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Improvement of Some Pharmaceutical Properties of Nocloprost by β- and γ-Cyclodextrin Complexation

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Abstract. Inclusion complexation of nocloprost, a potent antiulcer prostaglandin derivative, with α-, β-, and γ-cyclodextrins (CyDs) in aqueous solutions has been studied by the solubility method and ^{13}C-NMR spectroscopy. The steric requirement of host–guest interaction was reflected in the magnitude of the stability constants and the thermodynamic parameters of the inclusion complexes. Solid complexes of nocloprost with β- and γ-CyDs in a molar ratio of 1 : 2 were obtained on the basis of a *Bs*-type phase solubility diagram. The X-ray diffraction data suggested that nocloprost is included in the cylindrical channel formed by coaxial alignment of γ-CyD molecules to give a channel type structure. Release and thermal behavior of the solid complexes was examined and compared with nocloprost itself. The results indicated that the β-CyD complex may have great utility among the three CyDs, being a rapid dissolving form of nocloprost with improved thermal stability.

Key words. Nocloprost, cyclodextrin, inclusion complex, stability constant, thermodynamic parameter, solubility, release rate.

1. Introduction

One of the important characteristics of cyclodextrins (CyDs) is the formation of inclusion complexes with a variety of drug molecules in solution and in the solid state. In recent years, natural CyDs (α-, β-, and γ-CyDs) have been successfully utilized in the improvement of various drug properties, such as solubility, dissolution rate, stability, or bioavailability [1–4]. We have recently reported that CyDs are particularly useful in the improvement of the chemical lability and slow dissolution characteristics of some natural prostaglandin analogs through inclusion complex formation [5–7]. Since the prostaglandin molecules are essentially long-chain fatty acids containing a substituted cyclopentane ring system, the relatively hydrophobic environment of the CyD cavity seems to be favorable for the inclusion of the hydrophobic prostaglandin molecules. Nocloprost, [(5*Z*,13*E*)-(9*R*,11*R*,15*R*)-9-chloro-11,15-dihydroxy-16,16-dimethyl-5,13-prostadienoic acid], is a newly developed prostaglandin derivative, with a potent antiulcer activity. However, the low solubility in water of nocloprost, which exists as a viscous oil at room temperature,

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has limited the design of dosage form and has presented a substantial challenge to pharmaceutical scientists. Thus, the present study deals with inclusion complexation of nocloprost with three natural CyDs in anticipation of powdering and providing an improved release rate of nocloprost.

2. Experimental

2.1. MATERIALS

Nocloprost was provided by Schering AG (West Berlin, FRG). α-, β-, and γ-CyDs were donated by Nihon Shokuhin Kako Co. (Tokyo, Japan). Other materials and solvents were of analytical reagent grade, and deionized doubly-distilled water was used throughout the study.

2.2. APPARATUS

^{13}C-NMR spectra were measured on a JEOL JNM GX-270 spectrometer (Tokyo, Japan) in 0.1M borate buffer solution (pH meter reading of 9.3) at 25°C. The concentrations of nocloprost and CyD were 0.02M. The ^{13}C-chemical shifts were recorded with an accuracy of 0.024 ppm using tetramethylsilane as an external reference. The power X-ray diffraction pattern was taken by a Geiger Flex 2012 diffractometer (Rigaku Denki, Tokyo, Japan). The operating conditions were as follows: voltage: 30 kV; current: 10 mA; time constant: 2 s; scanning speed: 1°/min. Differential scanning calorimetry (DSC) and thermal gravimetry (TG) of the samples (about 5 mg) were accomplished with a Rigaku Thermoflex TG 8110 thermal analyzer (Tokyo, Japan) operating at a scanning rate of 10°C/min.

2.3. DETERMINATION OF NOCLOPROST

In order to determine nocloprost with high sensitivity, the drug was derivatized to give its phenacylester by using *p*-bromophenacyl bromide, and was then analyzed by high performance liquid chromatography (HPLC) according to a method described by Fitzpatrick [8]. For example, aqueous nocloprost solution (2.0×10^{-4}M, 0.5 mL), containing prostaglandin B_1 as an internal standard for HPLC analysis, was acidified by addition of 0.4M HCl (2 mL) and the drug was extracted with ethyl acetate (5 mL). After evaporation of the ethyl acetate (3 mL) under reduced pressure, the residue was redissolved in acetonitrile (120 μL). *p*-Bromophenacyl bromide solution (4.5×10^{-3}M in acetonitrile, 50 μL) and *N*,*N*-diisopropylethylamine (0.5 μL) were added and then the mixture was allowed to stand for 100 min at room temperature. A 15 μL portion of the resulting solution was subjected to HPLC under the following conditions: pump and detector: Hitachi 655A machine equipped with a 638 UV monitor (Tokyo, Japan); column: YMC-Pack ODS-AQ (10 μm, 6 mm i.d. × 150 mm, YMC, Japan); mobile phase: water–methanol (1 : 9); flow rate: 1.0 mL/min; detection: 254 nm. Components were quantitated by measuring peak height and comparing it with that of the internal standard.

2.4. SOLUBILITY STUDIES

Solubility measurements were carried out according to a method presented by Higuchi and Connors [9]. Excess amounts (about 2 mg) of nocloprost were added to aqueous solutions (2 mL) containing various concentrations of CyDs, and the mixture was shaken at 15, 25 or 35 ± 0.5°C. After equilibrium was attained (about 1 week), an aliquot was centrifuged and pipetted through a cotton filter. Nocloprost in the filtrate was determined by HPLC. No appreciable degradation of nocloprost was observed under the conditions of the solubility experiment.

2.5. PREPARATION OF SOLID COMPLEXES

The solid complex was prepared by mixing appropriate amounts of nocloprost and β-CyD in water. Amounts of the host and guest molecules were calculated from the descending curvature of the phase solubility diagram (see Figure 1). For example, 40 mg of nocloprost was added to 80 mL of aqueous β-CyD solution (1.5×10^{-2}M), sealed in a flask, and shaken at 25 ± 0.5°C for 1 week. The complex, which precipitated as a microcrystalline powder, was filtered and dried under vacuum at room temperature for 24 h. The powder corresponded to a 1 : 2

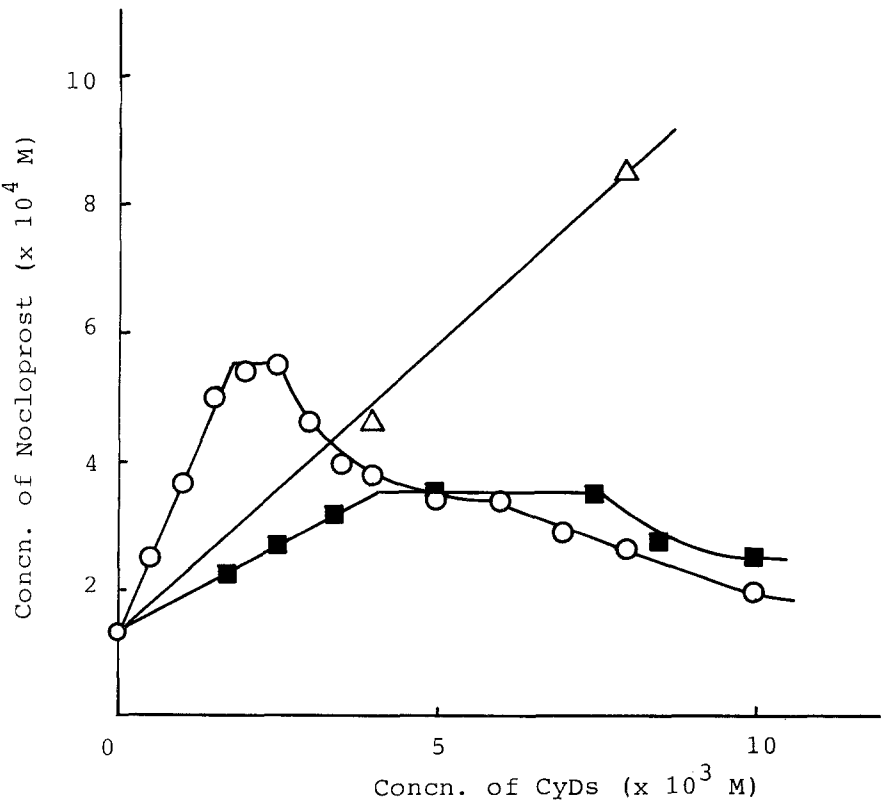

Fig. 1. Phase solubility diagrams of nocloprost–CyD systems in water at 25°C. △: α-CyD, ○: β-CyD, ■: γ-CyD.

guest : host molar ratio of the nocloprost–β-CyD complex. In a similar manner, the solid γ-CyD complex was prepared and the molar ratio was ascertained to be 1 : 2.

2.6. RELEASE STUDIES

Release rates of nocloprost from the CyD complex in powder and tablet form were measured by a dispersed amount method [10] and a paddle method, respectively. Powder: an excess amount of the complex powder (equivalent to 8 mg of the drug, < 100 mesh) was placed in 25 mL of water in a dissolution cell which was kept at 25°C, and the dissolution medium was stirred at 57 rpm. In the case of nocloprost alone, a hexane solution containing the drug was placed in the dissolution cell and evaporated to dryness, because nocloprost is a viscous oil. Tablet: the sample powder (53.4 mg, < 100 mesh), containing nocloprost (8 mg, diluent: lactose) or an equivalent amount of the complex, was compressed into a cylindrical tablet (diameter: 0.5 mm) at a pressure of 500 kg/cm^2. The tablet was placed in 25 mL of Japanese Pharmacopoeia XI (JP XI) first fluid (2.0 g NaCl and 24 mL of 10% HCl in 1000 mL water, pH about 1.2) or JP XI second fluid (250 mL of 0.2M KH_2PO_4 and 118 mL of 0.2N NaOH in 1000 mL of water, pH about 6.8) at 37°C and stirred at 150 rpm by a paddle.

At an appropriate interval, a 0.5 mL of solution was sampled by a pipet with a cotton plug and the drug was assayed by HPLC. The cumulative dilution caused by sampling was corrected by replacing the sample with an equal volume of the original medium.

3. Results and Discussion

3.1. INCLUSION COMPLEXATION IN AQUEOUS SOLUTION

The complexing behavior of nocloprost with three CyDs in water was studied by the solubility method [9]. The results are shown in Figure 1. In the case of α-CyD, the solubility of nocloprost increased in a linear fashion as a function of α-CyD concentration (up to 0.02M α-CyD), and the resulting solubility curve can be classified as type A_L [9], which may be due to the partial inclusion of nocloprost into the smaller α-CyD cavity. On the other hand, β-CyD as well as γ-CyD showed typical *B*s-type solubility curves [9], where the initial ascending portion is followed by a plateau region and then a decrease in the total concentration of nocloprost with precipitation of a microcrystalline complex. In the case of the β-CyD system, the phase solubility diagram could not be explained on the basis of a simple stoichiometric relationship. For example, a shoulder was observed at about 7×10^{-3}M β-CyD in the descending curve of the diagram. Furthermore, the stoichiometry of the β-CyD complex, which was estimated by analyzing the length of the plateau region [9], was inconsistent with that determined from the chemical analysis of the solid complex, i.e. 1 : 1 and 1 : 2 (guest : host), respectively. According to an examination of the Corey–Pauling–Koltun (CPK) model, the molecular dimension of nocloprost seemed to be too large to be entirely included within one CyD cavity. Therefore, it is reasonable to assume that at least one complex with a stoichiometry of more than 1 : 1 may be formed, in particular at a higher CyD

concentration range. In order to gain insight into the stoichiometry of the β-CyD system, the solid material which precipitated beyond the plateau region was analyzed. The chemical analysis of the nocloprost-β-CyD system gave the following results for $[CyD]_t$ (the concentration of β-CyD at which the solid material was isolated) and Xn (the molar ratio of β-CyD/nocloprost in the solid): 4.0×10^{-3}M, 1.4; 5.0×10^{-3}M, 1.7; 6.0×10^{-3}M, 1.8; 7.0×10^{-3}M, 1.9; 8.0×10^{-3}M, 1.95; 9.0×10^{-3}M, 2.0; 10.0×10^{-3}M, 2.0; 15.0×10^{-3}M, 2.0. These data indicate that the formation of the 1 : 2 complex of nocloprost and β-CyD predominates at the β-CyD concentration above 1×10^{-2}M, while the 1 : 1 complex may be formed at the initial increasing portion of the solubility diagram. In the case of the γ-CyD system, on the other hand, only the 1 : 2 solid complex precipitated in the γ-CyD concentration range of $5 \times 10^{-3} \sim 15 \times 10^{-3}$M, which was in accord with the stoichiometry estimated from the length of the plateau region of the diagram [9]. The one guest/two host inclusion complexation has been reported for the prostaglandin E_1–γ-CyD system [7] and 16,16-dimethyl-*trans*-Δ^2-prostaglandin E_1 methyl ester–β-CyD system [5].

The apparent stability constants (K') of the complexes were calculated, in term of Equation (1), using the data of the initial ascending portion of the solubility diagrams where the 1 : 1 complex may be formed predominantly. The K' value of the α-CyD complex was calculated, using at least seven values obtained in the α-CyD concentration range of $0 \sim 0.02$M. Table I summarizes the K' values at 15, 25 and 35°C.

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

As shown in Figure 2, van't Hoff plots of the K' values were linear in the temperature range employed, from which the thermodynamic parameters were calculated, and the results listed in Table I. The cavity size dependency of the guest–host interaction was clearly reflected in the magnitude of the K' values ($\beta > \alpha > \gamma$-CyDs).

In thermodynamic parameters all the enthalpy changes (ΔH) were negative, while the entropy changes (ΔS) were different in each case. The magnitude of these parameters is in the range observed for other CyD complexations [11]. The isoequilibrium temperature was estimated to be 250 K from the linear plot of ΔH versus ΔS (correlation coefficient = 0.99) [12]. This compensation rule is generally found in CyD complexation in aqueous solutions, suggesting that water molecules may play an important role in the complexation of nocloprost with CyDs

Table I. Apparent stability constants and thermodynamic parameters for complexations of nocloprost with α-, β-, and γ-CyDs.

System	Stability constant, K' (M^{-1})			ΔG	ΔH	ΔS
	15°C	25°C	35°C	(kJ/mol)	(kJ/mol)	(J/K mol)
α-CyD	1650	1130	840	−17.49	−25.56	−27.20
β-CyD	2900	2350	2000	−19.25	−14.02	17.57
γ-CyD	980	620	440	−16.05	−30.29	−47.70

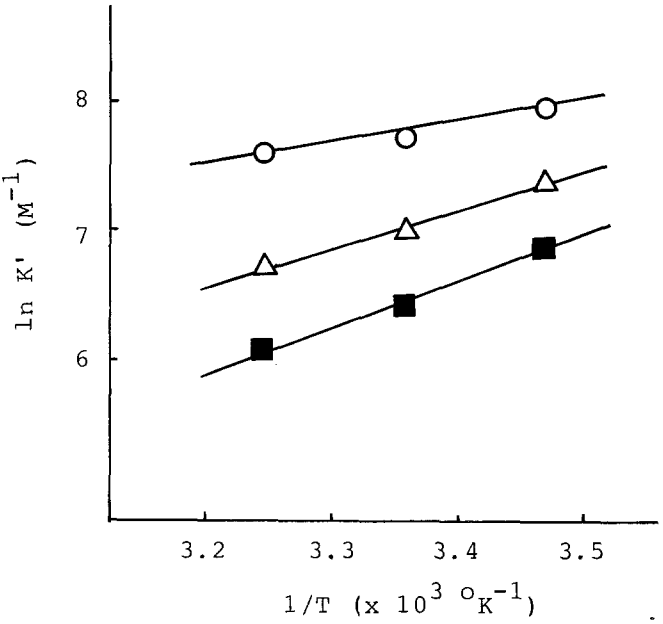

Fig. 2. Van 't Hoff plots of stability constants for nocloprost-CyD complexes. △: α-CyD complex, ○: β-CyD complex, ■: γ-CyD complex.

[13]. The positive ΔS value obtained for the β-CyD complex indicates that a number of ordered water molecules around the guest molecule and in the CyD cavity are set free due to the tight inclusion, i.e. typical hydrophobic interaction. On the other hand, the α- and γ-CyD complexes showed the unfavorable ΔS changes. This may be due to the lesser disordering of water molecules which are released from nocloprost and CyDs, since their K' values were smaller than that of β-CyD complex. Furthermore, the ordering of some water molecules may be facilitated around the guest and the CyD molecules, due to the partial inclusion of α-CyD and the loose inclusion of γ-CyD.

3.2. MODE OF INCLUSION

The ^{13}C-NMR technique and powder X-ray diffractometry were employed, in order to assess the inclusion mode of nocloprost–CyD complexes in aqueous solution and in the solid state, respectively.

Figure 3 shows the changes in ^{13}C-NMR chemical shifts of nocloprost upon complexation with β- and γ-CyDs. The NMR peaks of nocloprost were assigned by consulting the literature values of the NMR study of prostaglandins [14–17]. ^{13}C-NMR peaks of prostaglandins are known to shift up-field when they are located in a hydrophobic environment [14–17]. In the present system, the peaks tended to shift up-field, particularly in the case of β-CyD, suggesting that nocloprost is embedded in the CyD cavity. However, it was difficult to explain the magnitude and direction of the chemical shift changes simply in terms of hydrophobic interaction, because some carbons shifted to low-field. According to NMR spectroscopic [14,

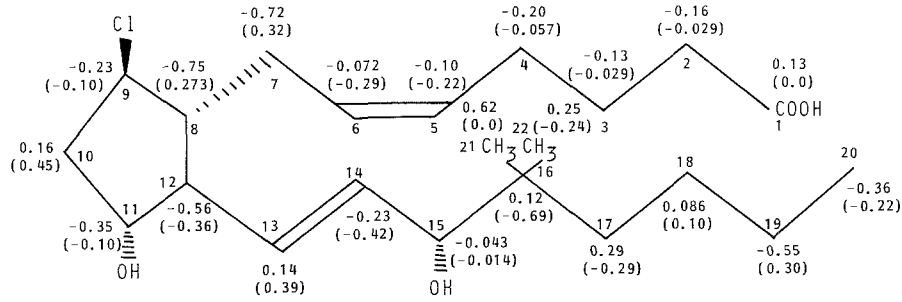

Fig. 3. ^{13}C-NMR chemical shift changes of nocloprost (0.02M) following the binding to β- and γ-CyDs (0.02M) in 0.1M borate buffer (pH 9.3) at 25°C. A chemical shift change in the downfield direction is expressed as a positive value and an opposite change is expressed as a negative value. Values in parenthesis are the γ-CyD-induced chemical shift changes.

15] and X-ray crystallographic studies [18], prostaglandin molecules are made up of three moieties, from a dynamic point of view, i.e. an alkyl moiety (C_1–C_6) in the α-chain, a five-membered ring and its neighborhood (C_7–C_{14}) and a terminal alkyl moiety (C_{15}–C_{20}) in the ω-chain. Therefore, by dividing the nocloprost molecule into the three parts, the absolute value of their chemical shift changes was considered, in order to assess the inclusion mode of CyDs. The averages of the chemical shift changes were as follows; 0.132 ppm (C_1–C_6), 0.393 ppm (C_7–C_{14}) and 0.290 ppm (C_{15}–C_{22}) for the β-CyD system and 0.104 ppm (C_1–C_6), 0.302 ppm (C_7–C_{14}) and 0.231 ppm (C_{15}–C_{22}) for the γ-CyD system. The relatively larger change in chemical shifts was observed around the five-membered ring and the terminal alkyl moiety in the ω-chain of nocloprost, while the change was smaller in the α-chain. These results suggest that β- and γ-CyDs preferentially include the five-membered ring as a main site, because this moiety is more hydrophobic due to the presence of the chlorine atom. Furthermore, one more CyD seems to include the alkyl moiety of the ω-chain as a second site. The inclusion mode of the complexes is proposed in Figure 4. In this inclusion mode, the five-membered ring may be inserted more deeply into the γ-CyD cavity, when compared with β-CyD, since the larger changes of the

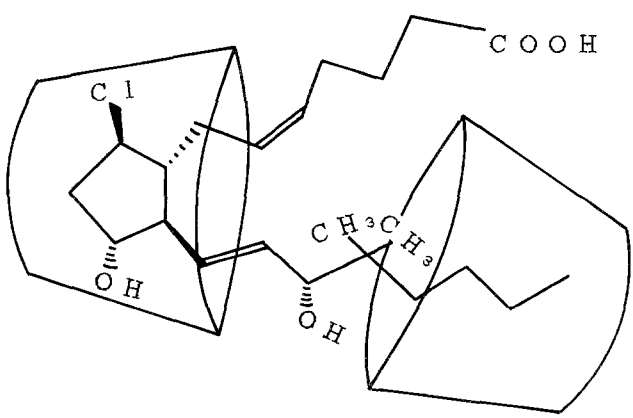

Fig. 4. Proposed inclusion mode for the nocloprost-β– and –γ-CyD complexes in water.

chemical shifts were observed up to the C_5, C_6, C_{13} and C_{14} carbons. Of course, nocloprost is more tightly included within β-CyD than γ-CyD, as is apparent from the magnitudes of the K' value and the chemical shift change. ^{13}C-Nuclear relaxation measurements are in progress to assess the molecular motion of nocloprost within the β- and γ-CyD cavities.

Figure 5 shows the powder X-ray diffraction patterns of the solid complexes of nocloprost with β- and γ-CyDs. The solid complexes were prepared on the basis of the *Bs*-type phase solubility diagram [9] (see the Experimental section), i.e. the solid material precipitated above the CyD concentration of 1×10^{-2}M is the solid complex with a stoichiometry of 1 : 2 (guest : host).

As shown in Figure 5, the diffraction patterns of both complexes were apparently different from those of β- and γ-CyDs, indicating a constitution of a new solid phase. The diffraction peaks at $2\theta = 12.2$, 14.8, 15.9 and 16.7° characteristic of γ-CyD disappeared and new peaks appeared at $2\theta = 6.1$, 10.6, 12.2, 16.1 and 22.2° in the γ-CyD complex. The diffraction pattern of the γ-CyD complex showed a hexagonal type of pattern similar to that observed for the *n*-propanol–γ-CyD complex [19]. The *n*-propanol–γ-CyD complex is known to have a channel structure with a hexagonal close packing of the cylinders that stacks the γ-CyD molecules coaxially. Therefore, the diffraction pattern of the complex was indexed on the basis of a two-dimensional hexagonal unit cell having $a = b = 33.46$ Å, where the *d*-spacing for the 200 reflection peak on the diagram was used to calculate the unit cell dimensions [19]. As shown in Table II, the calculated *d*-spacing was in excellent agreement with that observed. This suggests that nocloprost is included within the cylindrical channels formed by coaxial alignment of γ-CyD molecules, although the orientation of the guest molecule in the channel cannot be determined from the powder X-ray diffractogram. In the case of the β-CyD complex, it was difficult to index the diffraction peaks in terms of a hexagonal system, suggesting that the packing mode of the β-CyD complex may be different from that of the γ-CyD complex.

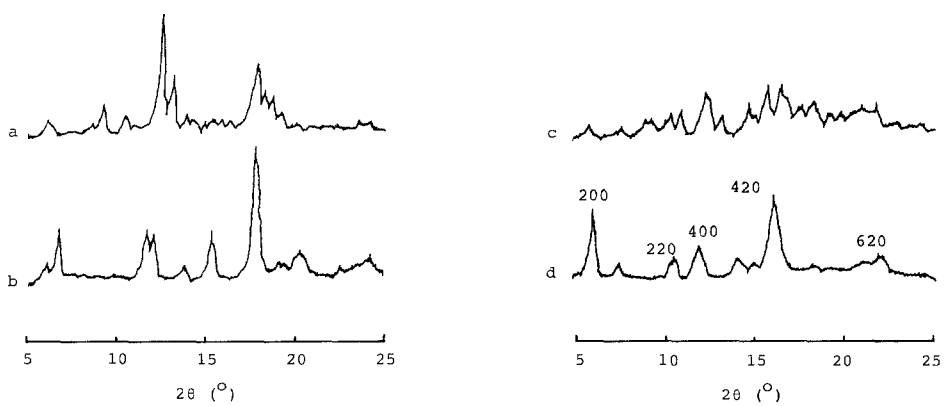

Fig. 5. Powder X-ray diffraction patterns of nocloprost–CyD systems. a: β-CyD; b: β-CyD complex; c: γ-CyD; d: γ-CyD complex.

Table II. Diffraction data for the nocloprost–γ-CyD complex.

hkl[a]	*d* (Å)	
	Observed	Calculated
200	14.49	14.49
220	8.35	8.37
400	7.25	7.24
420	5.50	5.47
620	4.04	4.02

[a] Hexagonal indices.

3.3. IMPROVEMENT OF THERMAL STABILITY AND RELEASE RATE

Figure 6 shows DSC and TG thermograms of the solid complex of nocloprost with β-CyD, in comparison with that of each component. The endo- and exothermic peaks due to the decomposition of nocloprost were observed around 160°C in the DSC thermogram, accompanied by a weight decrease in the TG thermogram. In sharp contrast, the β-CyD complex showed no appreciable peaks and weight decrease around the decomposition temperature of nocloprost. Similar phenomena were observed for the γ-CyD complex. These results suggest that the complexed form of nocloprost is highly stable in comparison with the drug itself.

Figure 7 shows the dissolution profiles of nocloprost and its β- and γ-CyD complexes in water at 25°C. It is apparent that the complexes dissolved much more rapidly than the drug itself. The rapid dissolution of the β-CyD complex may be due to the increase in solubility of the drug, as expected from Figure 1.

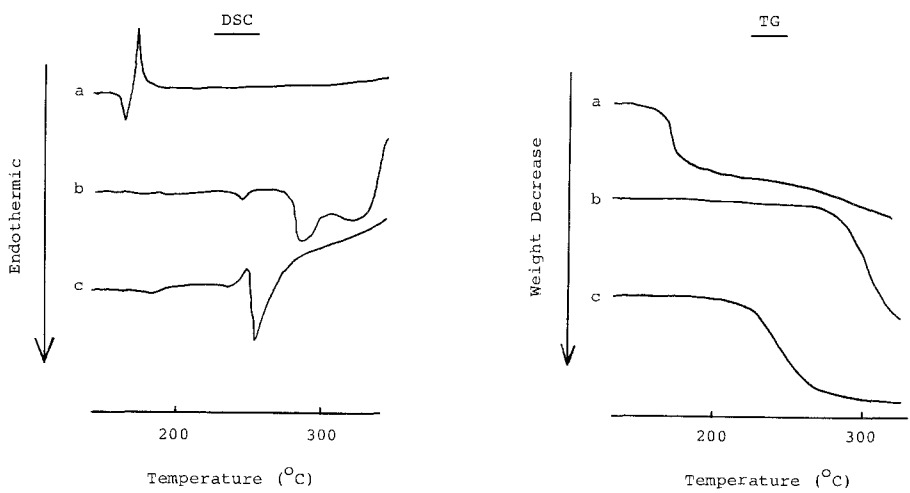

Fig. 6. DSC and TG thermograms of nocloprost–β-CyD systems. a: nocloprost; b: β-CyD; c: the β-CyD complex.

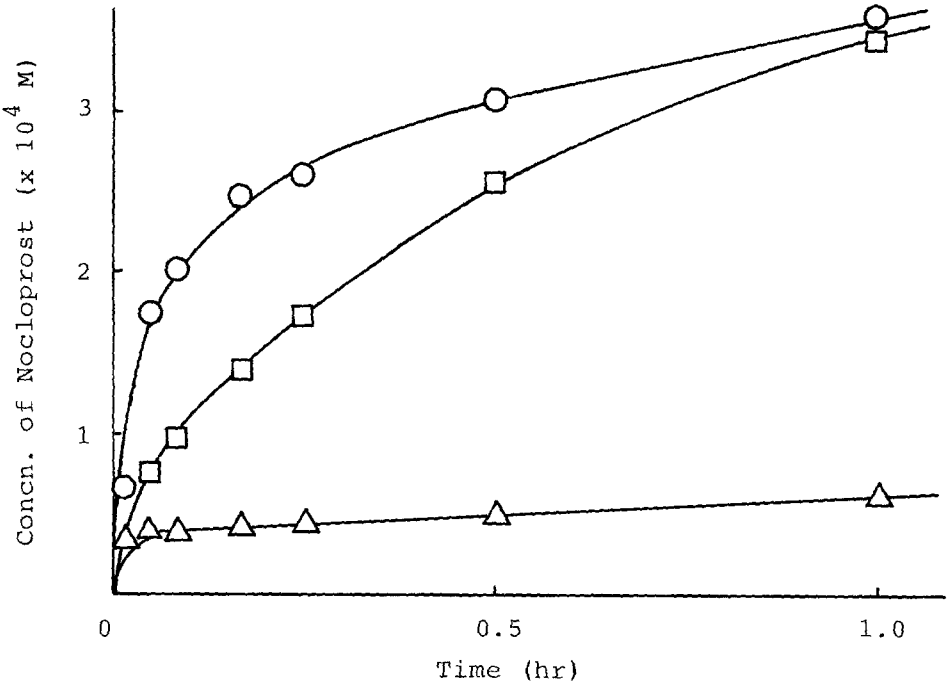

Fig. 7. Dissolution profiles of nocloprost and its CyD complexes in water at 25°C. △: nocloprost, ○: β-CyD complex, □: γ-CyD complex.

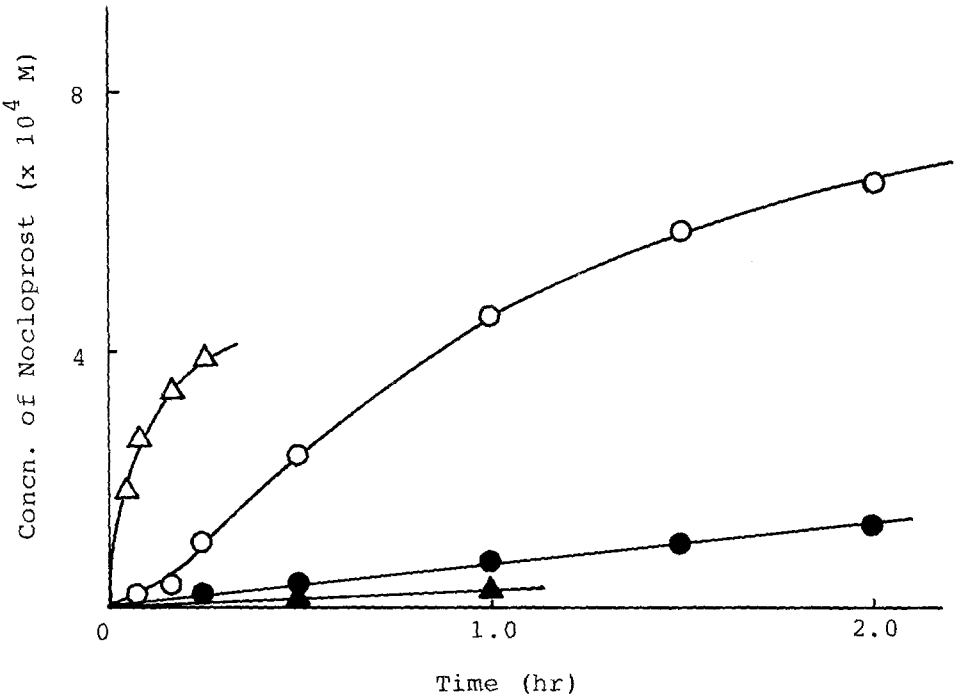

Fig. 8. Release profiles of nocloprost from tablets containing nocloprost and its β-CyD complex in artificial gastrointestinal fluid (pH 1.2 and 6.8) at 37°C. ▲: lactose tablet at pH 1.2; ●: complex tablet at pH 1.2; △: lactose tablet at pH 6.8; ○: complex tablet at pH 6.8.

Figure 8 shows the release behavior of nocloprost from the compressed tablets containing nocloprost (diluent : lactose) or its β-CyD complex in artificial gastrointestinal fluids. The release rate of nocloprost increased with increasing pH of the medium, owing to the ionization of the terminal carboxyl moiety of the drug (pK_a: about 5.0) [20]. Upon complexation with β-CyD, the amount of nocloprost released was apparently increased in both media, due to the increase in solubility. The rapid release of the drug from the lactose tablet at pH 6.8 may be attributable to the rapid disintegration of the tablet in the artificial gastrointestinal fluid.

All the data clearly indicate that β-CyD is the best host among the three natural CyDs for improving the solubility and dissolution rate of nocloprost. Furthermore, the solid form of the inclusion complex should facilitate drug formulation, which is difficult with uncomplexed nocloprost since it is a viscous oil.

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