

[Chem. Pharm. Bull.]
31(1) 286-291 (1983)

Enhancement of Oral Bioavailability of Spironolactone by β - and γ -Cyclodextrin Complexations¹⁾

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(Received July 12, 1982)

Inclusion complex formations of spironolactone (SP) with three cyclodextrins (α -, β -, γ -CyDs) in aqueous solution and in the solid state were studied by the solubility method, by spectroscopic methods (UV, CD, IR) and by X-ray diffractometry, and their modes of interaction were assessed. The solid complexes of SP with β - and γ -CyDs were obtained in molar ratios of 1:2 and 2:3, respectively, and their dissolution, membrane permeation and oral absorption properties were examined. The rates of dissolution and permeation through a cellophane membrane in water were significantly increased by inclusion complexation (γ -CyD complex > β -CyD complex > SP alone), depending upon the solubility of the test samples. The serum levels of SP following oral administration of CyD complexes were found to be greater than those after administration of SP alone. The results indicated that the γ -CyD complex rather than β -CyD complex may have great utility as a faster dissolving form of SP able to produce higher serum levels.

Keywords—spironolactone; α -cyclodextrin; β -cyclodextrin; γ -cyclodextrin; inclusion complex; stability constant; dissolution rate; permeation behavior through cellophane membrane; oral bioavailability; serum level of canrenone in dog

Spironolactone (SP, Fig. 1) is a steroidal aldosterone antagonist that has been widely used in the treatment of essential hypertension, edematous states, and primary hyperaldosteronism. Because of its low solubility in water (2.8 mg/100 ml at 25°C),²⁾ the bioavailability of SP preparations is known to vary significantly among brands and batches.³⁾

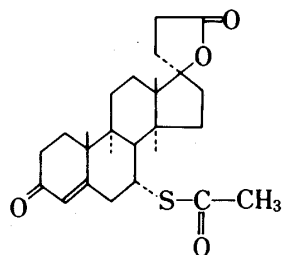


Fig. 1. The Chemical Structure of Spironolactone (SP)

Cyclodextrins (CyDs) have been extensively applied to improve the physicochemical properties of various drug molecules.^{4,5)} One of the important characteristics of CyDs is the formation of inclusion complexes in the solid phase and in solution, in which the drug molecule is included in the relatively hydrophobic cavity of CyDs.^{4,5)}

The present study dealt with inclusion complexations of SP with three CyDs (α -, β -, γ -CyDs) in an attempt to obtain improved dissolution characteristics of SP. In addition, a bioavailability study of the inclusion complexes was conducted by oral administration to dogs, with measurement of the serum levels of canrenone, the major effective metabolite⁶⁾ of SP.

Experimental

Materials—SP was a gift from Mitsubishi Yuka Pharmaceuticals (Ibaraki, Japan). Canrenone (mp 161°C) was prepared from potassium canrenoate according to the reported procedure,⁷⁾ and was recrystallized from ethyl acetate. α -, β -, and γ -CyDs were purchased from Nippon Shokuhin Kakō Co., Ltd. and recrystallized from water. All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout.

Apparatus—The circular dichroism (CD) and ultraviolet (UV) spectra were taken with a Jasco J-40S recording spectropolarimeter (Tokyo, Japan) and a Hitachi 556S double-beam spectrophotometer (Tokyo, Japan), respectively, at $25 \pm 0.5^\circ\text{C}$. The CD spectra were expressed in terms of molar ellipticity, $[\theta]$ ($\text{deg} \cdot \text{cm}^2 \cdot \text{dmol}^{-1}$). The infrared (IR) spectra were measured in KBr discs, using a Jasco DS-702 double-beam spectrophotometer (Tokyo, Japan). The powder X-ray diffraction patterns were taken by a Rigaku Denki Geiger Flex-2012 diffractometer (Tokyo, Japan). High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-3A with a Shimadzu SPD-1 UV absorbance detector (Kyoto, Japan).

Solubility Studies—Solubility measurements were carried out according to the method of Higuchi and Connors.⁸⁾ Excess amounts of SP were added to aqueous solutions containing various concentrations of CyDs and the mixtures were shaken at $25 \pm 0.5^\circ\text{C}$. After equilibration had been attained (approximately 10 d),⁹⁾ an aliquot was centrifuged and pipetted through a cotton filter. A portion of the sample (0.5 ml) was then diluted with 50% v/v ethanol–water and analyzed spectrophotometrically at the UV maximum (241 nm) of SP. An apparent stability constant, K' , was calculated from the initial straight line portion of phase solubility diagrams according to the following equation.⁸⁾

$$K' = \frac{\text{slope}}{\text{intercept} \cdot (1 - \text{slope})} \quad (\text{Eq. 1})$$

Preparation of Solid Complexes—The solid complexes were derived by mixing appropriate amounts of CyD and SP in water. Amounts were calculated from the descending curve of the phase solubility diagram (see Fig. 2). For example, 2.50 g of SP and 22.7 g of β -CyD were added to 1000 ml and sealed in a flask, then the mixture was stirred with a magnetic stirrer at 25°C for 7 d. The complex, which precipitated as a micro-crystalline powder, was filtered and dried under a vacuum at 60°C for 48 h. This powder corresponded to a 1:2 SP–CyD complex and had a molecular weight of 2687.

Dissolution Studies—The sample powder (150 mg, 100 mesh) was compressed into a cylindrical tablet (diameter 10 mm) at a pressure of about 200 kg/cm^2 . The release of SP was measured by using a rotating disc apparatus¹⁰⁾ in 25 ml of water at 91 rpm and 25°C . At appropriate intervals, 1 ml samples were removed from the flask, diluted with 75% ethanol–water solution and assayed spectrophotometrically. Corrections were applied for cumulative dilution caused by replacement of the samples with equal volumes of the original medium. The tablets maintained a constant shape through the measurement.

Membrane Permeation Studies—Permeation of SP through a cellophane membrane (type 36/32, Visking Co.) was examined by using a permeation cell.¹¹⁾ The sample powder (88 mg) of SP or its CyD complexes were put into 100 ml of water in a donor compartment while the same volume of water was placed in a receptor compartment. The solutions in the permeation cell were kept at 25°C by means of a thermostated water bath and stirred with a magnetic bar at a rate of 150 rpm . At predetermined intervals, a sample was pipetted from the receptor solution and the concentration of SP which had permeated from the donor cell was measured spectrophotometrically.

Determination of Canrenone in Dog Serum—Four male beagle dogs weighing 12–14 kg were fasted for 18 h prior to drug administration at intervals of more than one week. The administration sequence was based on a crossover matrix designed to minimize any residual or cumulative effects of the preceding dose. A test powder (5 mg/kg of body weight as SP) packed in a gelatin capsule was administered orally with 20 ml of water. At predetermined intervals, a 3 ml blood sample was taken from the cephalic vein. The blood samples were allowed to clot for 30 min and then were centrifuged for 20 min. The serum layer was removed and frozen until analysis. A 1 ml serum sample adjusted to pH 7.4 with 1 ml of 0.1 M phosphate buffer was extracted with 8 ml of chloroform, and 5 ml of the chloroform phase was evaporated to dryness under nitrogen on a water bath. The residue was dissolved in 100 μl of methanol, and 40 μl of the methanol solution was injected for HPLC. The chromatograph was operated at a flow rate of 2.3 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 $4.6 \text{ mm} \times 25 \text{ cm}$, Du pont), with methanol–0.1 M H₃PO₄ (65:35) as a mobile phase. A standard curve was prepared by carrying out the analysis on serum samples to which canrenone had been added at various concentrations ranging from 20 to 200 ng/ml.

Results and Discussion

Inclusion Complexation in Aqueous Solution

The complexation behavior of SP with three CyDs in aqueous solution was studied by the solubility method and by spectroscopic methods (UV and CD). Fig. 2 shows the phase

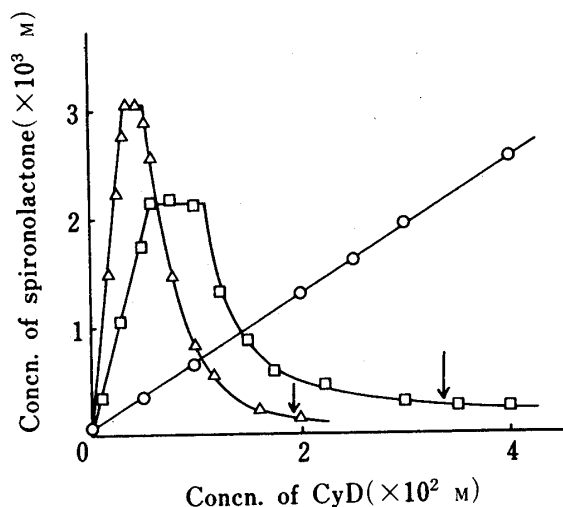


Fig. 2. Phase Solubility Diagrams of SP-CyD Systems in Water at 25°C

O, α -CyD system; Δ , β -CyD system; \square , γ -CyD system.

Arrows show the experimental conditions used for the preparation of solid complex (see text).

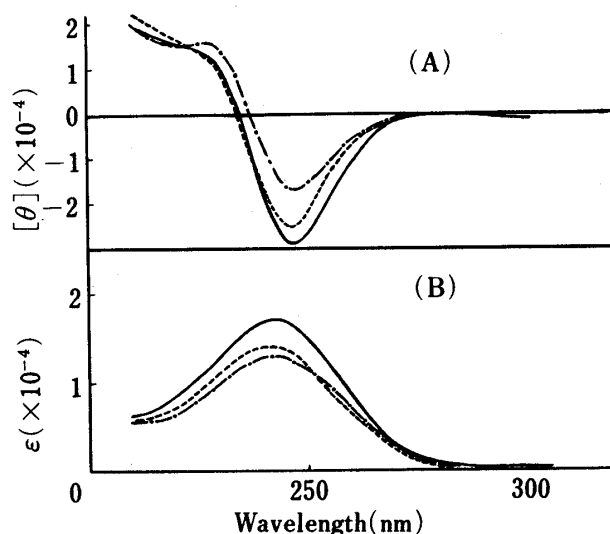


Fig. 3. Circular Dichroism (A) and UV Absorption (B) Spectra of SP in the Absence and Presence of CyDs in Water at 25°C

—, SP alone; ---, SP + β -CyD; ···, SP + γ -CyD.

Concentrations of SP and CyDs were 4.5×10^{-5} and 5.0×10^{-3} M, respectively.

solubility diagrams obtained for SP with three CyDs in water. In the case of α -CyD, the solubility of SP increased linearly as a function of α -CyD concentration and the solubility curve can be generally classified as being of type A_L.⁸⁾ On the other hand, β -CyD and γ -CyD systems showed typical B_s type solubility curves,⁸⁾ where the initial rising portions are followed by plateau regions, and then the total SP concentration decreased with the precipitation of micro-crystalline complexes. In sharp contrast, no precipitation was observed for the α -CyD system. The apparent stability constant (K'), as a tentative measure of inclusion complexation, was estimated from Eq. 1 based on the assumption that a 1:1 complex initially formed. The magnitude of K' values calculated from the initial rising portion of solubility diagrams was found to increase in the order β -CyD (27500 M^{-1}) $>$ γ -CyD (7600 M^{-1}) $>$ α -CyD (960 M^{-1}). These findings suggest that steric factors of the host and guest molecules are critical in these interaction.¹²⁾

Since the solubility studies indicated that SP would form relatively soluble complexes with β - and γ -CyDs, these interactions in aqueous solution were further examined by UV and CD spectroscopies. Figure 3 shows the UV and CD spectra of SP in the absence and presence of β - and γ -CyDs in water. SP exhibited an intense UV maximum and a negative CD peak at 241 and 247 nm, respectively. On the addition of β - or γ -CyD, the negative CD peak of SP decreased significantly, with a decrease in UV absorbance. The magnitudes of these spectral changes were well correlated with those of K' values, suggesting that the SP molecule may be included within the cavity of β - and γ -CyDs.

Inclusion Complexation in the Solid State

Since the β - and γ -CyD systems deposited micro-crystalline complexes of SP at high CyD concentrations (Fig. 2), the stoichiometries of the complexes in the solid phase were analyzed on the basis of data in the plateau region, and were estimated to be 1:2 for SP- β -CyD and 2:3 for SP- γ -CyD. The results were in good agreement with those obtained by isolation and analysis of the solid complexes. Inspection of a space-filling molecular model¹³⁾ also supported the 1:2 (SP: β -CyD) and 2:3 (SP: γ -CyD) interactions for the complete inclusion of SP, which fits tightly into β -CyD channels and more loosely into the larger interior space of

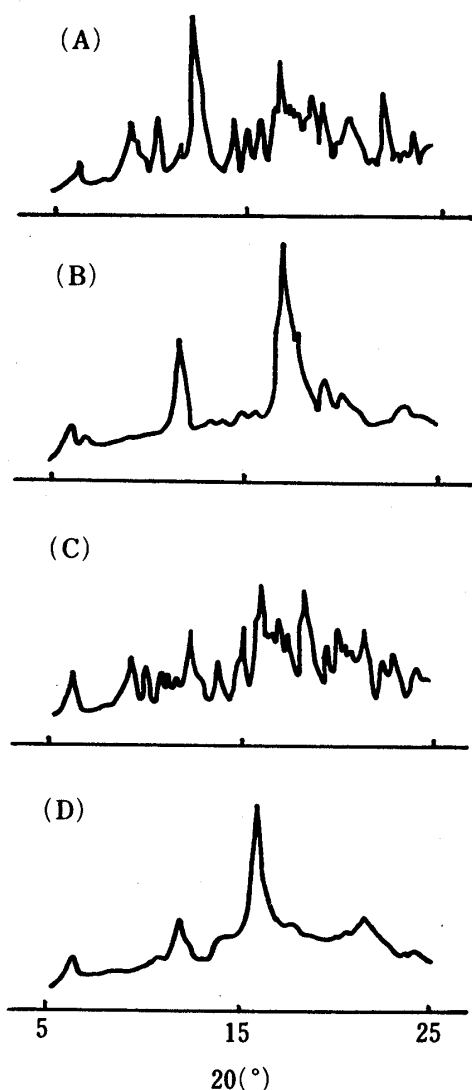


Fig. 4. Powder X-Ray Diffraction Patterns of SP-CyD Systems

(A), physical mixture of SP and β -CyD; (B), complex of SP with β -CyD; (C), physical mixture of SP and γ -CyD; (D), complex of SP with γ -CyD.

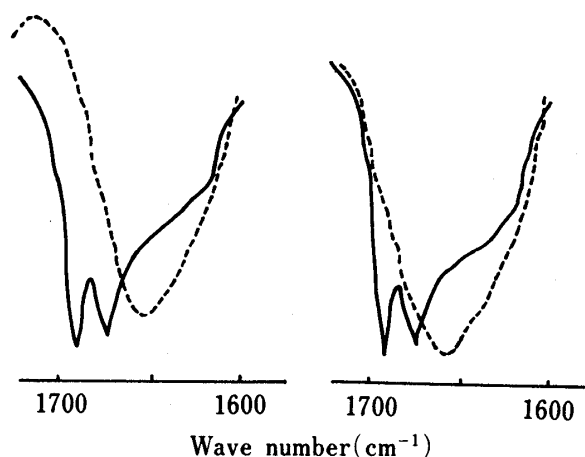


Fig. 5. IR Spectra of SP-CyD Systems (Left; β -CyD System, Right; γ -CyD System), measured by the KBr Disc Method

—, physical mixture of SP and CyD.
---, complex of SP with CyD.

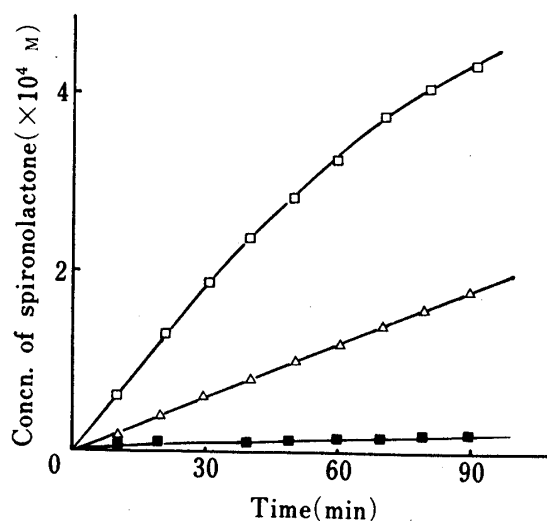


Fig. 6. Dissolution Profiles of SP and Its CyD Complexes in Water at 25°C, measured by the Rotating Disc Method

■, SP alone; Δ , β -CyD complex; \square , γ -CyD complex.

γ -CyD channels. Thus, these solid complexes corresponding to the descending region of the Bs type solubility diagram were used for further study. Figure 4 shows the powder X-ray diffraction patterns of SP-CyD complexes and their physical mixtures. The diffraction patterns of the physical mixtures were simply the superposition of each component pattern, while those of CyD complexes were apparently different and indicated the formation of a new solid phase. The CyD complexes gave somewhat diffuse diffraction patterns, suggesting that they are much less crystalline¹⁴⁾ than the physical mixtures. It was difficult to determine the crystal packing of the complexes because the diffraction patterns were too complicated to be reliably indexed by the powder method.¹⁵⁾ Figure 5 shows IR spectra of SP-CyD complexes and their physical mixtures in the carbonyl-stretching regions. In the cases of CyD complexes, the 1674 and 1692 cm^{-1} bands shifted to 1655 and 1658 cm^{-1} , respectively. The shift to shorter wave-number can be explained by the formation of intermolecular hydrogen bonds between SP and CyD.¹⁶⁾ The above results clearly indicate that the SP-CyD (β - and γ -CyDs) complexes exist in the solid state.

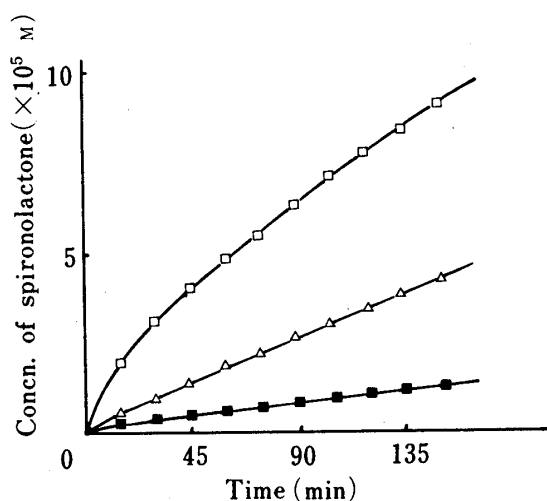


Fig. 7. Permeation Profiles of SP through a Cellophane Membrane in Water at 25°C from SP and Its β - or γ -CyD Complex

■, SP alone; Δ , β -CyD complex; \square , γ -CyD complex.

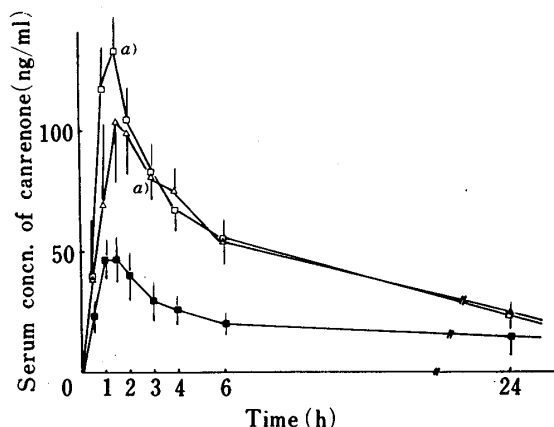


Fig. 8. Serum Concentrations of Canrenone following the Oral Administration of 5 mg/kg SP or Its CyD Complexes (equivalent to 5 mg/kg SP) to Dogs

■, SP alone; Δ , β -CyD complex; \square , γ -CyD complex. Each point represents the mean \pm S.E. of 4 dogs. a) $p < 0.01$ in (■) versus (Δ) or (\square).

Dissolution and Permeation Properties of the Complexes

Figure 6 shows a typical example of the dissolution profiles of SP and its β - and γ -CyD complexes from a rotating disc with constant surface area in water at 25°C; the release of SP was quantitatively measured by UV spectrophotometry. It is evident that the dissolution rate of SP was significantly improved by inclusion complexation, particularly with γ -CyD. The observed increase in dissolution rate may be due to an increase in solubility¹⁷⁾ and a decrease in crystallinity of the drug on inclusion complexation as expected from Fig. 2 and Fig. 4, respectively. Although γ -CyD complex dissolved much more rapidly than β -CyD complex, the former dissolution profile showed a negative curvature with time, in contrast to the latter profile. The curvature observed for the γ -CyD complex might be explained by the dissociation equilibrium of the complex in dissolution medium. That is, the γ -CyD complex has a smaller K' value than the β -CyD complex and may dissociate rather quickly during the dissolution process.

Figure 7 shows the permeation profiles of SP through a cellophane membrane, following dissolution from sample powder of SP or its CyD complexes in a donor cell. The faster the dissolution rate in the donor cell, the greater the net amount of SP that permeated into the receptor cell. The enhanced dissolution rate observed for γ -CyD complex may result in a good oral bioavailability of SP compared with β -CyD complex.

Bioavailability of SP-CyD Complexes

An *in vivo* absorption study was undertaken to determine whether or not the enhanced *in vitro* dissolution of SP from its β - or γ -CyD complex increases the GI absorption of the drug. Figure 8 shows the mean serum levels of canrenone, the major effective metabolite of SP, following the oral administration of SP and its CyD complexes to dogs. When the equivalent dose of SP (5 mg/kg) was administered to dogs, the β - and γ -CyD complexes produced maximum serum levels of 103 ± 28.3 ng/ml (mean \pm S.E.) and 131.0 ± 14.7 ng/ml at 90 min, respectively, which were about 2–3 times higher than that after administration of SP alone. The areas under the serum concentration–time curves (AUC) of the complexes up to 24 h were found to be 2.3–2.4 times greater than that of SP alone. On the other hand, no difference in T_{\max} (time to reach the maximum serum concentration) values between SP and its

CyD complexes could be seen¹; this is probably due to the rapid dissociation of the complex following the dissolution in the GI fluids. However, the enhanced bioavailability of SP produced by β - or γ -CyD complexation suggests that it may be possible to use a lower dose with fewer side effects in oral SP therapy. The γ -CyD complex may be practically applicable to injection preparations because of its high solubility in water.

References and Notes

- 1) A part of this work was presented at the 102nd Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982.
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- 17) From the B_s type phase solubility diagrams, the apparent solubilities of 1:2 SP: β -CyD and 2:3 SP: γ -CyD complexes (see arrows in Fig. 2) were estimated to be 1.42×10^{-4} and 2.57×10^{-4} M, respectively. These values are significantly greater than the intrinsic solubility of SP (5.50×10^{-5} M).