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Cyclodextrins as stabilizers for the preparation of drug nanocrystals by the emulsion solvent diffusion method

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Abstract

Cyclodextrins (CyDs) were employed as protective stabilizers for the preparation of surfactant-free nanocrystals of indomethacin (IMC) by using the emulsion solvent diffusion method. The effect of changing the type and concentration of CyDs on the formation of IMC nanocrystals was investigated. Dispersions were freeze-dried to characterize the size, shape, nanoparticle yield, crystallinity, and dissolution behavior of the obtained particles. Submicron-sized particles of IMC with average diameters in the range of 300–500 nm were obtained by incorporating α-, β-, or γ-CyD in the outer phase of the primary emulsions. Quantitative determination demonstrated that more than 80% of IMC was recovered as fine particles smaller than 0.8 μm. The powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) analyses of the freeze-dried samples confirmed the polymorphic change of IMC to the meta-stable form. A significant enhancement in the dissolution rate of IMC nanocrystals was observed when compared to the commercial powder.

Keywords: Cyclodextrin; Emulsion solvent diffusion; Nanocrystals; Indomethacin

1. Introduction

Recent advances in particle engineering technology have significantly enhanced the oral and parentral delivery of poorly water-soluble drugs. The technology of particle size reduction to the nano-scale usually results in a significant increase in drug solubility and dissolution rate with subsequent improvement of drug bioavailability (Müller et al., 2001). Based on the Noyes–Whitney equation, there is a direct relationship between the dissolution velocity and the effective surface area of drug particles. For fine drug powders, the effect of surface area is more pronounced and the dissolution velocity is highly enhanced. In addition, the saturation solubility of the drug can be highly increased by converting the drug particles to the nano-scale (Mosharraf and Nyström, 1995).

Recently, the techniques of particle size reduction to the submicron level have significantly advanced. The common methods for preparing drug nanocrystals depend on advanced milling techniques that utilize excipient stabilization (Müller and Peters, 1998; Merisko-Liversidge et al., 2003). Nanosuspensions produced by high pressure homogenization have recently been demonstrated to produce crystalline drug nanoparticles (Krause and Müller, 2001; Hecq et al., 2005). Nanoparticle precipitation by the anti-solvent method is also a direct and simple procedure for the preparation of drug nanocrystals (Zhang et al., 2006). However, it is usually difficult to control the particle size in the submicron region and the addition of surfactant as stabilizer is necessary to avoid the formation of microparticles. The hazards of organic solvent residuals emerged the use of supercritical fluid-based technologies as new preparation methods of drug nanocrystals. The commonly known processes are supercritical anti-solvent precipitation (Reverchon, 1999; Chattopadhyay and Gupta, 2001) and rapid expansion of supercritical solutions (Pathak et al., 2006). Co-grinding with hydrophilic stabilizers has been reported to produce fine drug particles in the submicron region; examples of co-grinding additives are cyclodextrins (Tozuka et al., 2004; Wongmekiat et al., 2007), polyvinyl pyrrolidones (Itoh et al., 2003), polyethylene glycols, and hydroxylpropyl methylcellulose (Sugimoto et al., 1998).

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of six to eight glucose units linked by α-1,4-glycosidic link-

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age. The molecular structure of CyDs approximates a truncated cone with a hydrophilic exterior surface and a non-polar interior cavity (Loftsson and Brewster, 1996). Because of this feature, CyDs can form inclusion complexes with drug molecules resulting in some changes in the physicochemical properties of guest molecules such as solubility and stability (Blanchard and Proniuk, 1999). One of the most important applications of CyDs is enhancing the solubility of poorly water-soluble drugs by complex formation (Becket et al., 1999; Veiga and Ahsan, 2000). However, there is growing evidence about the non-inclusion based aspects of interaction between CyDs and drug molecules, including formation of molecular aggregates and surfactant-like effects (Loftsson et al., 2004).

The aim of the present study was to prepare drug nanocrystals of a poorly water-soluble drug by applying the emulsion solvent diffusion method using CyDs as nanocrystal stabilizers. Indomethacin (IMC) is a non-steroidal anti-inflammatory drug (NSAID) that has very low aqueous solubility (solubility in water: 5 μg/ml) (Hancock and Parks, 2000) which is often used as a model for practically insoluble drugs. In the present method, the model drug was dissolved in a water-miscible organic solvent which was slowly poured into the aqueous CyD phase with moderate mechanical stirring. The obtained dispersions were freeze-dried for further physicochemical characterization of the obtained powdered particles.

2. Materials and methods

2.1. Materials

Indomethacin (IMC) was supplied from Sumitomo Pharmaceuticals Co. Ltd., Japan. Three different types of cyclodextrins (α-, β-, and γ-CyDs) were purchased from Nacalai Tesque, Inc., Japan. All other chemicals and solvents were of reagent grade.

2.2. Preparation of IMC nanocrystals

The emulsion solvent diffusion method was adapted in the current study for preparation of surfactant-free nanocrystals of IMC using CyDs as dispersion stabilizers. Typically, the ethanolic drug solution (200 mg/10 ml) was slowly added to 100 ml of aqueous CyD solution using a peristaltic pump (Tokyo Rikakikai Co. Ltd., Japan) at a flow rate of 2.0 ml/min while continuously stirring at 400 rpm with a propeller mixer. Different concentrations of α-, β-, or γ-CyD (0.1, 0.5, and 1.0%, w/v) were used in the outer phase of the emulsion. The formed dispersion was immediately centrifuged for 10 min at 4 °C and 20,000 rpm using a high speed refrigerated centrifuge (Kubota Co., Japan) and the obtained residue was redispersed in distilled water with sonication. This process was repeated twice and the final dispersion was subjected to freeze drying at −120 °C for 72 h (FD-81TS, Tokyo Rikakikai Co. Ltd., Japan).

2.3. Particle size and size distribution

The particle size of the freeze-dried samples was determined after dispersion in distilled water by dynamic light scattering method with a photon correlation spectroscopy (PCS) using Zetasizer 3000HS (Malvern Instruments, United Kingdom). The measured parameters by PCS are the average particle size diameter (ZAve) and the polydispersity index (PI). Fiber optics particle analyzer with laser diode light source and photomultiplier tube detector (FPAR-1000, Photal, Otsuka Electronics, Japan) was used additionally to measure the particle size distribution of the prepared samples.

2.4. Determination of IMC recovered as nanoparticles (nanoparticle yield)

The drug content of the freeze-dried samples was checked by UV-spectrophotometry to confirm the purity of the prepared samples. For the quantitative determination of IMC recovered as nanoparticles, aqueous dispersions of the prepared samples (25 mg/10 ml distilled water) were passed through 0.8 μm filter. The filtrates containing fine particles smaller than 0.8 μm were dissolved in 3 ml ethanol and appropriately diluted with phosphate buffer (pH 6.8). The concentration of IMC was determined spectrophotometrically at a wavelength of 320 nm (UV-1700, Shimadzu, Japan). The amount of drug in the filtrate relative to the total amount of drug in the dispersion was calculated and expressed as the percentage recovery (nanoparticle yield).

2.5. Scanning electron microscopy (SEM)

The morphology of the commercial IMC powder and the freeze-dried samples was examined by SEM (JSM-330A, Nihon Denshi, Japan). Prior to examination, the samples were mounted onto metal stubs using a double sided adhesive tape and sputtered with a thin layer of gold under vacuum. The scanning electron microscope was operated at an acceleration voltage of 15 kV.

2.6. Powder X-ray diffraction (PXRD)

PXRD analysis was performed using a Rigaku Geigerflex powder X-ray diffractometer (Rigaku Denki, Japan). The scanning rate was 4°/min over a 2θ range of 5–40°.

2.7. Differential scanning calorimetry (DSC)

Differential Scanning Calorimeter (DSC-6200, Seiko Instruments Inc., Japan), equipped with a liquid nitrogen cooling system, was used to measure the thermal behavior of the commercial IMC powder and the freeze-dried samples. In DSC analysis, 2–3 mg of sample powder was put in aluminum pan and examined at a scanning rate of 10 °C/min from 25 to 200 °C.

2.8. Dissolution test

A dissolution test for the commercial IMC powder and the freeze-dried samples was carried out according to the Japanese pharmacopoeia (XV). The prepared samples or the commercial drug powder (25 mg) were added to 900 ml of JP 2nd fluid (phosphate buffer pH 6.8) at a temperature of 37 ± 0.5 °C and paddle

Table 1
Effect of β-CyD concentration in the outer phase of primary emulsion on the average particle diameter (ZAve), polydispersity index (PI), and nanoparticle yield of the freeze-dried samples

β-CyD concentration (%, w/v)	ZAve (nm)	PI	Nanoparticle yield (%)
0.1	437.6	0.348	82.4
0.5	399.8	0.168	84.9
1.0	336.8	0.013	85.1

stirring at a rotation speed of 100 rpm. Five milliliters samples were withdrawn at specific time intervals, filtered through 0.2 μm filter, and the concentration of IMC was determined by UV-spectrophotometry.

3. Results and discussion

When the ethanolic solution of IMC was added to an aqueous phase in the presence of CyD by the emulsion solvent diffusion method, a homogenous milky dispersion was obtained. On the other hand, sticking of IMC particles on the propeller was observed when a control preparation was carried out in distilled water without CyD. The dispersion was centrifuged in order to yield an easily redispersible pellet of particles, and finally freeze-dried for further characterization. Prior to the freeze drying step, the dispersion was rapidly frozen to −120 °C in a stainless steel container, since it was reported that rapid freezing rates preserve the configuration of the liquid phase of the nanodispersion and keep the individual nanocrystals separate and redispersible (Lee and Cheng, 2006).

The particle size of the freeze-dried samples was determined using dynamic light scattering method after dispersion in distilled water by sonication for 5 min. Table 1 shows the average particle size (ZAve) and the polydispersity index (PI) values for IMC nanocrystals prepared by emulsion solvent diffusion in different concentrations of β-CyD solutions. Submicron-sized particles with average diameters in the range of 300–500 nm were successfully produced. The PI gives an indication about the width of particle size distribution, the lower the PI values the better the dispersibility of the particles. Increasing the concentration of β-CyD in the outer phase of the primary emulsion from 0.1 to 1.0% (w/v) resulted in considerable decrease in PI values.

Fig. 1 shows particle size distribution patterns of IMC after dispersing the freeze-dried samples. When distilled water was used to disperse the nanocrystals, larger particles in the range of 3–5 μm could be observed. However, samples dispersed in 0.1% polyvinyl alcohol (PVA-403) exhibited a monodispersed particle size distribution in the nanosize range. This observation emphasizes that these microparticles are aggregations of the submicron particles. The drug content of the freeze-dried samples was determined by UV-spectrophotometry and results indicated the purity of the prepared samples. Quantitative determination of the content of the submicron-sized particles in the prepared samples was performed. The amount of IMC recovered after filtration of the nanocrystal dispersion through 0.8 μm filter was determined and expressed as a percentage of the total IMC amount in the dispersion (nanoparticle yield). Table 1 shows a slight increase in the yield of nanocrystals by increasing the concentration of β-CyD during the preparation procedures. Furthermore, the effect of other cyclodextrins (α- and γ-CyDs) on the formation and stabilization of IMC nanocrystals by emulsion solvent diffusion method was investigated (Fig. 2). The results obtained in the case of α- and γ-CyDs are similar to those of β-CyD. Nanocrystals with average particle diameters in the range of 300–400 nm were obtained when α- and γ-CyDs were used as emulsion stabilizers. Since the cavity size difference of parent CyDs has no significant effect on the particle size of the prepared nanocrystals, it is suggested that an inclusion complex

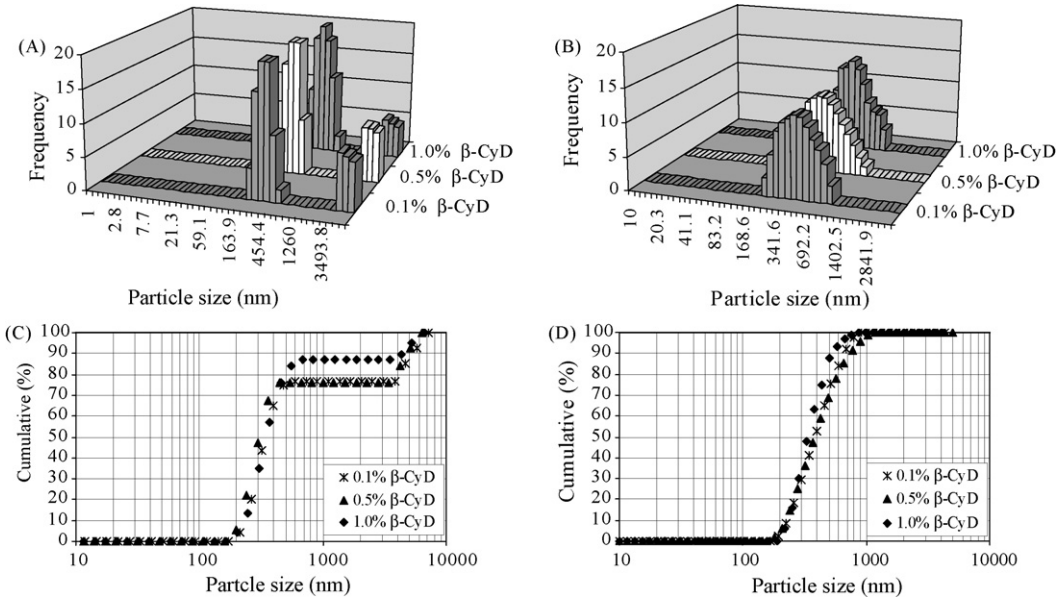

Fig. 1. Particle size distributions of freeze-dried samples prepared in different concentrations of β-CyD; dispersion in water: (A) and (C); and in 0.1% PVA solution: (B) and (D).

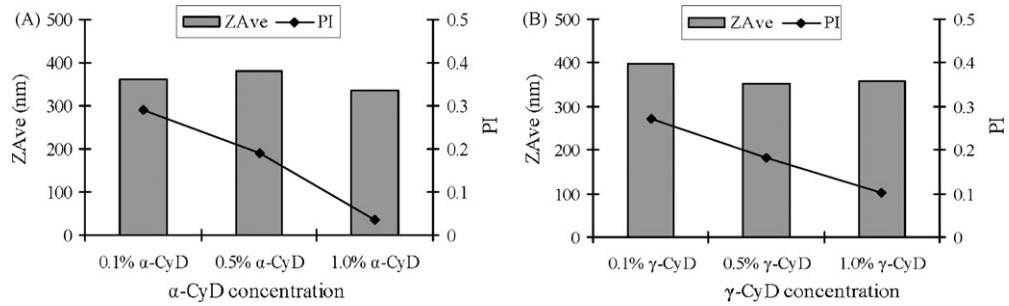

Fig. 2. Average particle size (ZAve) and polydispersity index (PI) of freeze-dried samples prepared in different concentrations of α-CyD: (A) and γ-CyD: (B).

formation mechanism is not a dominant factor for the stabilizing effect of CyD molecules. During the initial step of emulsion formation, the rapid diffusion of ethanol from the disperse phase to the aqueous bulk phase allowed the formation of fine IMC particles. The role of CyD was to prevent the growth and aggregation of these particles to the micro-order, probably by the formation of a CyD network through intermolecular interaction that could protect and stabilize the produced nanocrystals. It was reported that CyD molecules can self-associate in aqueous solutions to form nano-scale aggregates that have a minimum hydrodynamic radius of about 90 nm (Bonini et al., 2006). These aggregates can interact with hydrophobic drugs through non-inclusion complexation or by formation of micelle-like structures (Loftsson et al., 2004).

The particle size and morphology of freeze-dried samples of IMC particles as well as the commercial powder were characterized by the scanning electron microscopy (Fig. 3). The commercial IMC powder showed irregular-shaped particles with a relatively wide particle size distribution (Fig. 3A). The SEM photographs of freeze-dried samples of IMC prepared in different concentrations of β-CyD showed rod-shaped and fibrous aggregate of submicron size IMC particles (Fig. 3B–D). The particle size of the observed crystals is corresponding to the results of dynamic light scattering based on calculation of the diameter of the sphere that has the same volume.

The crystal property of commercial IMC powder and nanocrystals was evaluated by powder X-ray diffraction analysis (Fig. 4). The PXRD pattern of the commercial IMC crystals

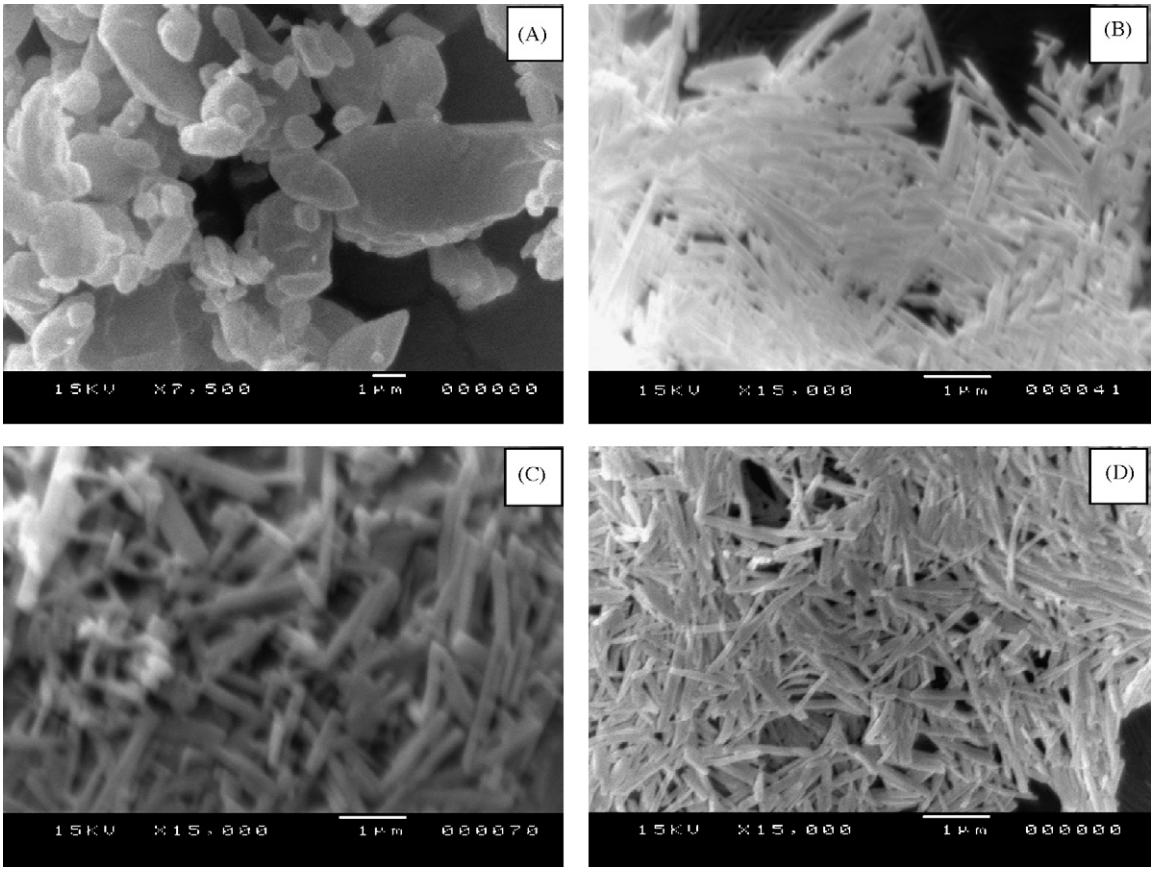

Fig. 3. SEM photographs of commercial IMC powder: (A) and freeze-dried samples prepared in different concentrations of β-CyD solutions; 0.1% β-CyD: (B), 0.5% β-CyD: (C), and 1.0% β-CyD: (D).

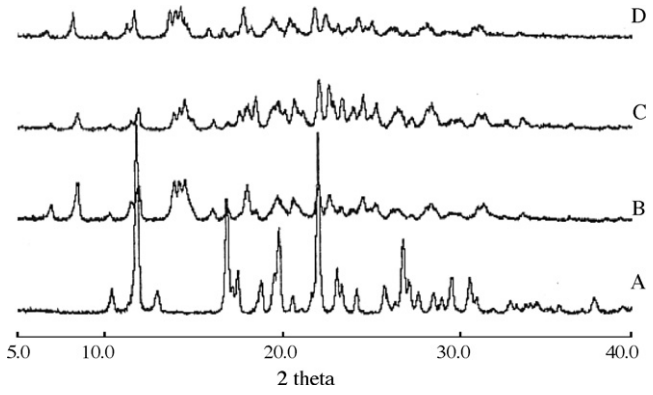

Fig. 4. PXRD patterns of commercial IMC powder: (A) and freeze-dried samples prepared in different concentrations of β-CyD solutions; 0.1% β-CyD: (B), 0.5% β-CyD: (C), and 1.0% β-CyD: (D).

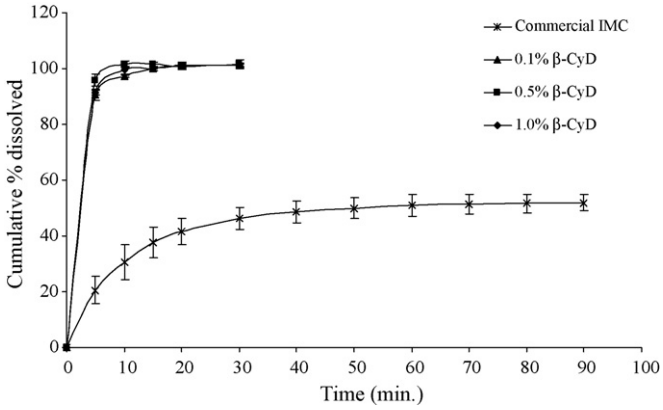

Fig. 6. Dissolution profiles of commercial IMC powder and freeze-dried samples prepared in different concentrations of β-CyD solutions.

showed diffraction peaks at 11.7, 17.0, 19.8, 22.0, and 26.8° (2θ) which corresponds to the stable form of IMC (γ-form). On the other hand, PXRD patterns of the freeze-dried nanocrystals of IMC showed low intensity peaks at 8.3, 11.8, 14.4, 18.0, and 22° (2θ). These patterns are identical with those of the meta-stable α-form of IMC and in a good agreement with previously published data (Takeuchi et al., 2005; Masuda et al., 2006). Further confirmation of the crystal properties was achieved by evaluation of the thermal behavior of the different samples using DSC (Fig. 5). As expected from PXRD, commercial IMC showed a sharp endothermic peak at 159.4 °C for the melting of the stable γ-form, while the melting peaks of the freeze-dried samples were observed at about 152.0 °C which corresponds to the meta-stable α-form. Similar PXRD and DSC patterns were obtained in the cases of α- and γ-CyDs (data are not shown) which further confirm polymorphic change of the drug to the meta-stable form. The formation of meta-stable polymorphs is well known to dramatically increase the apparent solubility and dissolution rate of poorly water-soluble drugs (Miller et al., 2007). The dissolution profiles of commercial IMC powder and the freeze-dried samples prepared by the emulsion solvent diffusion method are presented by Fig. 6. The dissolution of commercial IMC was slow and incomplete (about 50% dissolved within 90 min). In case of the freeze-dried samples, more than ninety percent of the IMC molecules dissolved within the first 5 min and complete dissolution was achieved within 10 min. The freeze-dried samples prepared in different concentrations of β-CyD solutions showed almost the same dissolution profile. Furthermore, the dissolution profiles of the freeze-dried nanocrystals prepared in the presence of α- and γ-CyDs (data are not shown) are similar to those of β-CyD. The great enhancement in the dissolution of IMC can be attributed to the particle size reduction to the nano-range with a significant increase in the surface area available for dissolution. Another factor that contributed to this enhancement is the polymorphic change to the meta-stable form which has higher solubility. Consequently, the emulsion solvent diffusion method in the presence of CyDs was successfully applied to produce drug nanocrystals of IMC. Ethanol, a non-toxic solvent, was used as a disperse phase and aqueous CyD solutions instead of surfactants were used as dispersion media of the primary emulsions. The role of CyD molecules during the emulsion solvent diffusion method was to keep the dispersion state of the formed primary particles and prevent particles aggregation and precipitation.

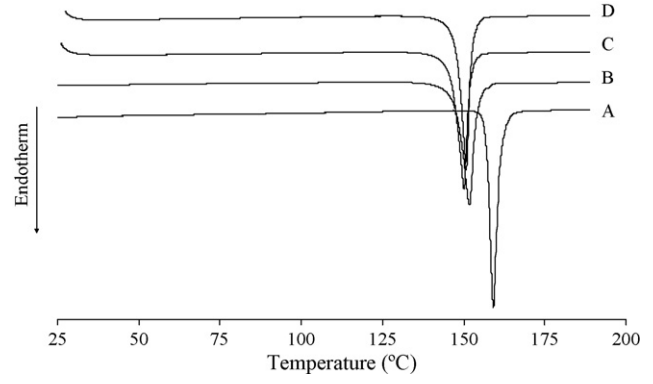

Fig. 5. DSC profiles of commercial IMC powder: (A) and IMC nanocrystals prepared in different concentrations of β-CyD solutions; 0.1% β-CyD: (B), 0.5% β-CyD: (C), and 1.0% β-CyD: (D).

4. Conclusion

The emulsion solvent diffusion method has been described for the effective production of drug nanocrystals of IMC for dissolution rate enhancement of a poorly water-soluble drug. In this method, CyDs were successfully employed as protective stabilizers of the prepared dispersions. This stabilizing effect is possibly attributed to the formation of CyD network by intermolecular interaction of CyD molecules that could prevent aggregation and crystal growth of the dispersed particles. The prepared IMC nanocrystals showed a uniform particle size distribution with an average diameter in the range of 300–500 nm. Compared to the commercial drug powder, fast and complete dissolution of IMC was achieved as a result of particle size reduction to the nano-order and polymorphic change to a meta-stable form.

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