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# Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrins

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## Summary

Hydroxyalkylated β-cyclodextrins (HA-β-CyDs), 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD), and hydroxyethyl-β-cyclodextrin (HE-β-CyD), were prepared and their physicochemical and biological properties and solubilizing abilities were studied and compared with those of parent β-cyclodextrin (β-CyD). HA-β-CyDs had much higher aqueous solubility (> 50 w/v%) and were less hygroscopic than the parent β-CyD. Their surface activities were between those of β-CyD and alkylated-β-cyclodextrins and were increased proportionately to their average degrees of substitution. The hemolytic activity (human erythrocytes) and local irritancy (rabbit muscle) of these compounds, and particularly of HE-β-CyD, were considerably less than those of natural cyclodextrin or dimethyl-β-cyclodextrin (DM-β-CyD). In contrast to surface activity, the hemolytic activity of HA-β-CyD decreased with the degree of substitution; possibly the difference in their ability to dissolve membrane components may be the reason. HA-β-CyDs were found to be powerful solubilizers of several drugs and no crystalline complexes were precipitated at high concentrations of solubilizer, a phenomenon which is often observed when β-CyD is used. HP-β-CyDs were somewhat better solubilizers than HE-β-CyDs and the preparations with the lower degrees of substitution were again better than those with the higher ones. The above data suggest that HA-β-CyDs are safer and more effective solubilizers for poorly water-soluble drugs than the parent cyclodextrin.

## Introduction

Chemically modified cyclodextrins have been utilized for the improvement of pharmaceutical properties, e.g. solubility, chemical stability, or bioavailability of drugs (Szejtli, 1982; Pitha et al., 1983; Uekama and Otagiri, 1987). Recently, hydrophilic derivatives of cyclodextrins such as HP-β-CyD or poly-β-CyD were found to have good solubilizing power and to lack toxicity (Müller and Brauns, 1985; Uekama et al., 1985; Pitha and Pitha, 1985; Pitha et al., 1986). In the present study, the relevant physicochemical properties of HP- and HE-β-CyDs, such as aqueous solubility, hygroscopic nature, optical rotation, and surface activity, were investigated and compared with those of parent cyclodextrins. To secure the safe use of HA-β-CyDs in parenteral formulation, the hemolytic activity against human erythrocytes and

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local irritating effects to muscular tissues were examined and compared with the natural and methylated cyclodextrins. Furthermore, the solubilization of several poorly water-soluble drugs by HA-β-CyD was studied.

## Materials and Methods

### *Materials*

β-Cyclodextrin was supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan). HP- and HE-β-CyDs with different degrees of substitution were prepared by condensation of β-cyclodextrin in aqueous alkali with propylene oxide and ethylene chlorohydrin, respectively, according to the method reported previously (Pitha and Pitha, 1985). The distribution and degree of substitution in HA-β-CyDs were evaluated by mass spectrometry (Fig. 1). The degree of substitution was calculated according to the formula:

$$\text{D.S.} = \Sigma(\text{peak height} \times \text{NS})/\Sigma\text{peak height}$$

NS: number of substitutions.

Other chemicals and drugs were from commercial sources, de-ionized double-distilled water was used.

### *Apparatus*

Optical rotation measurements: DIP-360 digital polarimeter (Jasco, Tokyo, Japan) with an accuracy of ±0.002°. Surface tension measurements: duNouy surface tensionmeter (Shimadzu Co., Kyoto, Japan) with an accuracy of $\pm 0.5\ \text{mN} \cdot \text{m}^{-1}$. Water content measurements: MKA-3P Karl-Fischer moisture meter (Kyoto Electronics Co., Kyoto, Japan) with an accuracy of ±0.3%. Secondary ion mass spectrometry (SIMS): double-focusing M-80B mass spectrometer (Hitachi, Tokyo, Japan) with xenon gas as a primary ion source and glycerol as a matrix; the primary and secondary ion accelerations were 8 and 3 kV, respectively. Plasma desorption mass spectra were measured on a spectrometer constructed by Dr. R.D. Macfarlane and located at NHLBI (Bethesda, MD); Cf-252 is used for the ionization in that case.

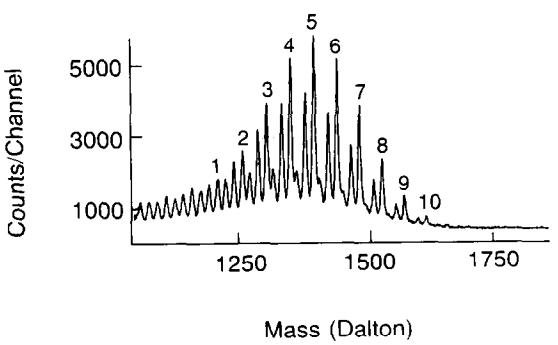


Fig. 1. Mass spectrum of HE-β-CyD (D.S. 5.0). The presence of both trace potassium and trace sodium ions in the sample caused the observed doublets; the dominant peaks in the doublet belong to the ions formed from cyclodextrin derivative and potassium cation.

### *Moisture sorption studies*

Powdered cyclodextrins (100 mg, 100 mesh) in a weighing bottle were placed in an incubator (Tabai Platinous Rainbow PR-1G, Tokyo, Japan) at 75% R.H. and 25°C. After about 3 days when the equilibrium was attained, the water content of the sample was determined by the Karl-Fischer method.

### *Hemolysis studies*

Human erythrocytes from freshly drawn blood were supplied by the Kumamoto Prefectural Red Cross Blood Center. Erythrocytes were separated by centrifugation ($1000 \times g$ for 10 min), washed 3 times with isotonic phosphate buffer (pH 7.4) and resuspended to give a hematocrit of 5%. Aliquots (0.1 ml) of the erythrocyte suspension were added to samples of cyclodextrin solutions (4 ml) and the mixtures were gently agitated for 30 min at 37°C. After centrifugation ($1000 \times g$ for 10 min) the optical density of the supernatant was measured at 543 nm. Results were expressed as percent of total hemolysis, which was obtained when erythrocytes were incubated in water only. Points of 50% hemolysis were calculated according to Reed and Muench (Ishii et al., 1983).

### *Intramuscular irritation studies*

These studies were carried out by the method of Shintani et al. (1967). Male albino rabbits weighing 2.5–3.0 kg were used. The studied com-

pounds were dissolved in normal sterile saline (1 ml) and injected into M. vastus lateralis using a 23-gauge 0.5 inch needle. Two days after the injection, rabbits were sacrificed, the muscle was exposed, cut longitudinally, and the lesions were scored as described (Shintani et al, 1967).

*Solubility studies*

Solubilities were measured using procedures established by Higuchi and Connors (1965). Drugs were added in excess to aqueous solutions containing solubilizers and the solutions were shaken at 25 °C. After equilibrium was reached (about 10 days) filtered aliquots were analyzed by spectrophotometry at suitable wavelengths. Apparent 1 : 1 stability constants ($K$) were calculated from the slope and intercept of the straight portion of the phase solubility diagrams according to the following equation (Higuchi and Connors, 1965): $K = \text{slope}/\text{intercept} \times (1 - \text{slope})$.

## Results and Discussion

*Physicochemical properties*

Optical activity, solubility in water, and surface activity of HE- and HP-β-CyDs were measured and are recorded in Table 1. The specific rotation, [α], of HP- and HE-β-CyDs tended to decrease with the increasing degree of substitution. Both HP- and HE-β-CyDs had very high water solubility (> 50%), as expected of amorphous compounds (Pitha and Pitha, 1985), but surprisingly were also less hygroscopic than the parent crystalline β-cyclodextrin (Uekama and Otagiri, 1987). The dependence of water content sorbed after 2 days at 75% R.H. and 25 °C on the degree of substitution of HA-β-CyDs is shown in Fig. 2. The water content of HP-β-CyDs decreased with the increasing degree of substitution, whereas that of HE-β-CyDs exhibited a biphasic pattern. Low hygroscopicity of HP-β-CyD may be of advantage in pharmaceutical applications since the moisture

TABLE 1

*Some physicochemical properties of cyclodextrin derivatives*

| Host molecule | Substituent | Degree of substitution | Aqueous [a] solubility (g/100 ml) | $[\alpha]_D^{25}$ [a] | Surface [b] tension (mN/m) |
|---|---|---|---|---|---|
| *Parent cyclodextrins* | | | | | |
| α-CyD | | | 15 | 151 | 71 |
| β-CyD | | | 1.85 | 163 | 71 |
| γ-CyD | | | 23 | 177 | 71 |
| *Methylated cyclodextrins* | | | | | |
| DM-β-CyD | $-OCH_3$ | 14 | 57 | 160 | 62 |
| TM-β-CyD | $-OCH_3$ | 21 | 31 | 158 | 53 |
| *Hydroxyalkylated cyclodextrins* | | | | | |
| 2-HE-β-CyD | $-OCH_2CH_2OH$ | 2.67 | > 50 | 138 | 71 |
| 2-HE-β-CyD | $-OCH_2CH_2OH$ | 4.56 | > 50 | 134 | 71 |
| 2-HE-β-CyD | $-OCH_2CH_2OH$ | 5.00 | > 50 | 129 | 70 |
| 2-HE-β-CyD | $-OCH_2CH_2OH$ | 6.07 | > 50 | 130 | 71 |
| 2-HE-β-CyD | $-OCH_2CH_2OH$ | 9.00 | > 50 | 121 | 68 |
| 2-HE-β-CyD | $-OCH_2CH_2OH$ | 10.55 | > 50 | 118 | 68 |
| 2-HP-β-CyD | $-OCH_2CH(OH)CH_3$ | 2.5 | > 50 | 144 | 69 |
| 2-HP-β-CyD | $-OCH_2CH(OH)CH_3$ | 5.6 | > 50 | 146 | 64 |
| 2-HP-β-CyD | $-OCH_2CH(OH)CH_3$ | 6.8 | > 50 | 139 | 61 |
| 2-HP-β-CyD | $-OCH_2CH(OH)CH_3$ | 8.0 | > 50 | 137 | 60 |
| 2-HP-β-CyD | $-OCH_2CH(OH)CH_3$ | 11.3 | > 50 | 98 | 52 |

[a] In water at 25 °C.

[b] Concentration of cyclodextrin derivatives was 0.1 w/v%.

TABLE 2

*Intramuscular irritation [a] of the M. vastus lateralis of rabbits by natural cyclodextrins and β-cyclodextrin derivatives*

| Cyclodextrin system | 10 mg/ml | 50 mg/ml | 100 mg/ml |
|---|---|---|---|
| α-CyD | 0.67 ± 0.17 | 1.50 ± 0.87 | 3.83 ± 0.17 |
| β-CyD | 0.00 ± 0.00 | 0.25 ± 0.14 [d] | – |
| γ-CyD | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.25 ± 0.14 * |
| DM-β-CyD | 2.33 ± 1.17 | 3.50 ± 0.29 | 4.33 ± 0.44 |
| HE-β-CyD [b] | 0.13 ± 0.13 | 0.20 ± 0.12 | 0.13 ± 0.13 * |
| HP-β-CyD [c] | 0.00 ± 0.00 | 0.38 ± 0.24 | 0.75 ± 0.14 * |

[a] Scored according to the method of Shintani et al. (maximum score 5).
[b] D.S. was 4.0.
[c] D.S. was 4.3.
[d] 20 mg/ml.
* $P < 0.001$ versus α-CyD.

sorption often initiates hydrolytic decomposition of drugs in solid state (Kikuchi et al., 1987). The surface activity of HA-β-CyDs, as estimated from surface tension, increased with increasing degree of substitution; that is probably due to the introduction of apolar hydroxyalkyl groups (Müller and Brauns, 1986).

*Hemolytic activity*

At higher concentrations the natural cyclodextrins cause hemolysis and shape changes of human erythrocytes even when isotonic solutions

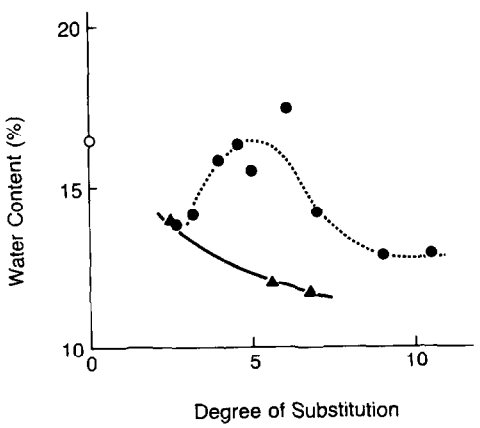


Fig. 2. Relationship between moisture sorption and degree of substitution of HA-β-CyDs. ○, β-CyD; ▲, HP-β-CyD; ●, HE-β-CyD.

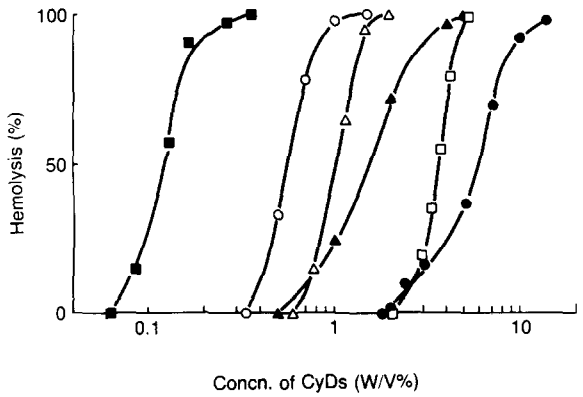


Fig. 3. Hemolytic effects of cyclodextrin derivatives on human erythrocytes in isotonic phosphate buffer (pH 7.4) at 37 °C. △, α-CyD; ○, β-CyD; □, γ-CyD; ■, DM-β-CyD; ▲, HP-β-CyD; ●, HE-β-CyD.

are used (Irie et al., 1982). Hemolytic activity of HP- and HE-β-CyDs were evaluated and compared with that of natural cyclodextrin and DM-β-CyD; results are in Fig. 3. Hemolytic activity of HA-β-CyDs, and particularly that of HE-β-CyDs, were lower than those of other cyclodextrins in spite of their rather high surface activities. In the series of β-cyclodextrin derivatives, the hemolysis started at 0.07% of DM-β-CyD, at 0.3% of β-cyclodextrin, at 0.5% of HP-β-CyD, and at 2% of HE-β-CyD. The cyclodextrin-induced hemolysis is probably due to the membrane disruption elicited by the dissolution and removal of membrane components (Irie et al., 1982). The observed decrease

TABLE 3

*Apparent stability constants ($M^{-1}$) for inclusion complexes of various drugs with β-cyclodextrin derivatives in water at 25 °C*

| Drug | β-CyD | DM-β-CyD | HP-β-CyD [a] | HE-β-CyD [b] |
|---|---|---|---|---|
| Diazepam | 220 | 770 | 170 | 140 |
| Digitoxin | 17000 | 84000 | 18000 | 17000 |
| Digoxin | 11000 | 37000 | 7300 | 5600 |
| Ethyl 4-biphenyl acetate | 3000 | 12500 | 4100 | 2400 |
| Prednisolone | 1600 | 7000 | 1800 | 820 |
| Progesterone | 13000 | 55000 | 17000 | 7500 |
| Testosterone | 7500 | 29000 | 12000 | 5100 |

[a] D.S. was 4.3.
[b] D.S. was 5.0.

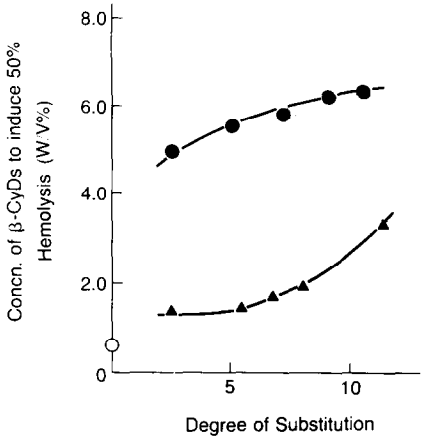


Fig. 4. Relationship between hemolytic activity and degree of substitution of HA-β-CyDs. ○, β-CyD; ▲, HP-β-CyD; ●, HE-β-CyD.

in hemolytic activity may thus be due to the decrease of ability of HA-β-CyDs to remove membrane components. The relationship between degree of substitution of HE- and HP-β-CyDs and the concentrations resulting in 50% hemolysis of human erythrocytes is shown in Fig. 4. The hemolytic activity of HP- and HE-β-CyDs decreased with increasing degree of substitution. That is probably also due to the decreased ability of HA-β-CyDs to remove components of biomembranes; the lower ability of HE-β-CyD to complex with drugs with bulky molecules (Table 3) seems to correlate with their lower hemolytic activity.

*Damage to muscle tissue*

The irritancy of HP- and HE-β-CyDs to muscle tissues (Table 2) is significantly lower than that of methylated cyclodextrins and that of natural cyclodextrins, except for γ-cyclodextrin. HE-β-CyD is particularly remarkable since there was no irritation to the muscle even at a concentration of 100 mg/ml. It is well known that the topical tissue damage by surface-active compounds parallels their in vitro hemolytic activity (Mima et al., 1962; Lee, 1976). The same dependency was observed in the present study. Thus, it is reasonable to assume that the damage to muscular tissue by cyclodextrins also occurs through the disruption of membrane.

The above results indicate that HA-β-CyD may be suitable for use in injection preparations and in preparations which are applied to mucous membranes.

*Solubilization of drugs by HA-β-CyDs*

The effects of parent cyclodextrin and its various derivatives on the solubility of several drugs was evaluated and the corresponding stability constants are summarized in Table 3. Compared to parent β-cyclodextrin, HE-β-CyD had a lower complexing ability, which may be due to the steric hindrance caused by the introduction of a hydroxyethyl group. To confirm the hypothesis, the effect of the degree of substitution on the ability of HE-β-CyDs to solubilize digoxin was studied; the complexing potential of HE-β-CyDs indeed decreased with increasing degree of substitution (Fig. 5). Nevertheless, the situation is a complex one; the steric hindrance of the hydroxypropyl group is expected to be greater than that of the hydroxyethyl group. However, HP-β-CyD showed a tendency to have higher complexing ability than β-cyclodextrin or HE-β-CyD. Obviously, both the steric effects and hydrophobic character of the substituent may play a role. The complexing ability of HP-β-CyDs was also affected by the degree of substitution; the lower the degree of substitution the better the complexing ability (data not shown). When the parent cyclodextrins are used as solubilizers, crystalline complexes of drugs often

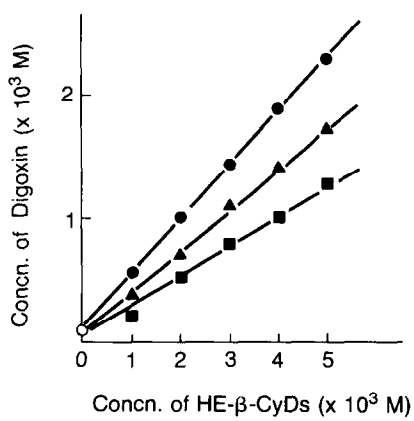


Fig. 5. Phase solubility diagrams of digoxin-HE-β-CyD systems in water at 25° C. ●, D.S. 2.6; ▲, D.S. 5.0; ■, D.S. 10.6.

precipitate at higher concentrations (Uekama et al., 1982). That type of precipitation was never observed when HP- and HE-β-CyDs were used, a circumstance which may be of great advantage in the use of HP- and HE-β-CyD as solubilizing agents and which may fully compensate for their lower complexing ability.

In conclusion, undesirable aspects of β-cyclodextrin usage, such as local irritation or poor aqueous solubility (Uekama and Otagiri, 1987), can be considerably diminished by substitution of β-cyclodextrin with a hydroxyalkyl group. Although the practical application of HA-β-CyD will have to wait until comprehensive toxicological studies are finished, the present data suggest that HA-β-CyDs may be of considerable pharmaceutical use.

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