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Some pharmaceutical and inclusion properties of 2-hydroxybutyl-β-cyclodextrin derivative

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ABSTRACT

2-Hydroxybutyl-β-cyclodextrins (HB-β-CyDs) with different degrees of substitution (D.S.) were prepared and their physicochemical and biological properties and solubilizing abilities were studied and compared with those of 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD). The surface activity of HB-β-CyD was higher than that of HP-β-CyD (D.S. 5.6) and increased with its concentration and D.S. The moisture sorption of HB-β-CyD (D.S. 5.5) was less than that of HP-β-CyD (D.S. 5.6), because of the introduction of hydrophobic hydroxybutyl groups in a molecule. The hemolytic activity (rabbit erythrocytes) decreased in the order of 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-CyD) > methyl-β-cyclodextrin (M-β-CyD) > HB-β-CyD (D.S. 5.5) > β-CyD > HP-β-CyD (D.S. 5.6). The hemolytic activity of HB-β-CyD increased with D.S. and HB-β-CyD induced echinocyte (or crenation), as well as DM-β-CyD does. It was suggested from the solubility study of membrane components that HB-β-CyD interacted predominantly with cholesterol in erythrocytes, resulting in the hemolysis. The inclusion ability of HB-β-CyD was higher than that of HP-β-CyD (D.S. 5.6), especially for poorly water-soluble drugs with long linear structures such as biphenylylacetic acid and flurbiprofen (FP). For example, HB-β-CyD formed the inclusion complex with FP in a molar ratio of 1:1, by including the biphenyl moiety in the host cavity. The dissolution rate of FP/HB-β-CyD (D.S. 5.5) complex was faster than that of HP-β-CyD (D.S. 5.6) complex. The results suggested that HB-β-CyDs have considerable pharmaceutical potential and can work as a fast-dissolving carrier for poorly water-soluble drugs.

1. Introduction

Cyclodextrins (CyDs), cyclic oligosaccharides consisting of usually 6–8 D-glucose units, form inclusion complexes with various molecules in aqueous solution and in solid state, and are successfully utilized for improvements of pharmaceutical properties of drugs. Chemically modified CyDs have been utilized for improvements of pharmaceutical properties, e.g. solubility, chemical stability or bioavailability of drugs (Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Uekama, 2004). For example, 2-hydroxypropyl-β-CyD (HP-β-CyD) has the higher aqueous solubility and greater solubilizing ability for poorly water-soluble drugs than the parent β-CyD. Moreover, physicochemical properties of HP-β-CyD, such as complexing ability and surface activity, were dependent on numbers of substituent groups or locations of the hydroxypropyl group in a molecule (Yoshida et al., 1988a,b, 1989, 1990). On the other hand, 2-hydroxybutyl-β-CyD (HB-β-CyD) employed in this study is a hydroxyalkylated β-CyD derivative that has one more methylene moiety in the substituent of HP-β-CyD. Recently, we reported that HB-β-CyD was useful for control of crystallization and polymorphic transition of solid drugs and provided an opportunity to isolate labile intermediate metastable polymorphs of chlorpropamide (Ishiguro et al., 2010). However, little is known about the pharmaceutical and inclusion properties of HB-β-CyD. In this study, we investigated some physicochemical and biological properties of HB-β-CyD, such as surface activity, moisture sorption and hemolytic activity, in comparison with those of HP-β-CyD. Further, the inclusion property of HB-β-CyD for poorly water-soluble drugs such as steroidal and non-steroidal anti-inflammatory drugs and the dissolution property of solid complexes were investigated, to evaluate utility of the host molecule as a drug carrier.

2. Materials and methods

2.1. Materials

Parent β-CyD and HP-β-CyD (degree of substitution (D.S.) of 2-hydroxypropyl groups 5.6) were supplied from Nihon Shokuhin

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Kako Co. (Tokyo, Japan). HB-β-CyDs (D.S. 3.8, 5.5 and 8.7) were prepared by the condensation of β-CyD in aqueous alkali with butylene oxide according to the method reported previously (Pitha and Pitha, 1985). The D.S. in HB-β-CyDs was evaluated by mass spectrometry (Yoshida et al., 1988a). 2,6-Di-*O*-methyl-β-CyD (DM-β-CyD) and methylated β-CyD (M-β-CyD) were purchased from Nakalai Tesque, Inc. (Kyoto, Japan). FP was obtained from Wako Pure Chemicals Co. (Kyoto, Japan). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

2.2. Surface tension and moisture sorption measurements

Surface tension was measured by the ring method using a duNouy surface tensionmeter (Ito Co., Tokyo, Japan). The moisture sorption studies were conducted as follows: CyD powder in a weighing bottle was dried under reduced pressure at 80 °C for 1 day, and then placed in a desiccator at 98% R.H. and 25 °C. At appropriate intervals, the water content of the sample was determined by change of the mass.

2.3. Hemolysis studies

Auricular venous blood was isolated from Japanese white male rabbits (Kyudo, Tosu, Japan). Red blood cells (RBC) were separated by centrifugation (1000 × *g* for 5 min), washed twice with phosphate-buffered saline (PBS, pH 7.4) and resuspended to give a hematocrit of 5%. The RBC suspension (0.1 mL) was added to CyD solution (1 mL) and the mixture was gently agitated for 30 min at 37 °C. After centrifugation (1000 × *g* for 5 min), the optical density of the supernatant was measured at 543 nm. Results were expressed as a percent of total hemolysis, which was obtained when erythrocytes were incubated in water only.

2.4. Scanning electron microscopy (SEM)

RBC was treated with CyDs using the same methods as used for hemolytic assay. After fixation of samples with 25% (w/v) glutaraldehyde at 25 °C for 30 min, RBC was resuspended with deionized double-distilled water. After samples were dried at 25 °C overnight, they were observed with a Hitachi TM-3000 SEM (Tokyo, Japan).

2.5. Solubilization for phospholipids and cholesterol

Egg phosphatidylcholine (1 mg; dissolved in chloroform at 5 mg/mL and evaporated to dryness with N_2) or cholesterol (5 mg) was added to sample of CyD solutions (1 mL) and shaken at 25 °C and 125 rpm for 1 week. After centrifugation (1000 × *g* for 5 min), an aliquot (0.5 mL) of the supernatant was withdrawn using a cotton-plugged pipette. The concentration of phospholipids and cholesterol were determined using a Phospholipids-test Wako® and Cholesterol-test Wako® (Wako Pure Chemical Industries, Osaka, Japan), respectively.

2.6. Solubility studies

Solubility measurements were carried out according to Higuchi and Connors (1965). Excess amounts (5 mg) of drugs were added to CyD solutions (1 mL) and shaken at 25 °C and 125 rpm for 1 week. After centrifugation (1000 × *g* for 5 min), an aliquot (0.5 mL) of the supernatant was withdrawn using a cotton-plugged pipette and analyzed for drugs by UV spectroscopy at suitable wavelengths. The stability constant (K_c) of CyD complexes was calculated by the equation of K_c = slope/[intercept (1 − slope)] (Higuchi and Connors, 1965) using slopes and intercepts of the initial straight-line portion of the phase solubility diagrams.

2.7. Spectroscopic studies

UV, circular dichroism (CD) and fluorescence spectra were recorded with a Hitachi U-2800A spectrometer (Tokyo, Japan), a Jasco J-720 circular dichroic spectropolarimeter (Tokyo, Japan) and a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan), respectively. ^{1}H and ^{13}C NMR spectra were measured with a Jeol JNM-A500 spectrometer (Tokyo, Japan) operating at 500 MHz, using a 5-mm sample tube at 30 °C. Chemical shifts were given as parts per million (ppm) down field from that of tetramethylsilane with an accuracy of 0.005 ppm.

2.8. Preparation of solid complexes

The solid complexes were prepared by the kneading method (Tsuruoka et al., 1981; Nagarsenker and Bhave, 1998). The amounts of flurbiprofen (FP) and HP-β-CyD (D.S. 5.6) or HB-β-CyD (D.S. 5.5) were weighed and triturated with ethanol/water (7:1, v/v) solution in a mortar and the slurry was thoroughly kneaded for 30 min while adding adequate volumes of the mixed solvent. After evaporation of the solvent, the solid complexes were dried at 50 °C for 1 day under reduced pressure. Powder X-ray diffraction patterns were measured by a Rigaku RINT 2500 X-ray diffractometer (Tokyo, Japan) with a Ni-filtered Cu-Kα radiation (1.542 Å), 40 kV, 40 mA, a scan range of 2θ = 3–30°, 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. Differential thermal analysis (DTA) was carried out using a Rigaku TG-DTA DTA 8120 (Tokyo, Japan), with a sample weight of 5 mg and a heating rate of 10 °C/min under air atmosphere.

2.9. Dissolution studies

The powders of FP/HP-β-CyD (D.S. 5.6) and HB-β-CyD (D.S. 5.5) complexes and their physical mixtures were compressed into a cylindrical tablet (diameter 10 mm) at a pressure of about 20 MPa (204 kg/cm^2) for 5 min using a IR tableting machine (RIKEN, Tokyo, Japan). The contents of FP in tablets of the HP-β-CyD (D.S. 5.6) and HB-β-CyD (D.S. 5.5) complexes were 21.5 and 20.6 mg, respectively. The dissolution rate of the drug from tablets was measured by the paddle method and the flow-through cell method. The paddle method was performed in water (900 mL) at 37 °C using a TBM-81 dissolution tester (Toyama Sangyo Co., Tokyo, Japan) with a paddle rotating at 50 rpm. At appropriate intervals, an aliquot (1 mL) of the dissolution medium was withdrawn using a cotton-plugged pipette, diluted appropriately with water, and analyzed for FP by UV spectroscopy at 246 nm. The flow-through cell method was carried out using a PTDZ1-J flow-through cell dissolution tester (Japan Machinery, Tokyo, Japan) equipped with 22.6 mm diameter tablet cell. A ruby bead of 5 mm diameter and glass beads of 1 mm were placed in the cell and the tablet was placed on the glass bead bed. Water of 37 °C was used as a dissolution medium and circulated in the cell with a flow rate of 4 mL/min. The dissolution medium was collected at appropriate intervals, filtrated with 0.8 μm membrane filter, diluted appropriately with water, and analyzed for FP by UV spectroscopy at 246 nm.

3. Results and discussion

3.1. Some physicochemical properties of HB-β-CyD

Fig. 1 shows surface tension of β-CyDs solutions measured by the ring method. The surface tension of parent β-CyD solution

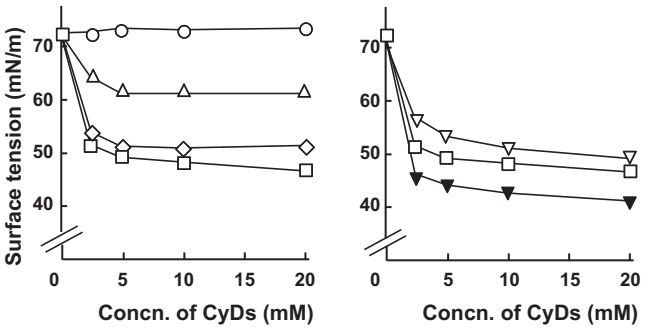

Fig. 1. Surface tensions of β-CyDs solutions at 25 °C. ○: β-CyD, △: HP-β-CyD (D.S. 5.6), ◇: DM-β-CyD, □: HB-β-CyD (D.S. 5.5), ▽: HB-β-CyD (D.S. 3.8), ▼: HB-β-CyD (D.S. 8.7). Each point represents the mean ± SE of three experiments.

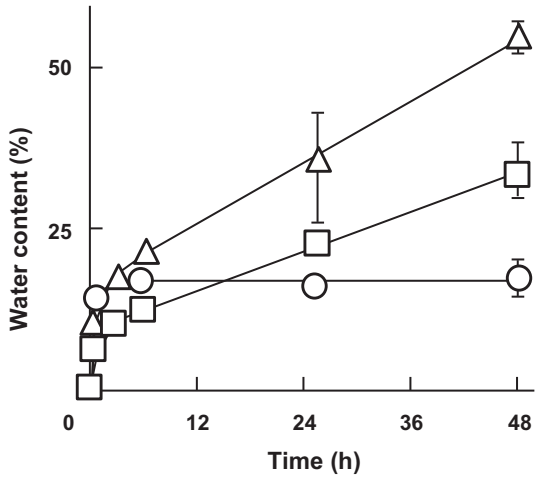

Fig. 2. Moisture sorptions of β-CyDs stored at 25 °C, 98% R.H. ○: β-CyD, △: HP-β-CyD (D.S. 5.6), □: HB-β-CyD (D.S. 5.5). Each point represents the mean ± SE of three experiments.

slightly increased with increasing concentrations. In contrast, HP-, DM- and HB-β-CyD solutions showed positive adsorption, the surface tension decreasing with increasing concentration. The surface activity of HB-β-CyDs as estimated from the surface tension was higher than those of HP- and DM-β-CyD and increased with increasing D.S., probably due to the introduction of hydrophobic hydroxybutyl moiety (Müller and Brauns, 1986). Fig. 2 shows the moisture sorption curves of β-CyDs at 98% R.H. and 25 °C. The water content of parent β-CyD increased up to 19% during 6 h storage and was maintained in this value for 48 h. This water content corresponded to about 15 water molecules, suggesting that one β-CyD molecule has 12 water molecules of crystallization (Lindner and Saenger, 1978) and 3 adsorbed water molecules. On the other hand, the water contents of HP-β-CyD (D.S. 5.6) and HB-β-CyD (D.S. 5.5) increased with time up to 55 and 34%, respectively, after the 2 days storage. The moisture sorption of HB-β-CyD (D.S. 5.5) was less than that of HP-β-CyD (D.S. 5.6), because of the presence of the hydrophobic hydroxybutyl group compared with the hydroxypropyl group.

3.2. Hemolytic activity of HB-β-CyD

Fig. 3 shows hemolytic effects of CyDs on 5% RBC suspension incubated for 30 min at 37 °C. The hemolysis started at the concentrations of about 0.8 mM of DM-β-CyD < 1 mM of M-β-CyD < 1.5 mM of HB-β-CyD (D.S. 5.5) < 3 mM of β-CyD < 4 mM of HP-β-CyD (D.S. 5.6), and the concentrations of 50% hemolysis increased in the same order. When compared with the different D.S. of HB-β-CyDs, the 50% hemolysis concentration increased in the order of D.S. 8.7 < D.S. 5.5 < D.S. 3.8, suggesting that the hemolytic activity of HB-β-CyDs increased with D.S. These results suggested that the hemolytic activity of HB-β-CyD (D.S. 5.5) was weaker than those of DM-β-CyD and methylated β-CyD, in spite of the higher surface activity of the former CyD, although the hemolytic activity of HB-β-CyD (D.S. 5.5) was higher than those of parent β-CyD and HP-β-CyD (D.S. 5.6).

Sheetz and Singer (1974) proposed the bilayer couple hypothesis, briefly, two types of morphological changes of erythrocytes, echinocyte (or crenation) and stomatocyte (or invagination), were caused by charged amphipaths due to intercalation into one side of lipid bilayer. We previously reported that unmodified CyDs and DM-α-CyD induced stomatocyte in human and rabbit erythrocytes due to the interaction with membrane components (Irie et al., 1982; Ohtani et al., 1989; Motoyama et al., 2006). On the other hand, we also revealed that M- and DM-β-CyD induced echinocyte (or crenation) in rabbit erythrocytes and their ability to extract membrane constituents was higher than that of β-CyD, being consistent with that of hemolytic activity (Motoyama et al., 2009). Hence, we investigated which type of morphological changes were induced by HB-β-CyD in RBC. As shown in Fig. 4B and C, RBC treated with 3 mM β-CyD and 4 mM HP-β-CyD (D.S. 5.6) showed the morphological change from discocyte to stomatocyte. On the other hand, RBC treated with 0.8 mM DM-β-CyD, 1.5 mM HB-β-CyD (D.S. 5.5) and 1 mM HB-β-CyD (D.S. 8.7) showed the morphological change from discocyte to echinocyte (Fig. 4D–F).

The hemolytic activity of CyDs is known to be affected by the solubilizing effect of CyDs on membrane components such as phospholipids, cholesterol and proteins (Ohtani et al., 1989; Irie and Uekama, 1997). To gain insight into the hemolytic activity of HB-β-CyD, solubility changes of phospholipids and cholesterol by the addition of β-CyDs were investigated. As shown in Fig. 5,

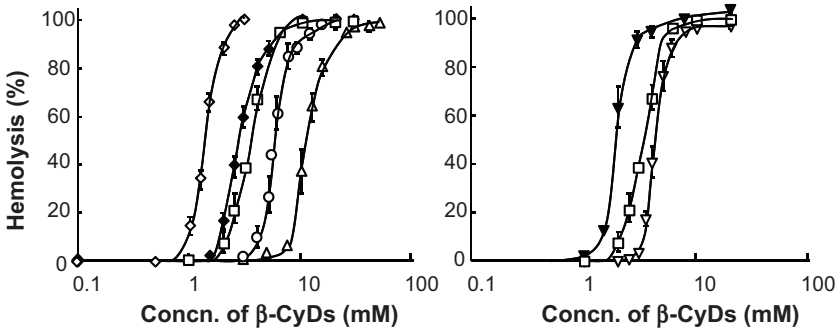

Fig. 3. Hemolytic activities of β-CyDs on rabbit erythrocytes in PBS (pH 7.4) at 37 °C. ○: β-CyD, △: HP-β-CyD (D.S. 5.6), ◇: DM-β-CyD, ◆: Methyl-β-CyD, □: HB-β-CyD (D.S. 5.5), ▽: HB-β-CyD (D.S. 3.8), ▼: HB-β-CyD (D.S. 8.7). Each point represents the mean ± SE of three experiments.

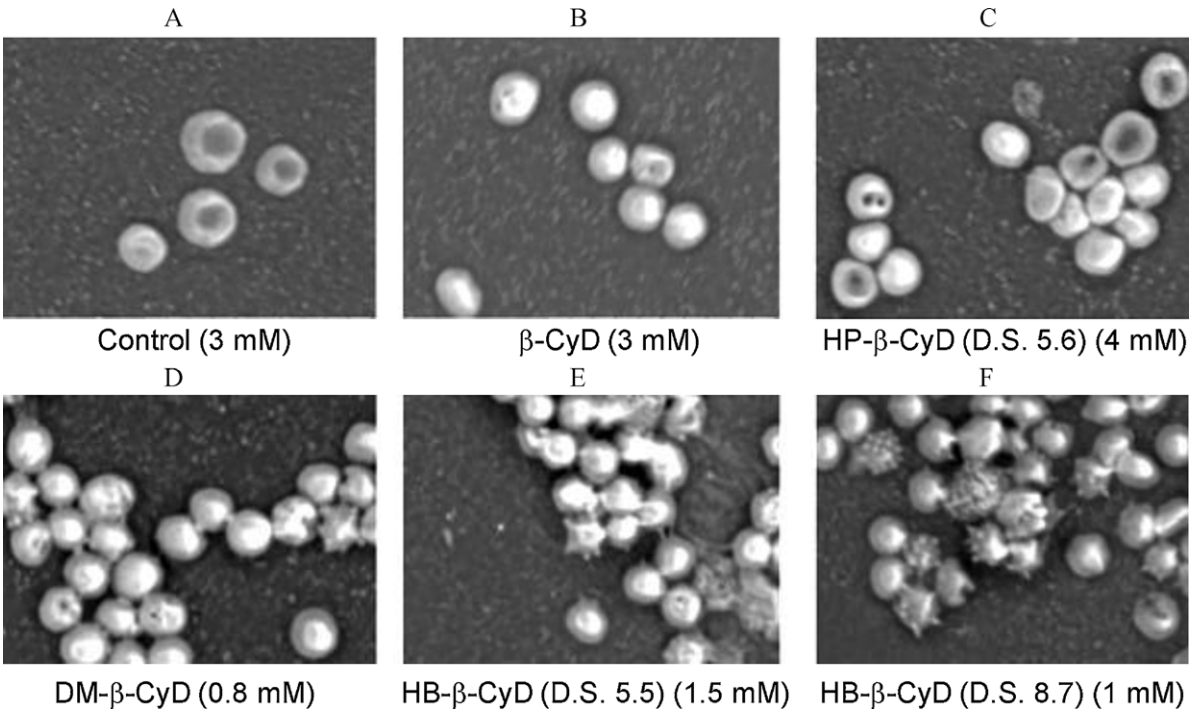

Fig. 4. SEM images of rabbit erythrocyte incubated with β-CyDs for 30 min at 37 °C.

DM-β-CyD significantly solubilized both phospholipids and cholesterol, whereas HB-β-CyD solubilized mainly cholesterol, although the cholesterol-solubilizing effect of HB-β-CyD decreased at the higher D.S. 8.7. These results suggest that the hemolytic activity of HB-β-CyDs results mainly from the interaction with cholesterol in membranes.

3.3. Solubilization of drugs by HB-β-CyD

The effects of HB-β-CyD on the solubility of several drugs were studied and were compared with those of parent β-CyD and HP-β-CyD (D.S. 5.6). Fig. 6 shows the solubilities of drugs in the absence and presence of β-CyDs (1.0×10^{-2} M). It was apparent that the addition of HB-β-CyD (D.S. 5.5) significantly increased the solubilities of FP, ketoprofen and 4-biphenylylacetic acid, and its solubilizing effect was higher than those of parent β-CyD and HP-β-CyD (D.S. 5.6). On the other hand, the solubilizing effect of HB-β-CyD (D.S. 5.5) on the steroidal drugs was almost the same or slightly lower, when compared with those of parent β-CyD and HP-β-CyD (D.S. 5.6). Fig. 7 shows the effect of D.S. of HB-β-CyD on the solubilization of the drugs. The solubilizing effect of HB-β-CyD on FP, ketoprofen and 4-biphenylylacetic acid was almost the same between different D.S. or slightly increased with increase in D.S., whereas that of HB-β-CyD on the steroidal drugs tended to decrease with D.S. These results suggest that the longer hydroxybutyl groups enhances the hydrophobic interaction of CyD with the drugs having long and narrow structures, whereas it weakens the interaction with bulky drugs such as steroids, probably due to steric hindrances.

3.4. Interaction between FP and HB-β-CyD in water

To gain insight into the inclusion property of HB-β-CyD, we investigated the interaction of FP with HB-β-CyD in aqueous solution by the solubility method (Higuchi and Connors, 1965) and various spectroscopic methods. Fig. 8 shows the phase solubility diagrams obtained for FP/β-CyD systems in water at 25 °C. The solubility plot for β-CyDs showed A_L-type solubility curves, i.e.,

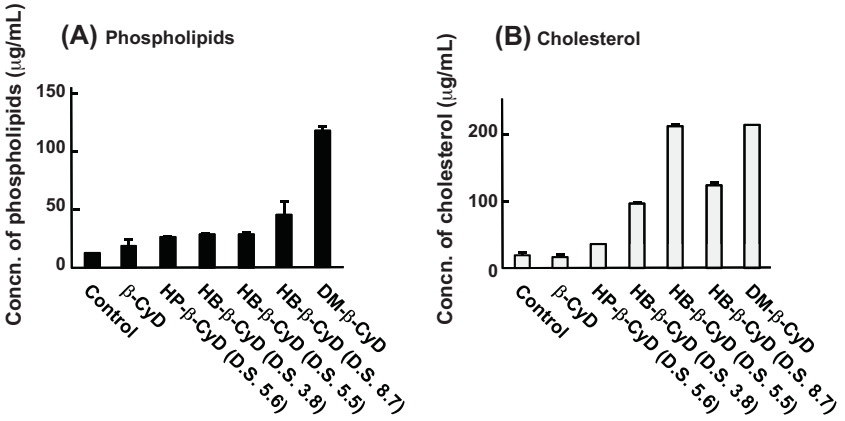

Fig. 5. Effects of β-CyDs (10 mM) on the solubilities of phospholipids (A) and cholesterol (B) in water at 25 °C. Each point represents the mean ± SE of three experiments.

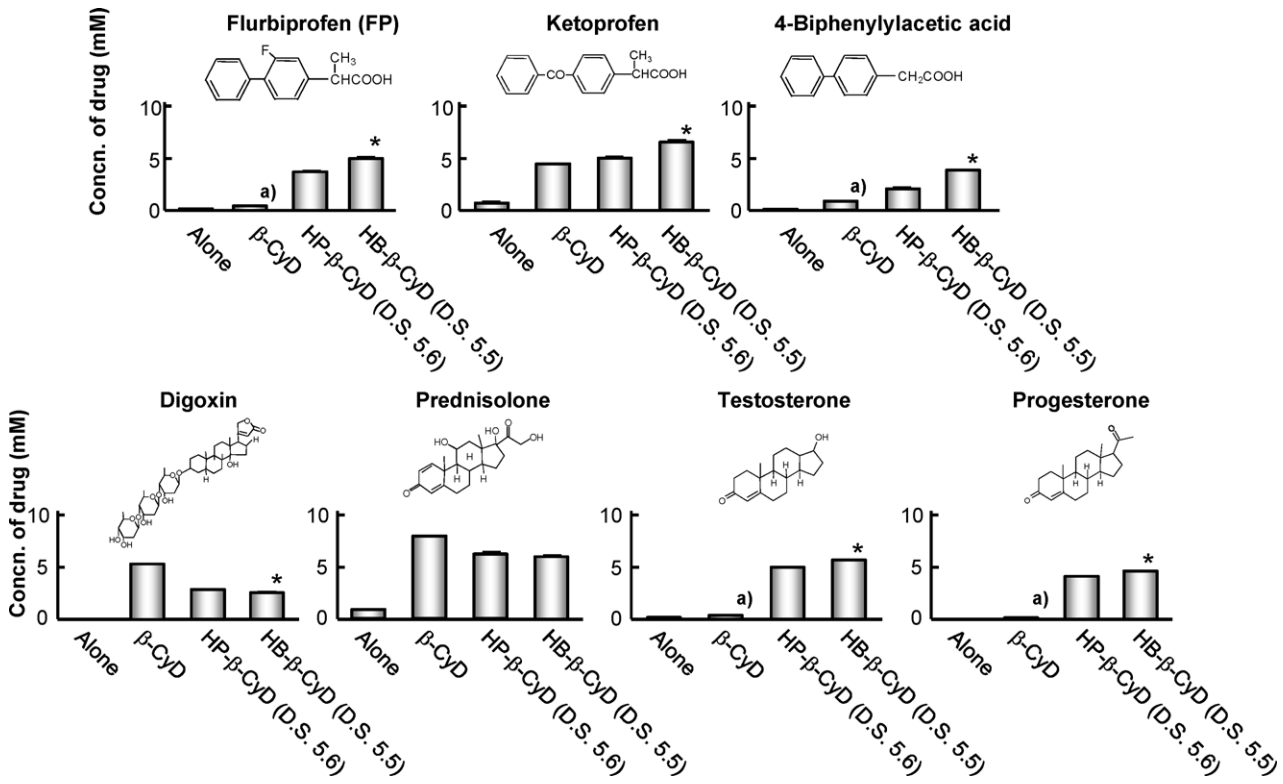

Fig. 6. Solubilizing effects of β-CyDs on poorly water-soluble drugs at 25 °C. Each point represents the mean ± SE of three experiments. *$p < 0.05$, compared with HP-β-CyD. (a) Plateau region of Bs type phase solubility diagrams.

the solubility of FP increased in a linear fashion as a function of CyD concentration under the experimental condition (Fig. 8A). The apparent 1:1 stability constant (K_c) of complexes was calculated from initial linear portion of the solubility diagrams (Table 1), and was in the order of β-CyD (5400 M^{-1}) < HP-β-CyD (D.S. 5.6; 6100 M^{-1}) < HB-β-CyD (D.S. 5.5; 10800 M^{-1}). The inclusion ability of HB-β-CyD to FP increased with D.S., as shown in Fig. 8B and Table 1.

The effect of β-CyDs on the UV, CD and fluorescence spectra of FP are shown in Fig. 9. The UV absorbance of FP at 246 nm decreased with the addition of β-CyDs in the order of FP alone > β-CyD > HP-β-CyD (D.S. 5.6) > HB-β-CyD (D.S. 5.5) (Fig. 9A), and the fluorescence

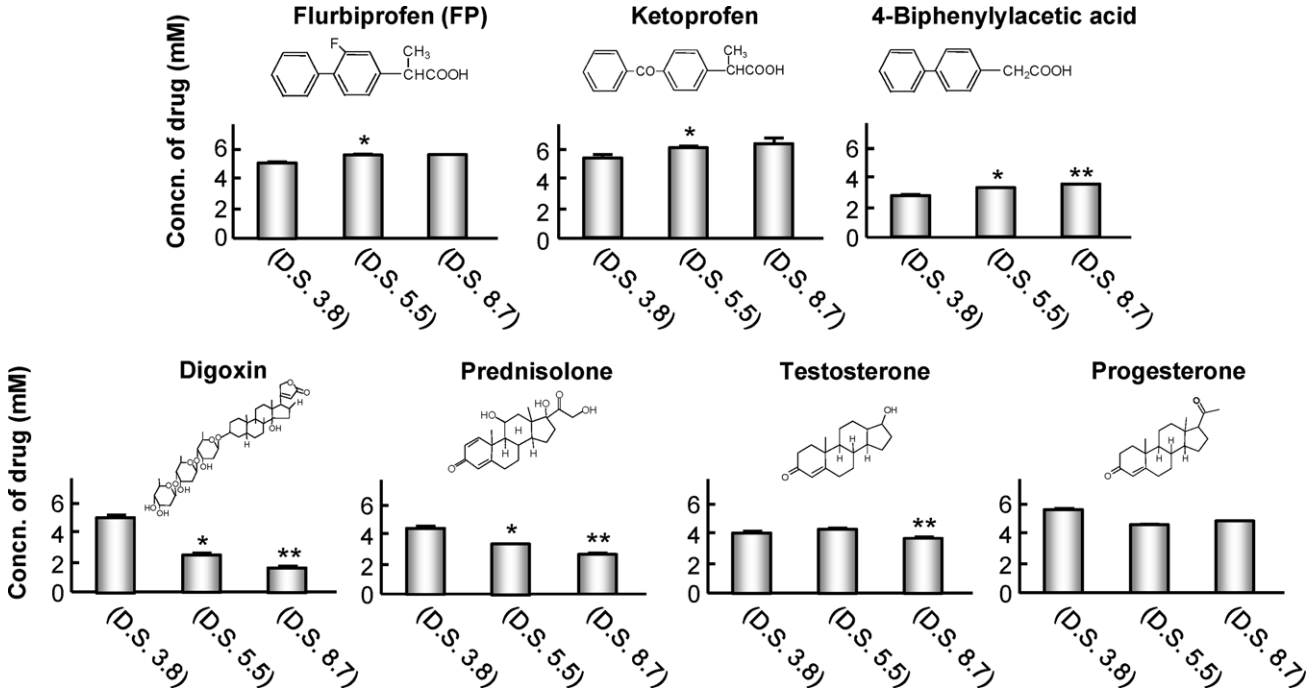

Fig. 7. Effects of hydroxybutyl moiety of HB-β-CyD on solubilities of poorly water-soluble drugs. Each point represents the mean ± SE of three experiments. *$p < 0.05$, compared with HB-β-CyD (D.S. 3.8), **$p < 0.05$, compared with HB-β-CyD (D.S. 5.5).

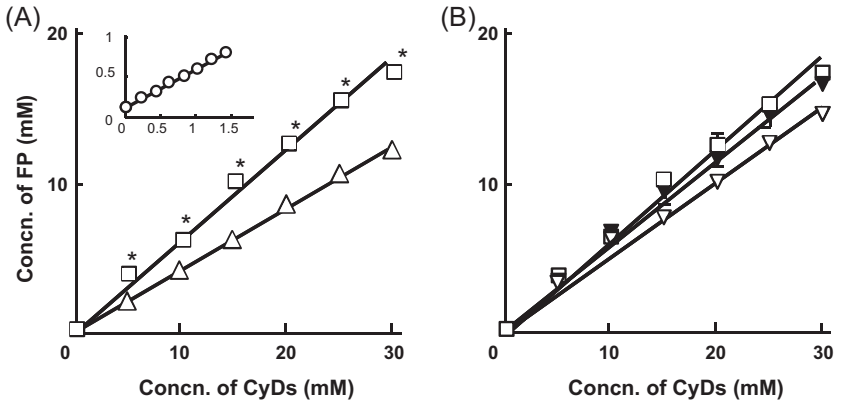

Fig. 8. Phase solubility diagrams of FP/β-CyD systems in water at 25 °C. ○: β-CyD, △: HP-β-CyD (D.S. 5.6), □: HB-β-CyD (D.S. 5.5), ▽: HB-β-CyD (D.S. 3.8), ▼: HB-β-CyD (D.S. 8.7). Each point represents the mean ± SE of three experiments. $^{*}p < 0.05$, compared with HP-β-CyD.

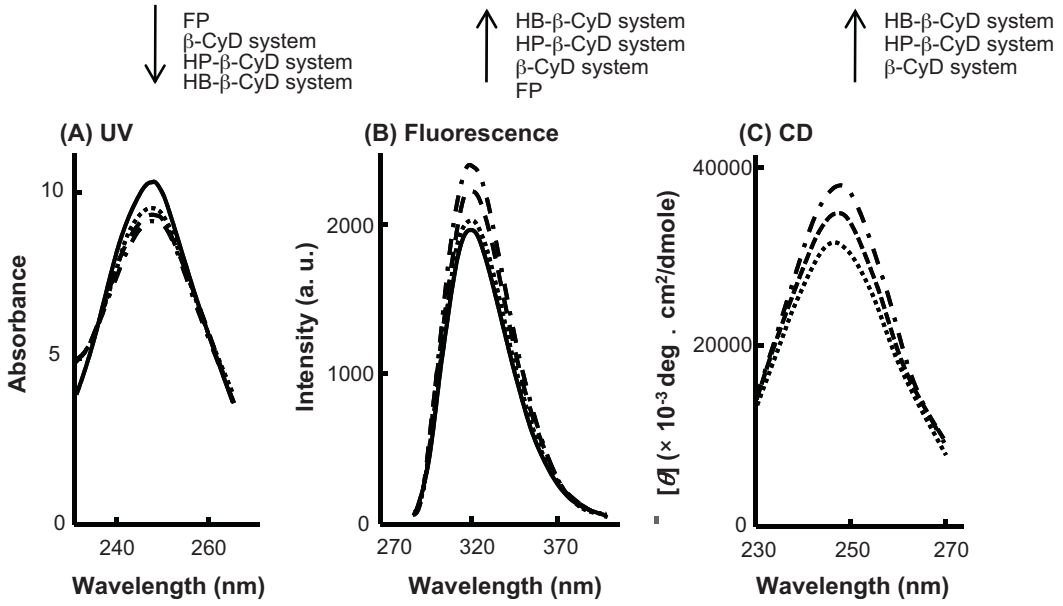

Fig. 9. UV (A), fluorescence (B) and CD (C) spectra of FP/β-CyD systems in 0.1 M phosphate buffer (pH 7.0) at 25 °C. Concentrations of FP and CyDs in UV and CD studies were 0.05 mM and 1.25 mM, respectively. Concentrations of FP and CyDs in fluorescence study were 1.25 mM and 31.3 mM, respectively; —: FP alone; · · · · · ·: β-CyD, - - - -: HP-β-CyD (D.S. 5.6), –.–: HB-β-CyD (D.S. 5.5).

intensity of FP increased in the order of FP alone < β-CyD < HP-β-CyD (D.S. 5.6) < HB-β-CyD (D.S. 5.5) (Fig. 9B). These order well correlated with the K_c values, as shown in Table 1. Fig. 9C shows CD spectra of FP with β-CyDs. The β-CyDs system gave positive CD band at 246 nm and their intensity was in the order of β-CyD < HP-β-CyD (D.S. 5.6) < HB-β-CyD (D.S. 5.5). It is well known that CyDs have no CD band at wavelengths longer than 220 nm and the inclusion of optically inactive compounds within the CyD's cavity generates extrinsic Cotton effects in the wavelength region of guest chromophores. Thus, the CD spectroscopic data indicate that FP is embedded in the asymmetric locus of β-CyDs cavities. Fig. 10

Table 1
Stability constants (K_c) of FP/β-CyD complexes in water at 25 °C.

	K_c (M^{-1})
β-CyD	5400 ± 300
HP-β-CyD (D.S. 5.6)	6100 ± 80
HB-β-CyD (D.S. 3.8)	7000 ± 200
HB-β-CyD (D.S. 5.5)	10,800 ± 400
HB-β-CyD (D.S. 8.7)	9500 ± 700

Each value represents the mean ± SE of three experiments.

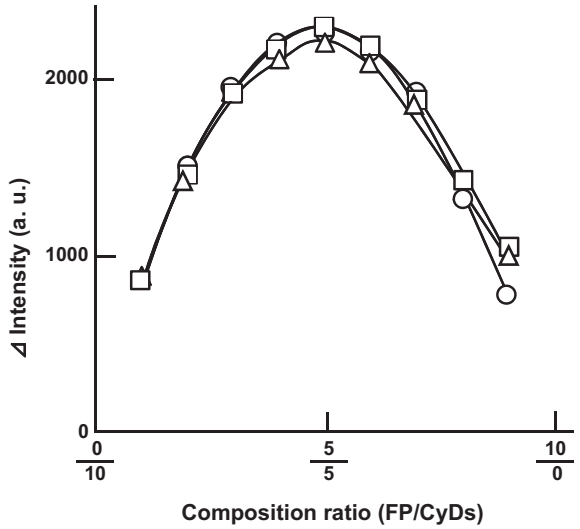

Fig. 10. Continuous variation plots of FP/β-CyD systems in 0.1 M phosphate buffer (pH 7.0) at 25 °C. ○: β-CyD, △: HP-β-CyD (D.S. 5.6), □: HB-β-CyD (D.S. 5.5).

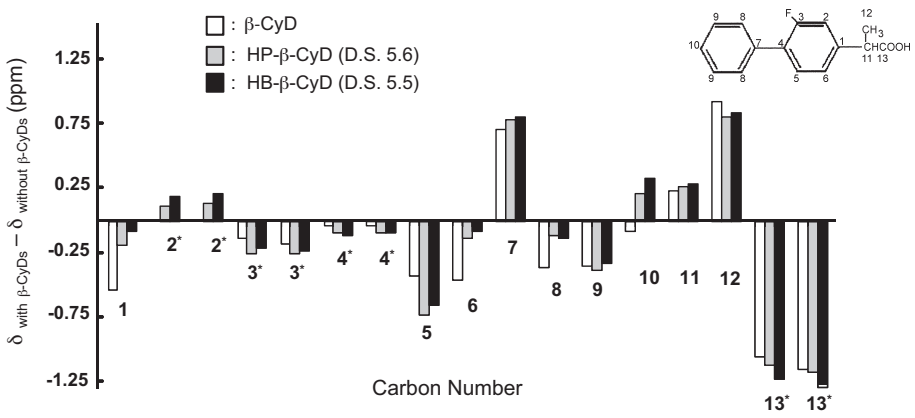

Fig. 11. Effects of β-CyDs (25 mM) on ^{13}C NMR chemical shifts of FP (25 mM) in 0.1 M sodium borate/D_2O buffer (pH meter reading = 9.3) at 30 °C. *These peaks were split due to the chiral carbon.

shows the continuous variation plots for the FP/β-CyD systems monitored by fluorescence intensity of FP at 320 nm. The maximal fluorescence intensity was observed at the molar ratio of 1:1, indicating that the guest forms inclusion complexes with β-CyDs in a molar ratio of 1:1 under the experimental conditions. These results suggest that HB-β-CyD (D.S. 5.5) forms a more stable inclusion complex with FP than β-CyD and HP-β-CyD (D.S. 5.6) in water. ^{13}C NMR spectroscopic studies were carried out to gain insight into the inclusion mode of the FP/HB-β-CyD (D.S. 5.5) complex in aqueous solution. Fig. 11 shows ^{13}C NMR chemical shift changes of FP induced by the addition of β-CyDs. The chemical shifts of the biphenyl ring (number 5 and 7) and propionic acid moiety (number 12 and 13) changed remarkably in the presence of these β-CyDs. The shift changes were similar between parent β-CyD, HP-β-CyD (D.S. 5.6) and HB-β-CyD (D.S. 5.5), suggesting that these β-CyDs includes preferably the biphenyl and propionic acid moieties of FP, as reported previously (Uekama et al., 1983, 1984). The peak splitting of the number 13 carbon by the addition of β-CyDs is ascribable to the chiral recognition of β-CyDs for racemic FP, i.e. R-(−)-FP or S-(+)-FP, as reported previously (Uekama et al., 1985).

3.5. Dissolution property of solid FP/HB-β-CyD (D.S. 5.5) complex

The solid FP complexes of HP-β-CyD (D.S. 5.6) or HB-β-CyD (D.S. 5.5) were prepared by the kneading method (Tsuruoka et al., 1981; Nagarsenker and Bhave, 1998), and their interaction was studied by powder X-ray diffractometry and differential thermal analysis (DTA). The 1:1 HP-β-CyD (D.S. 5.6) or HB-β-CyD (D.S. 5.5)

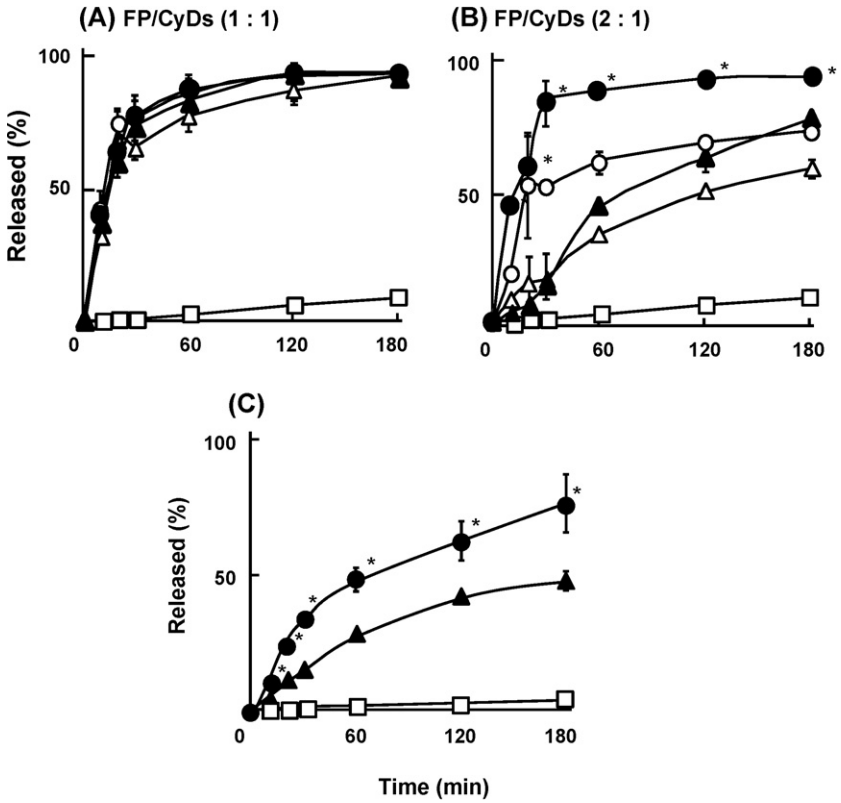

Fig. 12. Dissolution profiles of FP/HB-β-CyD (D.S. 5.5) and FP/HP-β-CyD (D.S. 5.6) complexes from tablets in water at 25 °C, measured by paddle method at 50 rpm (A, B) and flow-through cell method (C). ●: FP/HB-β-CyD (D.S. 5.5) complex, ○: FP/HB-β-CyD (D.S. 5.5) physical mixture, ▲: FP/HP-β-CyD (D.S. 5.6) complex, △: FP/HP-β-CyD (D.S. 5.6) physical mixture, □: FP alone. Each point represents the mean ± SE of three experiments. *$p < 0.05$, compared with FP/HP-β-CyD (D.S. 5.6) complex.

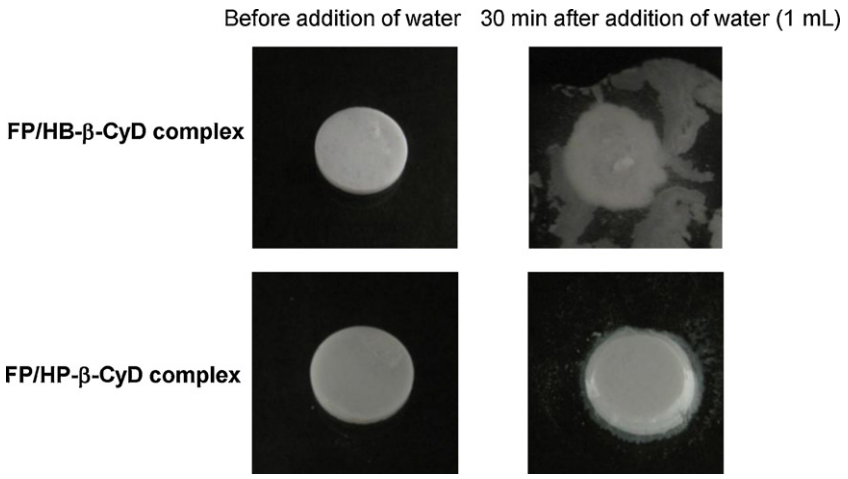

Fig. 13. Photographs of FP/HB-β-CyD (D.S. 5.5) and FP/HP-β-CyD (D.S. 5.6) complexes before and after addition of 1 mL water at 25 °C. Molar ratio of FP: CyDs was 2:1.

complexes showed halo-pattern in the powder X-ray diffractogram and no endothermic peaks due to the melting of FP in the DTA curve (data not shown), indicating the formation of amorphous complexes. Fig. 12A shows the dissolution profiles of FP from HP-β-CyD (D.S. 5.6) and HB-β-CyD (D.S. 5.5) complexes and physical mixtures (1:1 molar ratio), monitored by the paddle method. The dissolution of FP from these complexes and physical mixtures were faster than FP alone, and there was an insignificant difference in the dissolution rate between HP-β-CyD (D.S. 5.6) and HB-β-CyD (D.S. 5.5), achieving 80% of total dissolution in 60 min. Further, the difference in the dissolution of FP was insignificant between the 1:1 complex and the 1:1 physical mixture, probably because of the large volume of the dissolution medium (900 mL), the small amount (21.5 and 20.6 mg) of the drug in the tablets and the fast disintegration of the tablets due to the presence of large amounts of CyDs. Therefore, the 2:1 (guest:host) complex was prepared in the same way and its dissolution rate was studied, to gain insight into the dissolution of FP in the presence CyD. Fig. 12B shows the dissolution rate of FP from the 2:1 HB-β-CyD (D.S. 5.5) complex. The dissolution of the 2:1 HB-β-CyD (D.S. 5.5) complex was faster than that of the corresponding HP-β-CyD (D.S. 5.6) complex, i.e. the times of 50% release were 15 min and 70 min, respectively. The FP dissolution from the physical mixture of HB-β-CyD (D.S. 5.5) was also faster than that of HP-β-CyD (D.S. 5.6) (the times of 50% drug released were 20 min and 120 min, respectively), although their dissolutions were slower than the complexes. Fig. 12C shows dissolution behavior of FP/HP-β-CyD (D.S. 5.6) and FP/HB-β-CyD (D.S. 5.5) complexes from tablets, measured by the flow-through cell dissolution method. It was confirmed by this method that the HB-β-CyD (D.S. 5.5) complex dissolved faster than the HP-β-CyD (D.S. 5.6) complex.

Fig. 13 shows appearance of tablet surfaces of 2:1 FP/HP-β-CyD (D.S. 5.6) and FP/HB-β-CyD (D.S. 5.5) complexes before and after addition of 1 mL water. The HB-β-CyD (D.S. 5.5) complex disintegrated immediately after the addition of water. However, the HP-β-CyD (D.S. 5.6) complex formed a gel layer on the surface of the tablet, delaying the disintegration. The gel formation of HP-β-CyD (D.S. 5.6) may have resulted from the intermolecular interaction of the hydroxypropyl groups with its own or other CyD cavities. On the other hand, the hydrophilicity of HB-β-CyD (D.S. 5.5) may have decreased due to the introduction of the hydrophobic hydroxybutyl chain, leading to the immediate disintegration without formation of the gel layer. These results suggest that the fast dissolution of FP from the HB-β-CyD (D.S. 5.5) complex is attributable to prominent solubilization and disintegration of HB-β-CyD (D.S. 5.5). The present results suggest that HB-β-CyD is of considerable pharmaceutical use as a fast-dissolving carrier for poorly water-soluble drugs.

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