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Differences in the enhancing effects of water soluble β-cyclodextrins on the release of ethyl 4-biphenylyl acetate, an anti-inflammatory agent from an oleaginous suppository base

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Summary

The solid complexes of ethyl 4-biphenylyl acetate (EBA), an anti-inflammatory agent with β-cyclodextrin (β-CyD), heptakis-(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD) in a molar ratio of 1:2 were prepared and their in vitro releases from an oleaginous suppository base were examined. The release of EBA from the suppository base was significantly enhanced by the complexations with β-CyDs in the order of β- < DM-β- < HP-β-CyD, but in a differential manner. The complexes of EBA with DM-β- and HP-β-CyDs proved to partially dissociate into each component in the base, the former being more pronounced. In contrast to β- and HP-β-CyDs, the coexistence of EBA with DM-β-CyD in the base gave almost the same enhancing effect on the release of EBA as the complex does. The relative potency of β-CyDs to enhance the dissolution rate of EBA and to retain the drug in aqueous phase was in the order of β- < HP-β- < DM-β-CyD, which clearly fits the sequence of magnitude of stability constants of the complexes. The rather small enhancing effect of DM-β-CyD on the release of EBA than HP-β-CyD may be due to the considerable dissociation of the complex in the base, together with the increased viscosity of the suppository base. The present data suggest that HP-β-CyD is particularly useful in enhancing the release of EBA from the oleaginous suppository base tested.

Introduction

The usefulness of molecular entrapment of drugs with β-cyclodextrin (β-CyD) in pharmaceutical formulations has been realized (Szejtli, 1985; Uekama and Otagiri, 1987). The widespread uses of β-CyD as a drug carrier, however, are sometimes restricted by its low aqueous solubility (1.8 g/100 ml at 25 °C). The relatively low solubility of β-CyD in water may be ascribed to its stable crystal lattice, which consists of a hydrogen bonding network between the hydroxyl groups of β-CyD and water molecules (Saenger, 1984). Several approaches have been proposed to overcome this problem including alkylation and hydroxyalkylation of the hydroxyl groups of β-CyD (Szejtli, 1983; Pitha et al., 1988). These modifications may

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disrupt the hydrogen bonding network and/or convert crystalline β-CyD into amorphous mixtures of isomers; both of these factors could contribute to increased solubility of such derivatives. Among these derivatives, heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD) have been extensively studied and gained acceptance in pharmaceutical applications (Uekama and Irie, 1987; Yoshida et al., 1988).

As reported in our previous paper (Uekama et al., 1986), β-CyD has the potential to improve the rectal bioavailability of 4-biphenylylacetic acid (BA), an anti-inflammatory agent by enhancing the release of BA from the oleaginous suppository base in rats. In these continuing investigations, we examined the possible utility of water soluble β-CyDs as a more potent enhancer for the drug release from the rectal suppository. Ethyl 4-biphenylyl acetate (EBA), a lipophilic prodrug of BA (Shoji et al., 1986), was chosen here as a model compound because of its insufficient release from the suppository base and lower rectal bioavailability. This paper deals with the effects of complexation with three kinds of β-CyDs (β-, DM-β-, and HP-β-CyDs) on the in vitro release of EBA from an oleaginous suppository base, Witepsol H-5, and discusses the different mechanisms for the enhancement of drug release by β-CyDs with emphasis on the equilibrium dissociation of the complexes in the suppository base and in the aqueous phase.

Materials and Methods

Materials

EBA (Nippon Lederle Co., Tokyo), β- and HP-β-CyDs (Nihon Shokuhin Kako Co., Tokyo), DM-β-CyD (Toshin Chemical Co., Tokyo) and Witepsol H-5 and S-55 (Dynamit Nobel Chemicals, Troisdorf-Oberlar, F.R.G.) were used as supplied. The degree of substitution in HP-β-CyD was confirmed to be 5.8 by mass and nuclear magnetic resonance spectrometries (Pitha et al., 1986). Other materials were of reagent grade; deionized double distilled water was used.

Apparatus

The powder X-ray diffraction patterns were taken by a Rigaku Denki Geiger Flex 2012 diffractometer (Tokyo, Japan) under the following conditions: X-ray, Ni-filtered Cu-K_α radiation; voltage, 30 kV; current, 20 mA; detector, a proportional counter; time constant, 1 s; scanning speed, 1°/min. The melting points of the suppositories were measured by a Rigaku Denki model TAS 100 thermal analysis station (Tokyo, Japan) at a scanning rate of 10 °C/min. The water contents of the complexes were measured by a Kyoto Electronics MKA-3P Karl-Fischer moisture meter (Kyoto, Japan) with an accuracy of ±0.3%. The apparent viscosities of the suppositories were determined by a Contraves AG Low Shear 30 rotational rheometer (Zurich, Switzerland) with the rate of shear ranging from 0.081 to 0.277 s^{-1}.

Solubility studies

Solubilities were measured using procedures established by Higuchi and Connors (1965). Excess amounts of EBA were added to aqueous solutions containing various concentrations of β-CyDs, and the solutions were shaken at constant temperature (15–45 °C). After equilibrium was reached (about 10 days), an aliquot was centrifuged and pipetted through a cotton plug. The filtered aliquots were analyzed spectrophotometrically (model UV-160; Shimazu Co., Kyoto) at 253 nm. The apparent stability constant of the complex, K', assuming that a 1 : 1 complex is initially formed, was calculated from the initial straight-line portion of the solubility diagrams; the K' value for the HP-β-CyD complex is only approximate since HP-β-CyD is a mixture with a distribution of the degree of substitution (Pitha et al., 1988).

Preparation of solid complexes

The solid complexes of EBA with β-CyDs in a molar ratio of 1 : 2 were prepared according to the method reported previously (Tsuruoka et al., 1981). For example, EBA (1 g) and β-CyD (9.5 g) were triturated with a small amount of water (2 ml). The slurry was further kneaded thoroughly for 60 min, and then dried under reduced pressure at room temperature for 2 days. The water contents

of the complexes were 4.2, 1.2, and 1.5% for the β-, DM-β- and HP-β-CyD complexes.

Suppository release studies

The suppository (1 g) was prepared by dispersing EBA or its β-CyD complexes in Witepsol H-5, which was melted at 45 °C, to yield a drug concentration of 2.26% (w/w). The melt was then poured into aluminum suppository molds (Erweka, Frankfurt, F.R.G.) and allowed to cool at 25 °C. EBA remained stable during the suppository preparation, which was confirmed by high performance liquid chromatographic analysis (HPLC). The powder X-ray diffraction patterns of the complexes after being kept at 50 °C for 2 h were the same as those shown in Fig. 2. By the differential thermal analysis, an endothermic peak due to the melting of EBA was observed at 23 °C, while the complexes showed no appreciable peak at temperature below 50 °C. These observations clearly indicate that the complexes are stable on such heating during the preparation of the suppository.

The release rates of EBA and β-CyDs from the suppositories were measured using a suppository release apparatus (Toyama Sangyo Co., Osaka, Japan) according to the method of Muranishi et al. (1980). Each suppository was placed in the cylindrical chamber, which was lined from the inside with a membrane filter (pore size, 3.0 μm; Millipore, Bedford, MA) as a barrier for diffusion of the suppository base and lowered into a flask containing a normal saline (500 ml). The release phase was stirred with a magnetic stirrer at 100 rpm at 37 °C. The rotation rate of the steel rod in the suppository chamber was 25 rpm. The liquefaction time was defined as the moment in which the suppository has entirely liquefied in the chamber. Under the present conditions, the liquefaction times of the standard 2 g suppositories containing EBA or its β-CyD complexes were around 15 min. The liquefaction times of the 1 g suppositories listed in Table 2 were shorter than those of the standard suppositories, probably due to the specific surface area. At an appropriate interval, a 3 ml sample was withdrawn from the release phase and assayed for EBA as described above. The amounts of β-CyDs in the release phase were determined spectrophotometrically at 620 nm by the anthrone-sulfuric acid method (Dreywood, 1946). The cumulative dilution caused by sampling was corrected for by replacing the sample by equal volumes of the original medium.

Dissolution studies

The dissolution behavior of EBA and its β-CyD complexes in water was examined according to the dispersed-amount method (Nogami et al., 1969). An amount equivalent to 40 mg of EBA was weighed and put in a dissolution cell. The dissolution medium (100 ml of water) was maintained at 37 °C and stirred at 91 rpm. At an appropriate interval, 0.5 ml of solution was sampled by a pipet with a cotton plug, and assayed for EBA as described above. A correction was applied for the cumulative dilution.

Solubilities of EBA and β-CyDs in suppository base

The suppositories (1 g) containing EBA and its β-CyD complexes were melted at 37 °C and filtered through a hydrophobic membrane filter unit (pore size, 0.2 μm, Dismic-25, Toyo Roshi Kaisha, Tokyo). EBA and β-CyDs in the filtrate were extracted with 6.0 ml of methanol, and subjected to the HPLC analysis. The HPLC conditions for EBA were as follows: pump and detector, Hitachi 655A-11 type with 655A UV monitor (Tokyo); column, ERC-ODS-1161 (3 μm, 6φ × 100 mm, Erma Optical Works, Tokyo); mobile phase, methanol-0.1 M acetic acid (3 : 1 v/v%); flow rate, 1.0 ml/min; detection, 255 nm. The HPLC conditions for DM-β- and HP-β-CyDs were almost the same as those for EBA but with the following differences: detector, Shodex SE-51 refractive index detector (Showadenko, Tokyo, Japan); column, YMC pack R-ODS-5 (5 μm, 4.6φ × 250 mm, Yamamura Chemical, Kyoto, Japan); mobile phase, water-acetonitrile (9 : 11 v/v%) for DM-β-CyD and water-methanol (21 : 4 v/v%) for HP-β-CyD. β-CyD in the filtrate was extracted with 6.0 ml of water, and assayed by HPLC according to the method of Koizumi et al. (1985).

Measurement of partition coefficients

The apparent partition coefficient (PC) of EBA was determined after shaking 10 ml of saline containing 3.8 mM β-CyDs and 10 mg of the

suppository base containing 226 μg EBA for 24 h at 37 °C. To analyze the trace amount of EBA in the aqueous phase, a fluorescence spectrophotometer (Hitachi 650-10 LC, Tokyo, Japan) was employed: detection, 285 nm for excitation and 320 nm for emission. The other HPLC conditions were the same as described above. The PC was defined as the ratio of the equilibrium concentration of EBA in the lipid phase (μg/mg) to that in the aqueous phase (μg/ml).

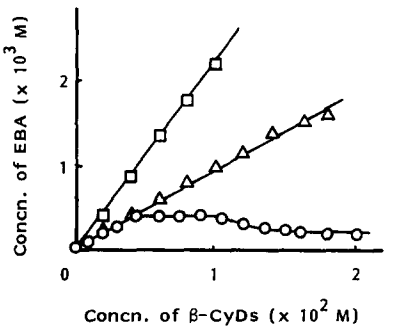

Fig. 1. Phase solubility diagrams of EBA-β-CyD systems in water at 37 °C. (○) β-CyD, (□) DM-β-CyD, (△) HP-β-CyD.

Results and Discussion

Complexations of EBA with β-CyDs

Fig. 1 shows a typical example of the phase solubility diagrams obtained for EBA and three β-CyDs in water at 37 °C. The solubility plot for β-CyD gave a B_S type solubility curve (Higuchi and Connors, 1965); the precipitation of the microcrystalline complex occurred at high β-CyD concentrations. The 1 : 2 stoichiometry observed for EBA with β-CyD on the basis of the data in the plateau region was in good agreement with that obtained by isolation and analysis of the crystalline complex. In sharp contrast, the solubility of EBA increased remarkably in a linear fashion as a function of DM-β- and HP-β-CyD concentrations, showing a feature of A_L type complex (Higuchi and Connors, 1965); i.e. highly water soluble complexes may exist in the solution. In the case of DM-β-CyD, however, a microcrystalline complex began to precipitate with a rise in temperature, due to the decrease in solubility of DM-β-CyD (Uekama and Irie, 1987). A 1 : 2 stoichiometry was also ascertained for the EBA-DM-β-CyD complex by the chemical analysis of the isolated solid complex.

As shown in Table 1, the apparent stability constant of the complex, K', increased in the order of β- < HP-β- < DM-β-CyD over the temperature range employed. The thermodynamic parameters were extracted from the temperature dependency of the K' values. The enthalpy changes were negative, while the entropy changes were considerably different in each case; all the interactions are enthalpy-driven and are not a result of a simple hydrophobic process. The large negative enthalpy changes indicate the strong host-guest interactions (Matsui et al., 1979). The variation in the entropy changes may arise from the difference in the behavior of the solvent, water on complex formation. The large negative entropy changes obtained for the DM-β- and HP-β-CyD systems could be accounted for by the restriction on conformational flexibility of the macrocyclic host molecule as well as the decrease in the freedom of the guest molecule (Harata et al., 1988). In addition, other factors such as the micellar dissociation of host molecule should be considered for the changes in these parameters, since DM-β- and HP-β-CyDs are surface active (Uekama et al.,

TABLE 1

Apparent stability constants (K') and thermodynamic parameters for complexation of EBA with β-CyDs

System	K' (M^{-1})				ΔG (298 K)	ΔH	ΔS (298 K)
	15 °C	25 °C	37 °C	45 °C	(kcal · mol^{-1})	(kcal · mol^{-1})	(cal · K^{-1} · mol^{-1})
β-CyD	3330	3050	2850	1630	−4.75	−4.34	1.37
DM-β-CyD	31500	12500	10000	5700	−5.59	−9.66	−13.66
HP-β-CyD	7950	4200	3000	1650	−4.94	−8.91	−13.32

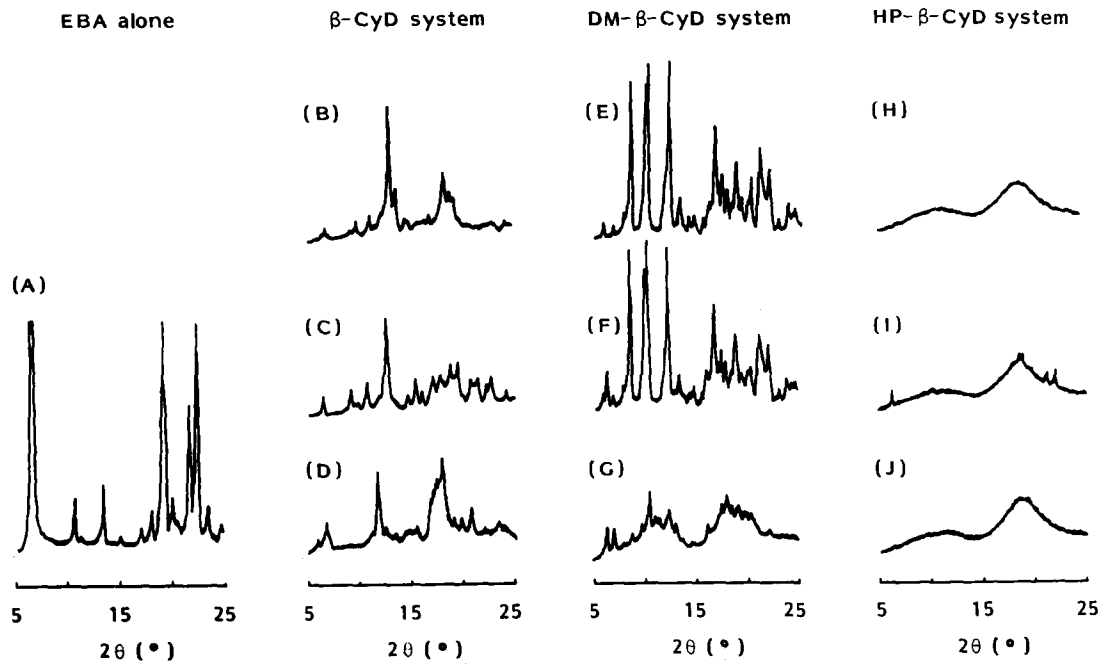

Fig. 2. Powder X-ray diffraction patterns of EBA-β-CyD systems. (A) EBA alone, (B) β-CyD alone, (C) physical mixture of EBA and β-CyD, (D) complex of EBA with β-CyD, (E) DM-β-CyD alone, (F) physical mixture of EBA and DM-β-CyD, (G) complex of EBA with DM-β-CyD, (H) HP-β-CyD alone, (I) physical mixture of EBA and HP-β-CyD, (J) complex of EBA with HP-β-CyD.

1985). In these systems, the greater negative enthalpy change, however, not only compensates for the effects of such a negative entropy change, but also makes the complex more stable than the β-CyD complex.

All the solid complexes of EBA with β-CyDs in a molar ratio of 1 : 2 were prepared by the kneading method (Tsuruoka et al., 1981) and used in the following studies. Attempts to crystallize the HP-β-CyD complex from aqueous solutions were unsuccessful. Fig. 2 shows the powder X-ray diffraction patterns of the EBA-β-CyD systems. The diffraction patterns of the physical mixtures were simply a superposition of each component, while those of the complexes were apparently different from each component and constituted a new solid phase. It is interesting to note that the crystalline EBA was converted completely into the amorphous form by the complexation with HP-β-CyD (Fig. 2J).

Drug release from suppository base

Fig. 3A shows the release profiles of EBA from the Witepsol H-5 suppositories containing EBA or its β-CyD complexes in saline at 37 °C. The release of EBA was remarkably enhanced by the complexations with β-CyDs; the order of the initial release rate was β- < DM-β- < HP-β-CyD complex. It is of interest that the enhancing effect of the physical mixture of EBA and DM-β-CyD was almost the same as that of the complex, whereas no remarkable enhancement was obtained for the β- and HP-β-CyD systems (Fig. 3B). It seems likely that the use of a suppository base

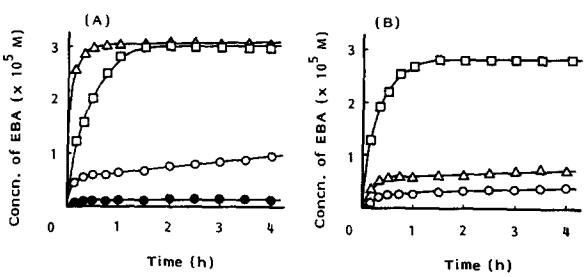

Fig. 3. Release profiles of EBA from oleaginous suppositories containing complexes (A) and physical mixtures (B) of EBA with β-CyDs in saline at 37 °C. (●) EBA alone, (○) β-CyD system, (□) DM-β-CyD system, (△) HP-β-CyD system.

which contains a surfactant provides the enhanced release of EBA similarly to DM-β-CyD. However, the level of drug release from Witepsol S-55, one of the surfactant bases, was found to be only twice that from Witepsol H-5 (data not shown); the use of the surfactant base was not so effective as the β-CyD complexations.

To gain insight into the mechanism of the enhanced release of EBA, the effects of the β-CyD complexations on the rate-limiting factors of the drug release were investigated. Schoonen et al. (1988) have stressed that the drug release from the fatty suppository is characterized by the presence of the interface between the molten base and the surrounding fluid. The drug release consists of three consecutive processes; drug diffusion into the lipid/water interface, dissolution of the drug at the interface, and drug transport away from the interface (Schoonen et al., 1979; Crommelin and De Blaey, 1980).

The drug diffusion into the lipid/water interface is known to be influenced by the rheological property of the suppositories (Yoshino et al., 1981). As shown in Table 2, the viscosity of the suppository was increased by the additions of the β-CyD complexes in the order of HP-β- < β- ≪ DM-β-CyD, although there was no noticeable difference in the melting point and liquefaction time between these suppositories. The increased viscosity in the lipid phase seems to be unfavorable for the diffusion of the complexes, particularly in the case of the DM-β-CyD complex.

To evaluate how the complex exists in the suppository base, the solubility of each component of the complex in the molten base was measured at 37 °C. It is evident from Table 2 that EBA was completely dissolved in the base, while the solubility of EBA in the base was significantly decreased after complexation with β-CyDs, due to the hydrophilic nature of the complexes as expected from Fig. 1. The most extreme example is the β-CyD complex; the dissolved fraction of EBA was only less than 2% of the total amount, the major fraction existed as well-dispersed fine particles. This indicates that EBA exists mainly in a complexed form with β-CyD in the base. This hypothesis was supported by the observation of X-ray diffraction patterns of the β-CyD complex in the suppository base (Fig. 4). The diffraction pattern of the freshly prepared suppositories showed the metastable and amorphous layered structure (Laine et al., 1988) and the base itself showed no characteristic peak between 8 and 20° at 2θ. The diffraction pattern of the β-CyD complex in the base was obviously different from that of the physical mixture in which EBA was completely dissolved in the base (Fig. 4A and B). This pattern was similar to that of the complex itself (Fig. 3D), where the two characteristic peaks were observed around 12 and 18° at 2θ. The small difference between these patterns may be ascribed to some interactions of β-CyD with the constituents of the base such as trilaurin and triolein. It is reasonable to assume that β-CyD includes the long acyl chains of these lipids (Miyajima et

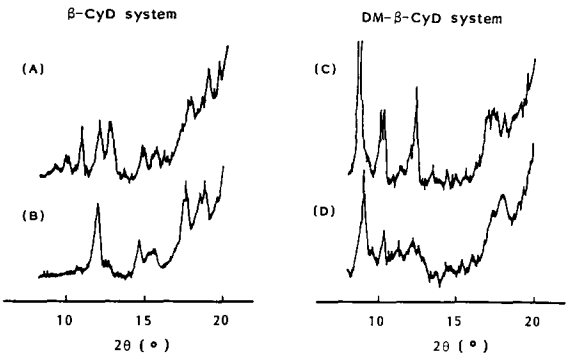

Fig. 4. Powder X-ray diffraction patterns of EBA-β-CyD systems in the suppository base. (A) Physical mixture of EBA and β-CyD, (B) complex of EBA with β-CyD, (C) physical mixture of EBA and DM-β-CyD, (D) complex of EBA with DM-β-CyD.

TABLE 2

Some physicochemical properties of the suppositories containing EBA or its β-CyD complexes

System	Melting point (°C)	Liquefaction time [a] (min)	Viscosity [a] (cP)
Without additives	36.3	8.1	175
EBA alone	36.4	8.0	130
β-CyD complex	36.5	7.2	689
DM-β-CyD complex	36.4	6.1	15105
HP-β-CyD complex	36.4	5.9	240

[a] At 37 °C.

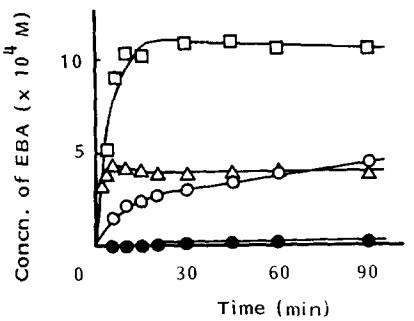

Fig. 5. Dissolution profiles of EBA or its β-CyD complexes in water at 37 °C, measured by dispersed amount method. (●) EBA alone, (○) β-CyD complex, (□) DM-β-CyD complex, (△) HP-β-CyD complex.

al., 1985); EBA may share the β-CyD cavity with these lipids to form higher order complexes.

In the case of the DM-β-CyD complex, considerable amounts of EBA and DM-β-CyD were dissolved in the base. The solubility ratio of EBA to DM-β-CyD was much higher than the molar ratio of the complex prepared. Similar tendency was observed to a lesser extent for the HP-β-CyD complex. In contrast to the β-CyD complex, the X-ray diffraction pattern of the DM-β-CyD complex in the base was somewhat different from that of the DM-β-CyD complex itself (Fig. 3G). These results indicate that the DM-β- and HP-β-CyD complexes partially dissociate into each component in the base, the former being more pronounced. It seems likely that these β-CyD derivatives have higher affinity to the fatty base and could interact more strongly with the constituents of the base than the parent β-CyD, due to their amphiphilic nature (Müller and Brauns, 1986). This might result in the expelling of EBA from the cavity of DM-β- or HP-β-CyD by the constituents of the base, and consequently the loss of the enhancing efficacy of the drug release.

It is well known that the dissolution of the drug in the aqueous side of the lipid/water interface is the rate-limiting step for the release of poorly water soluble drugs (Schoonen et al., 1979). Fig. 5 shows the dissolution profiles of EBA or its β-CyD complexes in water at 37 °C. It is apparent that all the complexes dissolved much more rapidly than EBA alone; the relative potency of β-CyDs was in the order of β- < HP-β- < DM-β-CyD, which clearly fits the magnitude of stability constants of the complexes. The enhanced dissolution rate of EBA may be due to the increase in solubility and wettability along with the decrease in crystallinity caused by inclusion complexation (Otagiri et al., 1984).

Fig. 6 shows the release profiles of β-CyDs from the suppositories containing EBA-β-CyD complexes in comparison to the release behavior of EBA. β-CyDs were completely released from

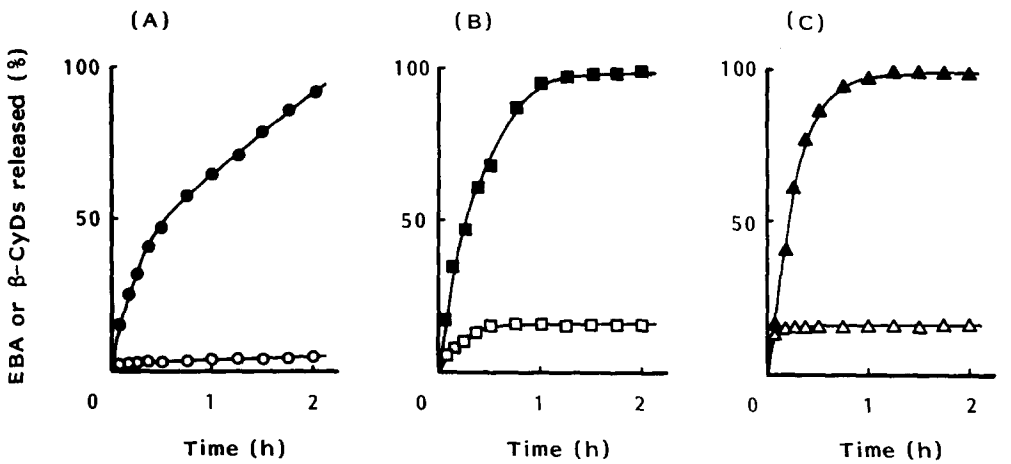

Fig. 6. Release profiles of EBA and β-CyDs from oleaginous suppositories containing EBA-β-CyD complexes in saline at 37 °C. (A) β-CyD complex, (B) DM-β-CyD complex, (C) HP-β-CyD complex. The open and closed symbols represent the release profiles of EBA and β-CyDs, respectively.

TABLE 3

Solubilities of EBA and β-CyDs in the oleaginous base and apparent partition coefficients of EBA between the oleaginous base and saline at 37 °C

	Solubility (mg/g)		Apparent partition coefficient [a]
	EBA	β-CyDs	
EBA alone	22.6 (100) [b]	–	> 4100
β-CyD complex	0.4 (1.8)	0.01 (0.01) [b]	7.2
DM-β-CyD complex	8.7 (38.5)	13.90 (5.54)	0.3
HP-β-CyD complex	1.8 (8.0)	2.40 (0.86)	5.7

[a] Ratio of equilibrium concentration of EBA in the oleaginous base (μg/mg) to that in the saline (μg/ml).
[b] The percentage to the total amount.

the base within 4 h and the release rate was much faster than that of EBA. It should be noted that the release of EBA was no longer observed after the depletion of β-CyDs from the base. The difference in the release profile between EBA and β-CyDs indicates that the uptake of EBA into the suppository base takes place following the dissociation of the complex at the lipid/water interface. As shown in Table 3, the apparent partition coefficient of EBA between the base and saline was decreased by the addition of β-CyDs, and corresponds to the magnitude of the stability constants of the complexes. This implies that the free form of EBA, which is in equilibrium with the complexed one, may be predominantly incorporated into the suppository base.

In conclusion, β-CyDs were found to significantly enhance the release of EBA from the oleaginous suppository base in the order of β- < DM-β- < HP-β-CyD in a differential manner. The rather small enhancing effect of DM-β-CyD on the release of EBA, compared with HP-β-CyD, may be due to the considerable dissociation of the complex in the base, together with the increased viscosity of the suppository.

The present data suggest that HP-β-CyD is particularly useful in enhancing the release of EBA from the oleaginous suppository base. An additional advantage of HP-β-CyD is its low toxicity (Pitha et al., 1988; Brewster et al., 1988), therefore this approach may hold promise for improving the rectal bioavailability of EBA.

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