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Amorphous spironolactone-hydroxypropylated cyclodextrin complexes with superior dissolution and oral bioavailability

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Abstract

Inclusion complex formations of spironolactone (SP) with four cyclodextrins (parent β- and γ-CyDs and hydroxypropylated β- and γ-CyDs) in aqueous solution and in the solid state were investigated by the solubility method, spectroscopic methods, thermal analysis, powder X-ray diffractometry, and cross polarization/magic angle spinning ^{13}C-nuclear magnetic resonance spectroscopy. Although the stability constant of the parent CyD complexes was larger than that of the corresponding hydroxypropylated CyD complexes, the solubilizing effect of hydroxypropyl CyDs was greater than that of parent CyDs. The solid complexes of SP were prepared by the spray-drying method in molar ratios of 1:2 and 2:3 (guest/host) with β-CyDs and γ-CyDs, respectively. The CyD complexes maintained an amorphous state for lengthy time periods (over 2 months at 75% relative humidity and 60°C), with the exception of the γ-CyD complex which was converted to a crystalline complex after 1 month storage. The dissolution rate of hydroxypropyl CyD complexes was much faster than that of the parent CyD complexes, the rate being in the order of hydroxypropyl-β-CyD $\gg$ hydroxypropyl-γ-CyD > γ-CyD > β-CyD > metastable SP forms > stable SP form. Plasma levels of canrenone, a major effective metabolite of SP, were monitored to estimate the absorption-enhancing effect of hydroxypropyl CyDs. The area under the plasma concentration-time curve after oral administration of the hydroxypropyl-β-CyD complex in dogs was 3.6 times that of SP alone, and this enhancement was higher than those of the parent CyD complexes reported previously (Seo, H., Tsuruoka, M., Hashimoto, T., Fujinaga, T., Otagiri, M. and Uekama, K., Enhancement of oral bioavailability of spironolactone by β- and γ-cyclodextrin complexations. *Chem. Pharm. Bull.*, 31 (1983) 286–291). © 1997 Elsevier Science B.V.

Keywords: Spironolactone; Hydroxypropylated-cyclodextrin; Amorphous inclusion complex; Crystallization; Solubility; Dissolution; Bioavailability

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1. Introduction

Spironolactone (SP), a synthetic steroid lactone which has specific competitive antagonism to the action of aldosterone and other mineralocorticoids, is of clinical value in the treatment of congestive heart failure, hepatic ascitis, primary aldosteronism and essential hypertension (Weuner and Mudge, 1985). SP is reported to exhibit incomplete bioavailability as well as a significant difference in the bioavailability from various brands and batches. These bioavailability problems could be related to the slow dissolution characteristics of SP (Levy, 1962; Clarke et al., 1977). Various methods have been reported for improvement of the dissolution property of SP, for example, micronization (McInnes et al., 1982) and inclusion complexation with cyclodextrins (CyDs) (Anderson and Bundgaard, 1983; Seo et al., 1983; Yusuff and York, 1991).

In a previous study (Seo et al., 1983), we reported that SP forms inclusion complexes with parent β- and γ-CyDs and its solubility and bioavailability are improved by the complexation. However, the solubility change of SP by the complexation of parent CyDs was limited because of the formation of less soluble complexes. Recently, hydroxypropyl CyDs have received attention because of their amorphous property and higher solubility, compared with parent CyDs (Müller and Brauns, 1985; Pitha, 1988; Uekama et al., 1992). Such characteristics of hydroxypropyl CyDs are particularly useful for the conversion of crystalline drugs to amorphous forms with improved dissolution. Therefore, the present study dealt with inclusion complexation of SP with hydroxypropyl CyDs (HP-CyDs), in the hope of achieving greater dissolution rate of SP than for that observed when complexed with parent CyDs. Furthermore, the crystallization behavior of SP from amorphous HP-CyD complexes was investigated, in comparison with various SP polymorphs and solvates.

2. Materials and methods

2.1. Materials

SP (Wako Pure Chemical, Osaka, Japan) and HP-β- and γ-CyDs (an average degree of substitution: 4.8; Nippon Shokuhin Kako Co., Tokyo, Japan) were used as received. β- and γ-CyDs (Nippon Shokuhin Kako Co.) were used after recrystallization from water. Other chemicals were of analytical grade and deionized double-distilled water was used throughout the study.

2.2. Apparatus

Powder X-ray diffraction patterns were taken on a Rigaku Denki, Rint-2500-VL diffractometer (Tokyo, Japan), operated at the conditions of Ni-filtered Cu-Kα radiation, a voltage of 40 kV, a current of 40 mA, a time constant of 1.25 s, and a scanning speed of 1°/min. Differential scanning calorimetric (DSC) and thermal gravimetric (TG) analyses were carried out by Perkin-Elmer DSC 7 and TG thermal analyzers (USA) with a data analysis system, operated at the conditions of sample weights of 5 mg for DSC and 10 mg for TG, a scanning rate of 10°C/min and a temperature range of about 50–350°C. Ultraviolet (UV) and circular dichroism (CD) spectra were taken on a Hitachi U-3200 spectrophotometer (Tokyo, Japan) and a Jasco J-40S recording spectropolarimeter (Tokyo, Japan), respectively, at 25°C. Cross polarization/magic angle spinning (CP-MAS) ^{13}C-nuclear magnetic resonance (NMR) spectra of solid complexes were recorded with a Jeol JNM EX-270 spectrometer and CP-MAS accessory (Tokyo, Japan), operated at 270 MHz and 25°C, CP with a radio-frequency field strength of about 56 kHz, a contact time of 5 ms, and a MAS rate of 6 kHz. The ^{13}C-chemical shifts were measured with respect to the resonance of adamantane (29.7 ppm downfield from the resonance of tetramethylsilane). The chemical shifts of SP and CyDs were assigned according to the reports of Highet et al. (1980) and Gidley and Bociek (1988), respectively.

2.3. Preparation of SP polymorphs and solvates

SP polymorphs, stable form (form II) and metastable form (form I), were prepared by slow

and rapid recrystallizations from acetone, respectively, while the solvates, acetonitrile (form III), ethanol (form IV), ethyl acetate (form V) and methanol (form VI), were prepared by recrystallization from the corresponding solvents (Agafonov et al., 1991).

2.4. Preparation of solid complexes

Solid complexes of SP with CyDs were prepared by the spray-drying method, using a Pulvis GA32 Yamato spray-dryer (Tokyo, Japan) under the following conditions: air flow rate, 0.40 m^3/min; air pressure, 1.0 kgf/cm^2; inlet and outlet temperatures, 100°C and 65°C, respectively. A weighed quantity of SP was dissolved in a minimum volume of ethanol/water mixture (40:60, v/v). The calculated quantities of CyDs corresponding to 1:2 and 2:3 (SP/β-CyDs and SP/γ-CyDs) molar ratios were dissolved individually in these mixed solvents, and then spray-dried. These guest/host mol ratios were chosen on the basis of the stoichiometry determined from the Bs type phase solubility diagrams of parent β- and γ-CyD systems described later. The complexes were stored in a desiccator, and after sieving the powder fractions of a particle size of about 150 μm were used in the following study.

2.5. Phase solubility studies

The solubility measurements were carried out according to the method described by Higuchi and Connors (1965). The screw-capped vials containing SP (50 mg) in excess amount and CyDs at various concentrations (2×10^{-3}–2.4×10^{-2} M, 5 ml) were shaken at 25°C. After equilibrium was attained (about 12 days), the solutions were properly diluted with water and analyzed spectrophotometrically at 242 nm for total SP content. The apparent 1:1 stability constant of the complexes, K_c, was calculated from the initial linear part of phase diagrams using the following equation (Higuchi and Connors, 1965)

$$K_c = \text{slope}/\text{intercept}\,(1 - \text{slope}) \quad (1)$$

2.6. Ageing studies

The test powders (2–3 g) were placed in glass containers, in desiccators at three temperature levels (50, 60 and 80°C) and 0% and 75% relative humidities (RH) which were attained by phosphorous pentaoxide powder and saturated solutions of sodium chloride (74.9, 75.1 and 76.4% RH at 50, 60 and 80°C, respectively (Carr and Hariss, 1949). At appropriate time intervals, samples were withdrawn and subjected to powder X-ray measurement.

2.7. Dissolution studies

The dissolution rates of SP, its polymorphs and solvates and CyD complexes were measured according to the dispersed amount method (Uekama et al., 1988). The equivalent amount of 100 mg SP or 200 mg for HP-β-CyD complex was put into 25 ml of Japanese Pharmacopoeia XIII (JP XIII) second fluid (pH 6.8) and stirred at 91 rpm at 37°C. At appropriate time intervals (5, 10, 20, 30, 60, 120 and 180 min), 0.5 ml was withdrawn, then diluted with the dissolution medium, and analyzed spectrophotometrically at 242 nm for SP concentration. The cumulative dilution caused by sampling was corrected by replacing the sample with an equal volume of the original medium.

2.8. In vivo absorption studies

Three male beagle dogs weighing 12–14 kg were fasted for 24 h prior to drug administration at intervals of more than 1 week. The administration sequence was based on a crossover matrix designed to minimize any residual or cumulative effects of the preceding dose. The test powder of SP or SP-HP-β-CyD complex (equivalent to SP 50 mg/body) was administered orally with 20 ml of water. At predetermined intervals, (0.5, 1, 1.5, 2, 3, 4, 6, 12, 18 and 24 h), 3 ml of blood sample was taken from the cephalic vein by heparinized syringe, then centrifuged for 20 min to obtain 1 ml of plasma for analysis. A 1.0-ml of plasma

sample was adjusted to pH 7.4 with 1 ml of 0.1 M phosphate buffer, then mixed with 8 ml of chloroform, shaken for 10 min and centrifuged. A 5-ml aliquot of the organic phase was evaporated to dryness under reduced pressure. The residue was dissolved in 100 μl of methanol, 40 μl of which was analyzed for canrenone, a major effective metabolite of SP (Sadee et al., 1972) by high-performance liquid chromatography (HPLC). The chromatograph was operated at a flow rate of 1.5 ml/min and the eluent was monitored spectrophotometrically at the UV maximum (280 nm) of canrenone. The separation utilized a column of Zorbax C8 (5 μm in 4.6 mm × 25 cm, Du Pont), with methanol/0.1 M H_3PO_4 (65:35, v/v) as a mobile phase. A standard curve was prepared by carrying out the analysis on plasma samples treated similarly and canrenone had been added at various concentrations ranging from 0.5 to 20 μg/ml.

3. Results and discussion

3.1. Inclusion complexation in solution and in solid state

Fig. 1 shows the phase solubility diagrams of SP with parent and hydroxypropylated β- and γ-CyDs in water. α-CyDs were not employed in this study, because of its weak interaction with the SP molecule due to the smaller cavity size, as reported previously (Seo et al., 1983). The solubility of SP increased linearly as a function of HP-β- and HP-γ-CyD concentrations and the solubility curves can be classified as A_L type (Higuchi and Connors, 1965). On the other hand, the parent β- and γ-CyD systems showed B_S type solubility curves, where the initial rising portions are followed by plateau regions and then the total SP concentration decreased with the precipitation of microcrystalline complexes. The solubilizing ability of HP-CyDs was markedly higher than that of parent CyDs at higher concentrations ($> 10 \times 10^{-3}$ M), although the latter was slightly higher than the former at lower CyD concentrations ($< 5 \times 10^{-3}$ M). These results suggest that HP-CyDs are of greater advantage than parent CyDs for solubilization of SP, because of the absence of precipitation of the complexes and the higher intrinsic solubility of HP-CyDs in water (> 50% w/v). The apparent 1:1 stability constants (K_c) were calculated by Eq. (1), assuming the 1:1 inclusion complex formation in low CyD concentra-

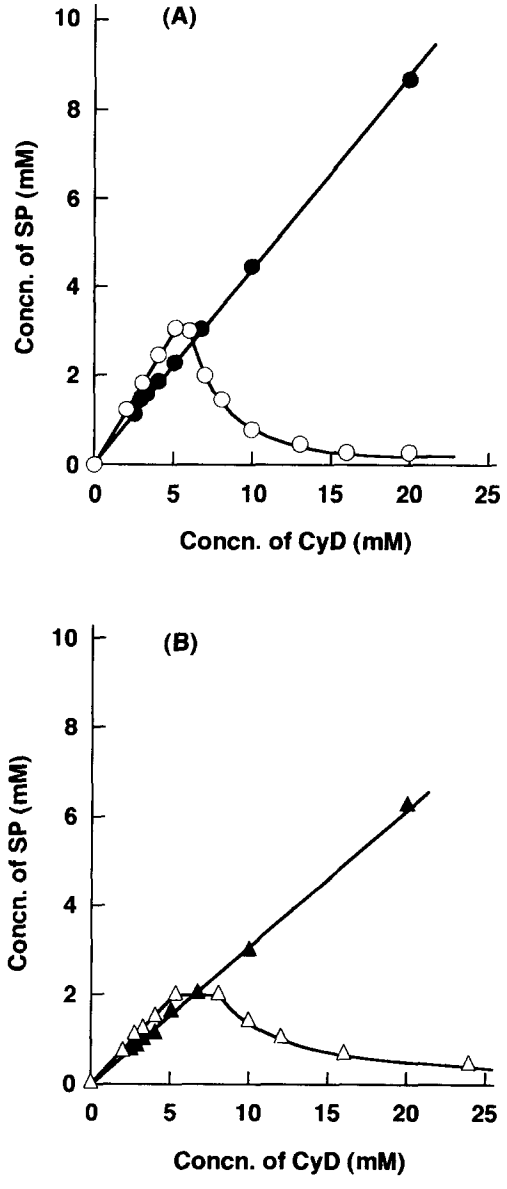


Fig. 1. Phase solubility diagrams of SP-β-CyDs (A) and SP-γ-CyDs (B) systems in water at 25°C. (○) β-CyD; (●) HP-β-CyD; (△) γ-CyD; (▲) HP-γ-CyD.

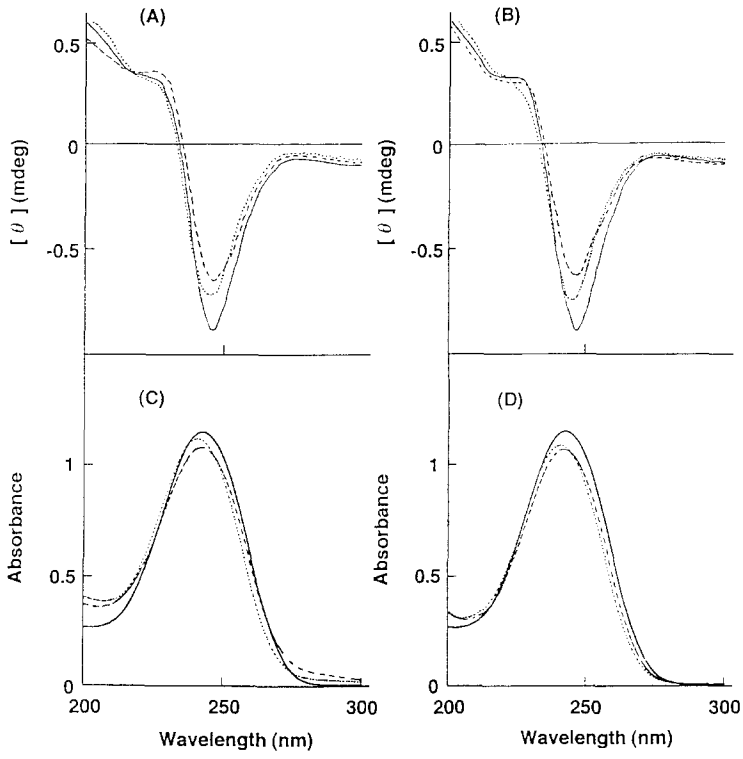


Fig. 2. Circular dichroism (upper) and UV absorption (lower) spectra of SP (4.5×10^{-5} M) in the absence and presence of CyDs (5.0×10^{-3} M) in water at 25°C. (A, C): ——, SP alone; — - - —, with β-CyD; - - -, with γ-CyD. (B, D): ——, SP alone; — — —, with HP-β-CyD; - - -, with HP-γ-CyD.

tions, and were in the order of β-CyD (24 800 M^{-1}) > HP-β-CyD (15 700 M^{-1}) > γ-CyD (9920 M^{-1}) > HP-γ-CyD (7000 M^{-1}), indicating that β-CyD cavity is the more favourable site for inclusion of SP than γ-CyD cavity. This was in agreement with the results of Yusuff and York (1991) and Seo et al. (1983). The stoichiometry of the β- and γ-CyD complexes was determined to be 1:2 (guest/host) and 2:3, respectively, by the analysis of the length of the plateau region in B_S type diagrams as well as by chemical analysis of the isolated complexes. The same stoichiometry was assumed for HP-β-CyD (1:2) and HP-γ-CyD (2:3) complexes on the basis of the above result, because the inclusion behavior of hydroxyalkylated CyD derivatives is the same as with parent CyDs as concerns stoichiometry and thermodynamics (Bettinetti et al., 1991). Fig. 2 shows UV and CD spectra of SP in the absence and presence of CyDs in water. SP exhibited an intense UV maximum at 242 nm and a negative CD peak at 247 nm. In the presence of CyDs, the negative CD peak of SP decreased significantly, with a decrease in UV absorbance. Also, in case of γ-CyD and HP-γ-CyD, the peaks of UV and CD were shifted slightly to shorter wavelengths. The magnitude of these spectral changes was well correlated with that of K_c values, suggesting that the SP molecule is included within the cavity of β-CyD, γ-CyD, HP-β-CyD and HP-γ-CyD.

SP-CyD complexes were prepared by the spray-drying method in molar ratios of 1:2 (guest/host) and 2:3 for HP-β- and HP-γ-CyDs, respectively, on the basis of the phase solubility data of parent CyDs, and their interactions in the solid state were investigated. SP-CyD complexes showed no peaks but only halo-pattern in powder X-ray diffractograms, indicating that the complexes are in

an amorphous state. On the other hand, the physical mixtures of each component showed sharp diffraction peaks at $2\theta = 8.4°$, 16.1°, 16.7°, 17.5° and 20.5° due to SP crystals. Furthermore, the endothermic peak due to the melting of SP at 209°C disappeared due to the inclusion complexation with CyDs in DSC curves, suggesting that the drug is monomolecularly dispersed in the CyD cavities, whereas the physical mixtures showed the endothermic peak at this temperature. Fig. 3 shows CP/MAS ^{13}C-NMR spectra of the SP-CyD complexes or their physical mixtures. SP crystals showed many signals in the range of 10–200 ppm, and these signals were assigned according to the spectrum in deuterated chloroform (Highet et al., 1980). The signals of parent β- and γ-CyDs were assigned as follows: 103 ppm for C1, 82 ppm for C4, 71–76 ppm for C2, 3, 5, and 61 ppm for C6. Three additional signals due to the methyl, methine and methylene carbons of the 2-hydroxypropyl group in HP-CyDs were observed at 20, 67 and 82 (shoulder) ppm, respectively. The NMR spectra of physical mixtures were simply superposition of spectra of each component. In the case of the complexes, on the other hand, the signals due to the guest molecule markedly broadened or disappeared from the spectra. The line-broadening of NMR signals may be explained from the viewpoint of static and dynamic mechanisms that arise from chemical-shift dispersion and molecular motion, respectively (Inoue et al., 1984). The first mechanism occurs when the SP molecule occupies many magnetically non-equivalent positions in CyD cavities and gives many signals of each carbon resulting in line-broadening. On the other hand, the second mechanism involves the molecular motion of SP in CyD cavities, such as exchange or interchange of SP molecule between several non-equivalent sites at a rate close to their chemical shift differences, irregular phase coherences in ^{13}C-transverse magnetisation by anisotropic chemical-shifts, or molecular motions with a rate close to proton decoupling field. The dynamic mechanism seems to be a less significance for the line-broadening, because the space-filling molecular model suggested that SP molecule is tightly included within the β- and γ-CyD cavities in the 1:2 and 2:3 complexes, respectively, and its molecular motion is thus severely restricted in the cavities (Seo et al., 1983). On the other hand, the static mechanism may be mainly operated in the broadening, since the complexes are prepared by the spray-drying method and are in amorphous state, as is apparent from powder X-ray diffraction patterns and DSC curves, giving many orientations of the complexes in the solid state and even SP in the cavities.

3.2. Ageing

The crystallization behavior of SP from the amorphous CyD complexes was investigated at various temperatures (50, 60, and 80°C) and relative humidities (0 and 75% RH). Fig. 4 shows the changes in the powder X-ray diffraction pattern of SP-CyD complexes during storage at 60°C and 75% RH, as an example. The spray-dried SP-CyD complexes showed halo-patterns, and in the case of parent β- and HP-CyD complexes this pattern did not change for at least 2 months even at the storage conditions of 60°C and 75% RH. On the other hand, the γ-CyD complex showed many diffraction peaks 2 months after the storage, and this diffraction pattern was in disagreement with those of any polymorphs and solvates of SP, but in good agreement with that of the crystalline SP-γ-CyD complex isolated from the descending portion of the phase solubility diagram (see Fig. 1). At the storage condition of 60°C and 75% RH, amorphous SP, metastable form (form I) and solvates (methanol, ethanol, acetonitrile, ethyl acetate) were quickly converted to the stable crystalline form (Form II) within 30 min. These results suggest that HP-CyDs are useful particularly for conversion of crystalline drugs to an amorphous state and also for maintenance of the amorphous state for long time. This effect of HP-CyDs may be much higher than that of parent CyDs, because the latter quickly forms a crystalline complex or alternatively after dissociation, each component crystallizes separately.

3.3. Dissolution behavior

The dissolution curves obtained for SP-CyD complexes in JP XII second fluid are shown in

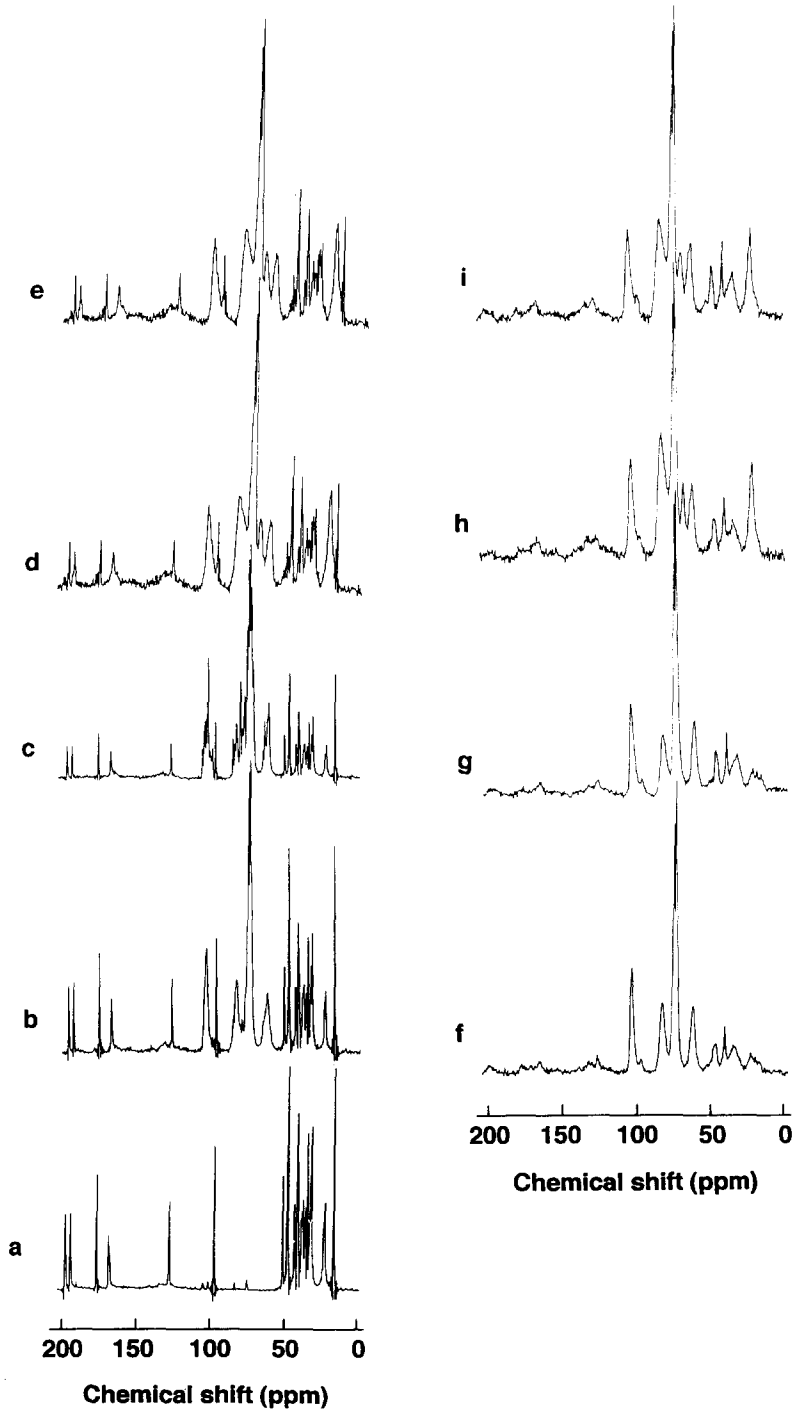


Fig. 3. ^{13}C-CP/MAS NMR spectra of SP-CyD systems. (a) SP alone, (b) β-CyD physical mixture, (c) γ-CyD physical mixture, (d) HP-β-CyD physical mixture, (e) HP-γ-CyD physical mixture, (f) β-CyD complex, (g) γ-CyD complex, (h) HP-β-CyD complex, (i) HP-γ-CyD complex.

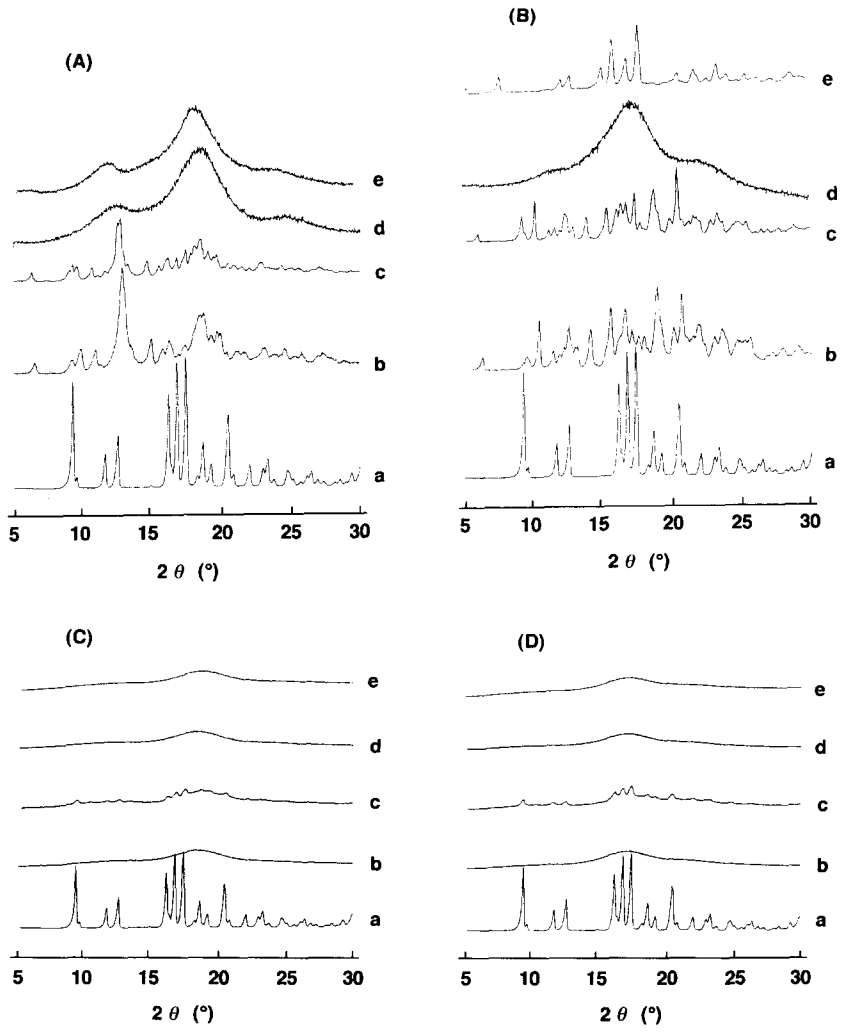
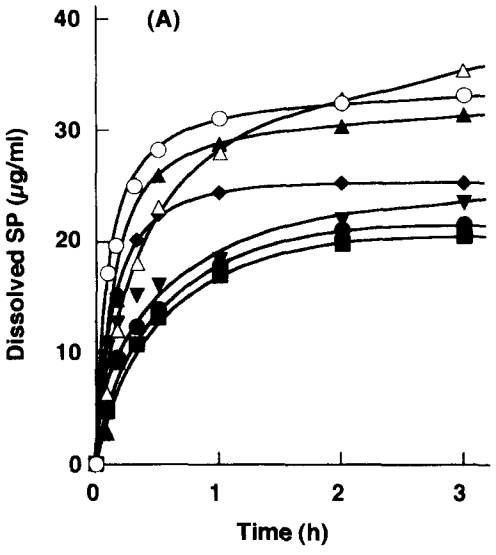


Fig. 4. Powder X-ray diffraction patterns of SP in β-CyD systems (A), γ-CyD systems (B), HP-β-CyD systems (C) and HP-γ-CyD systems (D). (a) SP alone, (b) CyDs alone, (c) SP-CyDs physical mixtures, (d) SP-CyDs complexes, (e) SP-CyDs complexes, stored at 60°C, 75% RH for 2 months.

Fig. 5, in comparison with SP polymorphs and solvates. It is evident that the dissolution rate of SP was significantly improved through the complex formation, particularly with HP-β-CyD complex followed by HP-γ-CyD > γ-CyD > β-CyD complexes. In the case of the SP-HP-β-CyD complex, the dissolved amount of SP reached 6 mg/ml after about 30 min, and this amount was much higher than the amount (0.93 mg/ml) calculated from the solubility diagram when the host of the complex is completely dissolved. This result suggests that HP-β-CyD inhibits the crystallization of SP from the super-saturated solution. Under the experimental conditions, the amounts of SP dissolved from its polymorphs and solvates were below 40 μg/ml. Fig. 6 shows the dissolution profiles of the SP-CyD complexes after storage at 60°C and 75% RH for 2 months. It is apparent that there is no deterioration in the dissolution rate from the HP-CyD complexes, which may be due to the longer maintenance of the amorphous state.

3.4. Absorption behavior

The in vivo absorption study was carried out to determine whether or not the enhanced in vitro



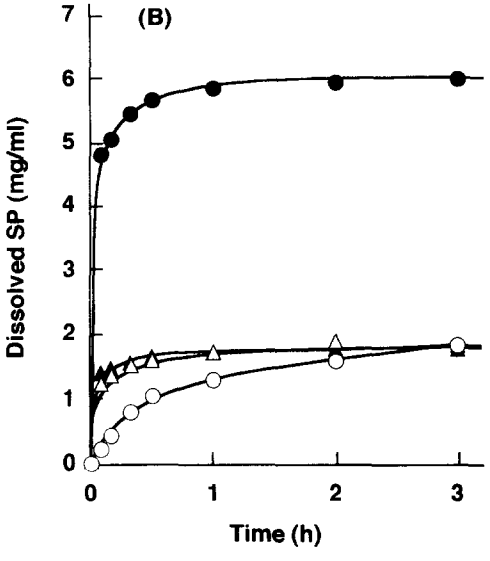


Fig. 5. Dissolution profiles of SP polymorphs and solvates (A) and CyD complexes (B) (equivalent to 100 mg of SP) in JP XIII second fluid at 37°C, measured by dispersed amount method at 91 rpm. (A) (●) form I, (■) form II, (◆) form III, (▲) form IV, (▼) form V, (○) form VI, (△) amorphous form. (B) (○) β-CyD complex, (△) γ-CyD complex, (●) HP-β-CyD complex, (▲) HP-γ-CyD complex.

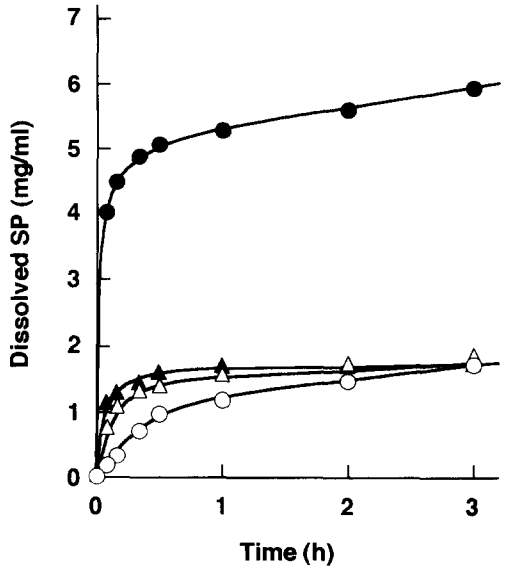


Fig. 6. Dissolution profiles of SP-CyD complexes (equivalent to 100 mg of SP) after storage at 60°C, 75% RH for 2 months, in JP XIII second fluid at 37°C, measured by dispersed amount method at 91 rpm. (○) β-CyD complex, (△) γ-CyD complex, (●) HP-β-CyD complex, (▲) HP-γ-CyD complex.

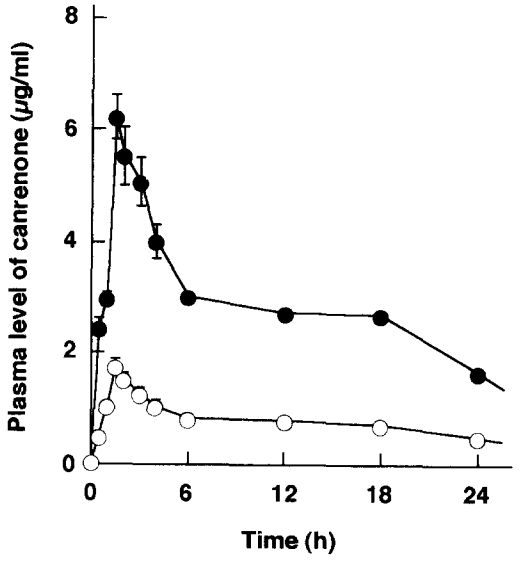


Fig. 7. Plasma concentrations of canrenone following the oral administration of SP or its HP-β-CyD complex (equivalent to SP 50 mg/body) to dogs. (○) SP alone, (●) HP-β-CyD complex.

Table 1
Pharmacokinetic parameters of SP after oral administration of powders containing SP or its HP-β-CyD complex (equivalent to SP 50 mg/body) to dogs

System	T_{max} (h)	C_{max} (μg/ml)	MRT (h)	AUC (h·μg/ml)
SP	1.5 ± 0.0	1.8 ± 0.1	10.5 ± 0.7	19.6 ± 1.8
HP-β-CyD complex	1.5 ± 0.0	6.2 ± 0.4	10.4 ± 0.5	70.4 ± 5.2

Each value represents the mean ± S.D. of 3 dogs.

dissolution of SP from the HP-β-CyD complex reflects in the gastrointestinal (GI) absorption of the drug. Fig. 7 shows the mean plasma levels of canrenone, a major effective metabolite of SP (Sadee et al., 1972), following the oral administration of SP and its HP-β-CyD complex (equivalent dose of SP 50 mg/body) to dogs and their pharmacokinetic parameters are summarized in Table 1. SP and HP-β-CyD complex produced maximum plasma levels (C_{max}) of 1.8 ± 0.1 μg/ml (mean ± S.D.) and 6.2 ± 0.4 μg/ml, respectively, 90 min after the administration. So, the value of C_{max} for SP-HP-β-CyD complex was about 3.4 times that of SP alone. The area under the plasma concentration-time curves (AUC) of the complex up to 24 h was found to be 3.6 times greater than that of SP alone. On the other hand, no difference in the time values to reach the maximum plasma concentration (T_{max}) between SP and its complex could be observed. This is probably due to the rapid dissociation of the complex following the dissolution in the GI fluids. While, mean residence times (MRT) of SP and its complex were 10.5 ± 0.7 h and 10.4 ± 0.5 h, respectively. This indicates that the drug and its complex remained in the body for the same period, but the complex in significantly higher concentration. The enhanced bioavailability of SP produced by HP-β-CyD complexation suggests that it may be possible to use a lower dose with little side effects in oral SP therapy. Furthermore, the SP/HP-β-CyD complex may become of some practical use as regards injection preparations due to its high solubility in water.

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