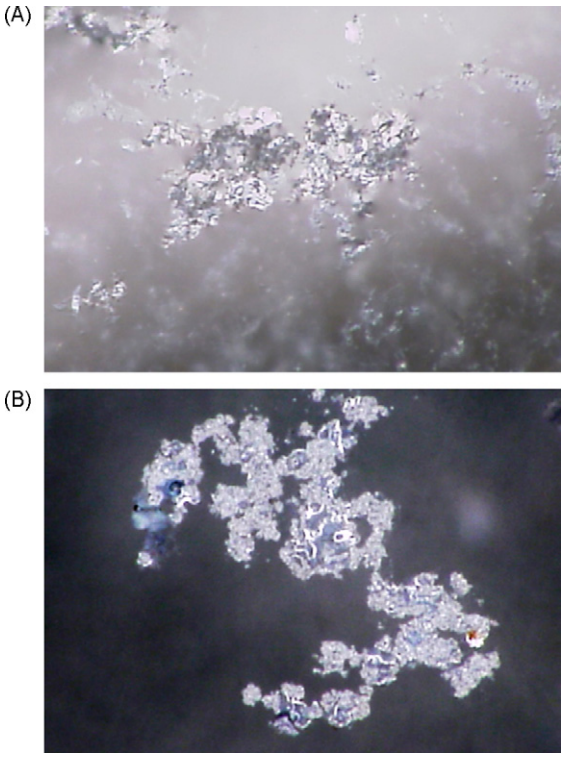


Fig. 2. Photographs of Form V crystals (A) and amorphous form (B) of indomethacin, precipitated in the absence and presence of DM-β-CyD (5 mM), respectively, in pH 5.0 phosphate buffer at 4 °C.

from aqueous, buffered (pH 5.0) 2 mM indomethacin solutions in the absence and presence of various CyDs (5 mM). It is of interest to note that the solid precipitated from aqueous DM-β-CyD solution is in amorphous state, showing a halo-pattern in powder X-ray diffractogram. On the other hand, indomethacin crystallized into Form V crystals of the drug reported by Lin, giving the diffraction peaks at $2\theta = 7.7°$, 12.8°, 19.6° and 23.4° (Lin, 1992). Fig. 2 shows macroscopic appearances of amorphous and Form V forms of indomethacin precipitated from solutions with and without DM-β-CyD, respectively, Form V crystals being thin plate-like and the amorphous form being very small powder-like. These results indicate that indomethacin precipitates in amorphous state from aqueous DM-β-CyD solutions.

To gain insight into the mechanism for the precipitation of amorphous indomethacin from DM-β-CyD solution, we compared the crystallization behavior of the drug from aqueous sodium phosphate buffer solutions (pH 5.0) without and with 5 mM HP-β-CyD, DM-α-CyD and DM-β-CyD. The content of Form V crystals in the obtained total crystal mass was determined from the diffraction intensity at $2\theta = 19.6°$ (see Fig. 1) and that of the amorphous form was estimated by subtracting the Form V content from the total amount of the drug. Fig. 3A–D show changes in contents of Form V and amorphous forms in the total crystal mass obtained during storage (1 day to 1 week) at 4 °C. From indomethacin solutions without CyDs or with HP-β-CyD (Fig. 3A and B, respectively), the drug precipitated as Form V crystals from the initial stage of the crystallization (after 1 day). In the case of DM-α-CyD solution, the amorphous form initially precipitated, but rapidly transformed to Form V crystals within about 3 days (Fig. 3C). By contrast, in the presence of DM-β-CyD (Fig. 3D), indomethacin precipitated exclusively as amorphous form, and the amorphous state was maintained for about 5 days. These results indicated that DM-β-CyD inhibits the transition of amorphous form of the drug to Form V crystals under the experimental conditions. DM-α-CyD and HP-β-CyD may suppress the transition to some extent at higher CyD concentrations. However, because viscosity of CyD solution affects the transition, we did not study in detail the effect of CyDs at higher concentrations. Further, the crystallization of indomethacin at higher CyD concentration was difficult due to the solubilization of the drug by CyDs.

Then, the effect of CyD concentration on the crystallization of 2 mM indomethacin in aqueous sodium phosphate buffer solution (pH 5.0) was investigated, employing HP-β-CyD and DM-β-CyD that provided Form V crystals and amorphous form of the drug, respectively. As shown in Fig. 4, HP-β-CyD gave Form V

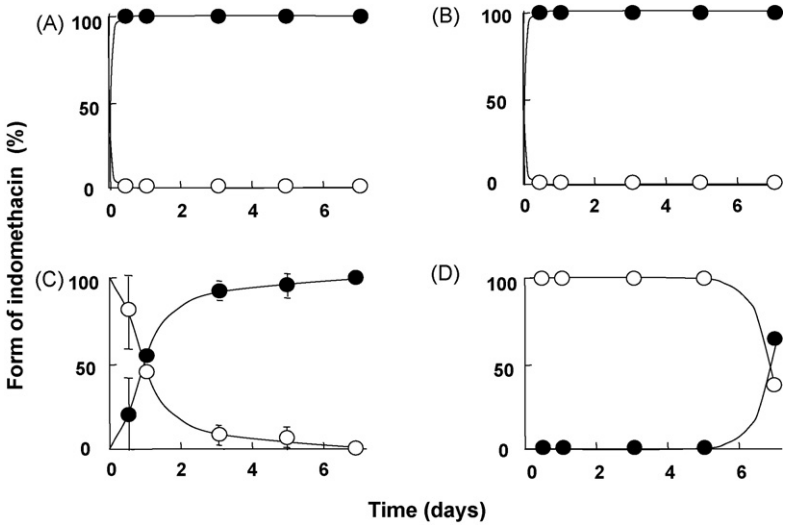


Fig. 3. Time courses for appearance and disappearance of amorphous indomethacin in the absence (A) and presence of 5 mM HP-β-CyD (B), DM-α-CyD (C) and DM-β-CyD (D) in phosphate buffer (pH 5.0) at 4 °C. Each point represents the mean ± S.E. of 3 experiments. (◯) Amorphous form; (●) Form V.

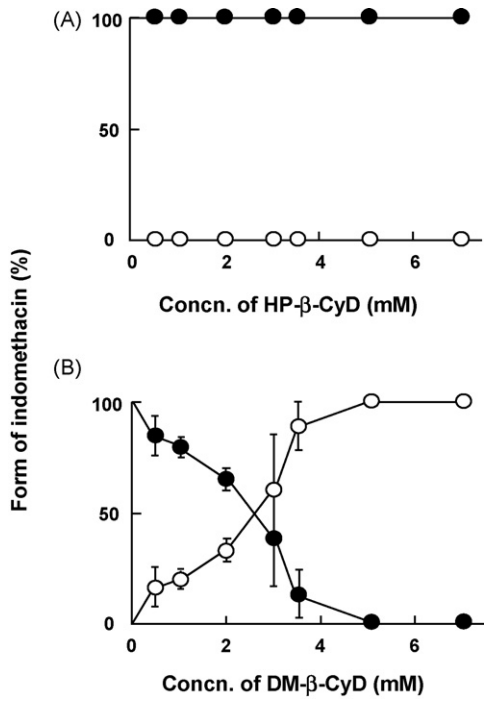


Fig. 4. Effects of CyD concentrations on crystallization of indomethacin (2 mM) in the presence of HP-β-CyD (A) and DM-β-CyD (B) in phosphate buffer (pH 5.0), stored at 4 °C for 1 day. Each point represents the mean ± S.E. of 3 experiments. (◯) Amorphous form; (●) Form V.

crystals of the drug in all CyD concentrations studied (1–7 mM). In the case of DM-β-CyD, on the other hand, the content of the amorphous form increased with increasing CyD concentration, and the conversion of the amorphous form to Form V crystals was completely inhibited above 5 mM DM-β-CyD concentration, thus giving exclusively amorphous indomethacin.

Next, we investigated how amorphous indomethacin transforms to Form V crystals, when excess amounts (10 mg) of the amorphous form was added to the saturating indomethacin solution (0.02 mM, 25 mL) of the most stable Form I crystal (Form γ crystal (Borka, 1974; Tong and Zografi, 2001)) of the drug in the absence and presence of DM-β-CyD (5 mM). In the absence of DM-β-CyD as shown in Fig. 5A, the added amorphous indomethacin converted to Form V crystals within 1 day. In the presence of DM-β-CyD, however, there was no conversion of the amorphous form to Form V crystals under the experimental conditions, as shown in Fig. 5B. Fig. 5C shows changes in concentration of indomethacin in the solutions during the conversion in Fig. 5A and B. The added amorphous indomethacin dissolved in the absence of DM-β-CyD, because the solubility of the amorphous form is larger than that of Form V crystals. Therefore, the indomethacin concentration increased within about 5 h, but shortly afterwards it decreased due to the crystallization of Form V, because the concentration is supersaturated with respect to Form I. On the other hand, when the solution contained 5 mM DM-β-CyD, the added amorphous indomethacin dissolved to a drug concentration of 0.5 mM that remained, as no transformation of the added amorphous form to Form V crystals occurred. These results indicate that the conversion of amorphous indomethacin to Form V crystals proceeds according to

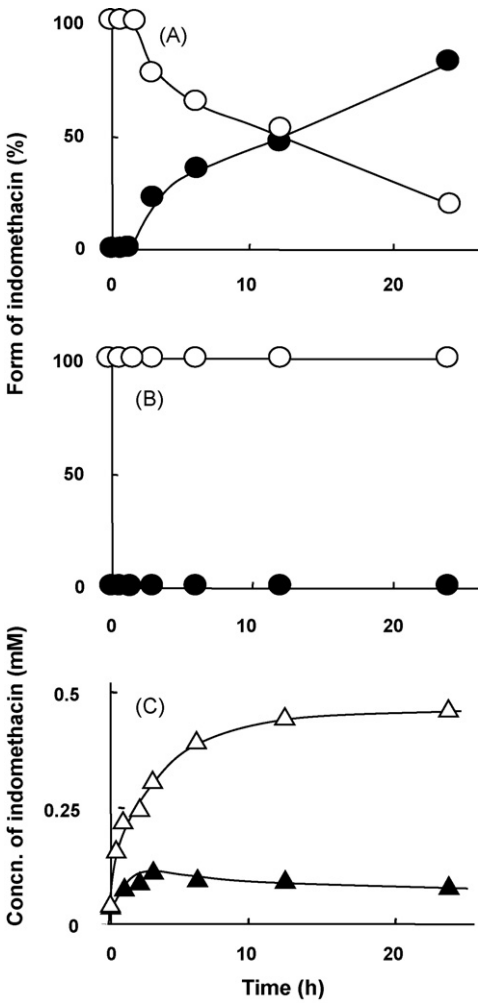


Fig. 5. Time courses for crystallization of amorphous form of indomethacin to Form V crystal in the absence (A) and presence of DM-β-CyD (B) in phosphate buffer (pH 5.0) at 4 °C and changes (C) in indomethacin concentration in solution during the crystallization. (◯) Amorphous form; (●) Form V. Excess amount (10 mg) of amorphous indomethacin was added to a saturated solution of Form I crystal (0.02 mM, 25 mL) of the drug in the absence (▲) and presence (△) of DM-β-CyD (5 mM).

the solution-mediated polymorphic transition mechanism (Tse et al., 1994), and DM-β-CyD inhibits this transition process.

To investigate the effects of inclusion competitors on the crystallization behavior of indomethacin, we added *p*-hydroxybenzoic acid and its ethyl and butyl esters to 5 mM aqueous indomethacin solutions with and without 5 mM DM-β-CyD. In the absence of DM-β-CyD, these competitors did not affect the crystallization of the drug, i.e. precipitation of Form V crystals. On the other hand, in the presence of DM-β-CyD the transformation of the amorphous form to Form V crystals was accelerated by the addition of these competitors (Fig. 6), and was faster when the competitor with a large stability constant of its DM-β-CyD complex was added, e.g. 50% inhibitory concentrations of the competitors were 3.5, 3.0 and 2.0 mM for *p*-hydroxybenzoic acid (the stability constant of competitor/DM-β-CyD complex determined by the solubility method = 120 M^{-1}) and its ethyl (310 M^{-1}) and butyl esters (5800 M^{-1}), respectively.

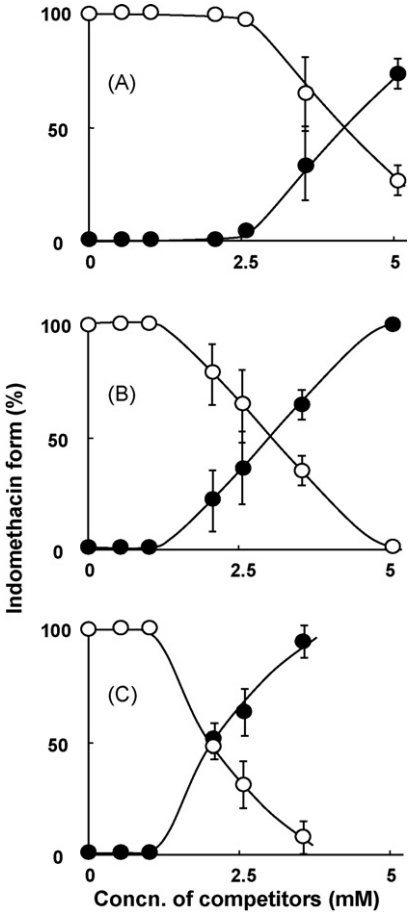


Fig. 6. Contents of amorphous form and Form V crystals of indomethacin solids precipitated in the presence of different concentrations of competitors (*p*-hydroxybenzoic acid (A) and its ethyl (B) and butyl (C) esters) in DM-β-CyD solution (5 mM) in pH 5.0 sodium phosphate buffer at 4 °C. (◯) Amorphous form; (●) Form V.

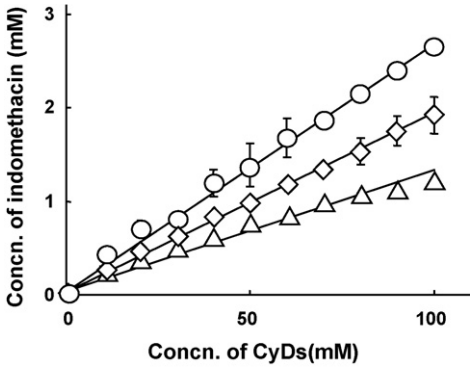


Fig. 7. Phase solubility diagrams of indomethacin/CyD systems in phosphate buffer (pH 5.0) at 25 °C. (◊) With DM-α-CyD; (◯) with DM-β-CyD; (△) with HP-β-CyD.

The interaction of indomethacin with CyDs in aqueous solution was studied by the solubility method and ^{1}H NMR spectroscopy. Fig. 7 shows the phase solubility diagrams obtained in water at 25 °C. In all CyDs, the solubility of indomethacin increased linearly with CyD concentrations, showing A_L type diagrams and the stability constant ($K = 1030\,M^{-1}$) of the indomethacin/DM-β-CyD complex was much higher than those of DM-α-CyD ($K = 720\,M^{-1}$) and HP-β-CyD complexes ($K = 490\,M^{-1}$). The solubilizing ability of DM-β-CyD is much higher than that of DM-α-CyD. In ^{1}H NMR spectroscopic studies, relatively large chemical shift-changes were observed for H6 and H7 protons in the six-membered ring of the indole moiety and the *p*-chlorobenzoyl ring of indomethacin in the presence of DM-α-CyD and DM-β-CyD, as shown in Fig. 8. The continuous variation plots (Fig. 8C) made by monitoring the ^{1}H NMR shift-changes indicated the formation of 1:1 complex of indomethacin with DM-α-CyD and DM-β-CyD (Fig. 8C). Fig. 9 shows 2D-ROESY spectra of indomethacin/DM-CyD systems in pH 9.3 borate buffer at 30 °C. The H14, 15, 17 and 18 protons

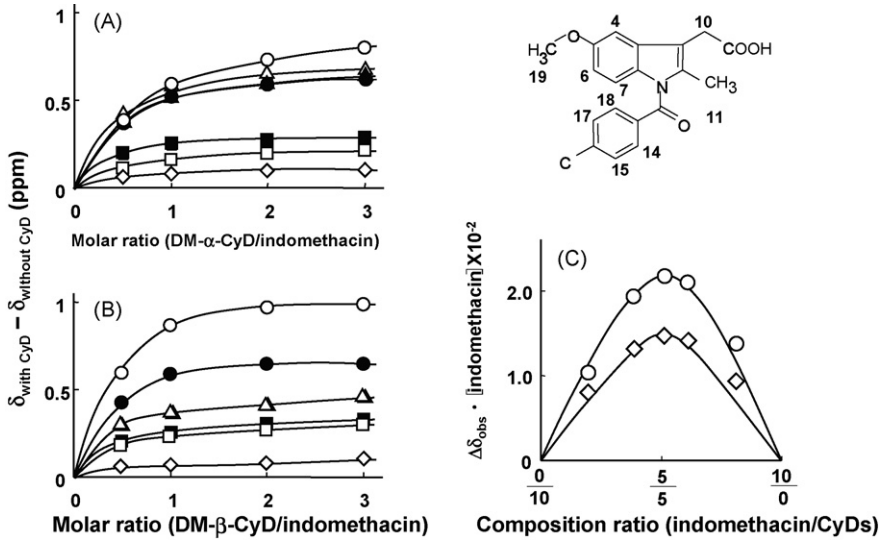


Fig. 8. Effect of DM-α-CyD (A) and DM-β-CyD (B) on ^{1}H NMR chemical shifts of indomethacin in 0.1 M sodium borate/D_2O buffer (pH 9.3) at 30 °C. (●) H6, (◯) H7, (□) H4, (△) H15, 17, (▲) H14, 18, (◊) H11, and (■:) H19. (C) Continuous variation plots of indomethacin/CyD systems in 0.1 M sodium borate/D_2O buffer (pH 9.3) at 30 °C. (◊) DM-α-CyD (H7); (◯) DM-β-CyD (H7).

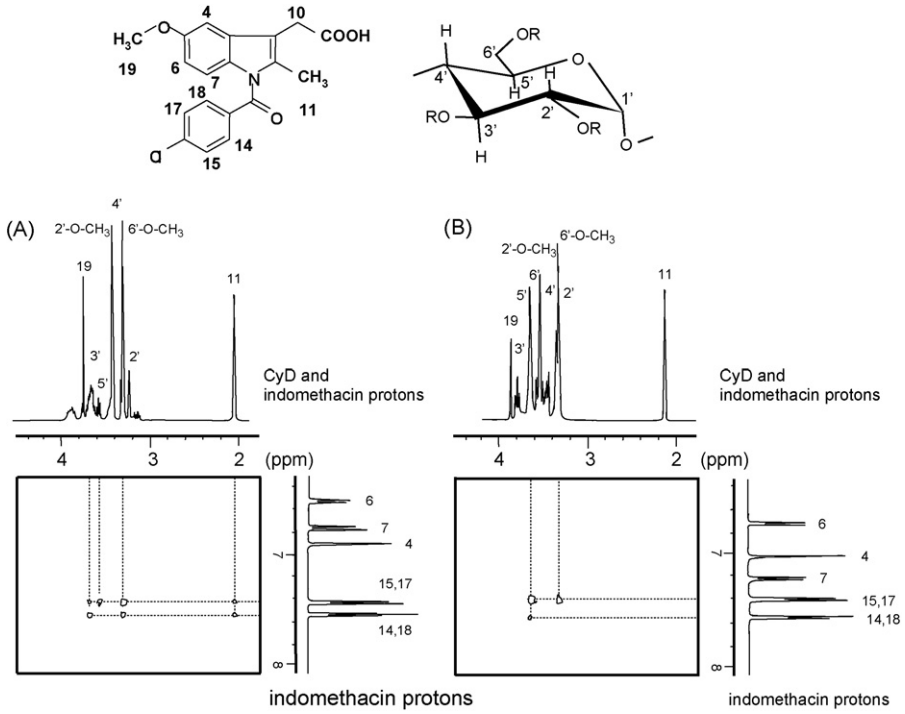
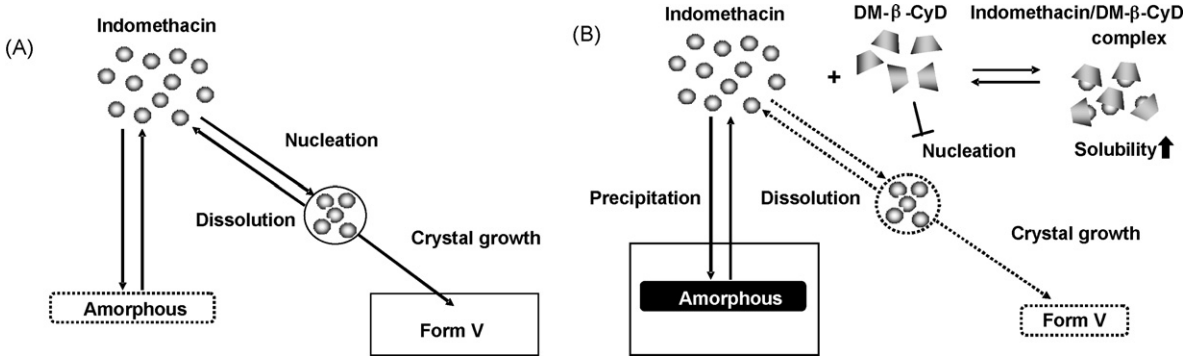


Fig. 9. 2D-ROESY spectra of indomethacin/DM-α-CyD (A) and indomethacin/DM-β-CyD (B) systems in pH 9.3 borate buffer at 30 °C. The concentrations of indomethacin and DM-CyDs were 50 mM.



Scheme 1. Possible crystallization pathways of indomethacin in the absence (A) and presence (B) of DM-β-CyD in solution in phosphate buffer at 4 °C.

of *p*-chlorobenzoyl moiety of indomethacin gave cross-peaks with the H3′ and H5′ protons of DM-α-CyD protons and the H5′, 6′-OCH_3 protons of DM-β-CyD, suggesting that the *p*-chlorobenzoyl ring of indomethacin is preferably included in the DM-α-CyD and DM-β-CyD cavities.

Scheme 1 shows a possible mechanism for the selective precipitation of amorphous indomethacin from DM-β-CyD solutions. In the absence of DM-β-CyD, amorphous indomethacin with higher solubility precipitates from aqueous solution first according to Ostwald's Rule (Ostwald, 1897). However, once Form V crystal nuclei are formed even in small amounts, the indomethacin solution becomes supersaturated state with respect to Form V crystals, and therefore Form V nuclei rapidly grows and the amorphous form dissolves, as shown in Scheme 1A. In the presence of DM-β-CyD, on the other hand, an additional equilibrium of inclusion complexation is introduced to the system, and this equilibrium may compete with the nucleation and crystal growth processes, inhibiting the crystallization of the amorphous form to Form V crystals, as shown in Scheme 1B.

4. Conclusions

The present study indicates that DM-β-CyD complexation is useful for preparation of amorphous indomethacin of Ostwald's metastable forms that occur at an early stage of the drug crystallization. The method described here for control of polymorphic transformation by CyD complex formation will provide an opportunity to isolate labile intermediate metastable polymorphs and will be a valuable tool for the detection and preparation of new polymorphs that are undiscovered so far.

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