COMPARATIVE STUDY ON INCLUSION COMPLEXATIONS OF ANTIINFLAMMATORY DRUG FLURBIPROFEN WITH β-CYCLODEXTRIN AND METHYLATED β-CYCLODEXTRINS

Teruko Imai, Tetsumi Irie, Masaki Otagiri, Kaneto Uekama*
Faculty of Pharmaceutical Sciences, Kumamoto University,
5-1, Oe-honmachi, Kumamoto 862, Japan
Masaki Yamasaki
Department of Biochemistry, Medical School,
Kumamoto University, 2-2-1, Honjo, Kumamoto 860, Japan

ABSTRUCT. Some physicochemical properties of methylated β-cyclodextrins, i.e., heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CyD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CyD) were compared with those of natural β-cyclodextrin (β-CyD). Inclusion behaviors of β-CyD and methylated β-CyDs in water and in solid state were studied by solubility analysis, spectroscopies (UV, CD, ^{13}C-NMR and IR), X-ray diffractometry and thermal analysis, using an antiinflammatory drug flurbiprofen(FP) as a guest molecule. The spectral data suggest that the inclusion mode of FP—TM-β-CyD is somewhat different from those of FP—β-CyD and FP—DM-β-CyD. The solid complexes of FP with β- and methylated β-CyDs were obtained in molar ratio of 1:1, and their dissolution behavior and release from suppository base were examined. The data are presented suggesting that DM-β-CyD is particularly useful for improving the pharmaceutical properties of FP in various dosage forms.

1. INTRODUCTION

Recently, chemically modified cyclodextrins have received considerable attention in many fields (1-6), because their physicochemical properties are different from those of natural cyclodextrins. For example, the methylated β-cyclodextrins such as heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CyD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CyD) are much more soluble in both water and organic solvents compared with parent β-cyclodextrin (β-CyD). In addition, TM-β-CyD ring is remarkably distorted from the regular heptagonal symmetry of β-CyD owing to the steric hindrance involving the methyl groups (7). Therefore, it is interesting to compare the inclusion behavior of methylated β-CyDs with that of β-CyD. The present paper deals with the inclusion complexations of antiinflammatory drug flurbiprofen (FP), 2-(2-fluoro-4-biphenylyl)propionic acid, with β-CyD and methylated β-CyDs in solution and in solid phase in the hope of improvement of some

Journal of Inclusion Phenomena **2**, 597–604. 0167-7861/84.15.

pharmaceutical properties of FP. In this study, FP was employed as an adequate guest molecule, because its inclusion complexes with natural CyDs have been recently described (8,9).

2. EXPERIMENTAL

2.1. Materials

FP was kindly supplied from Mitsubishi Yuka Pharmaceutical Co., Ltd. and used without further purification. β-CyD and DM-β-CyD were purchased from Nihon Shokuhin Kako, Ltd. and Toshin Chemical Co., Ltd., respectively, and recrystallized from water. TM-β-CyD was synthesized by the method of Hakomori (10) and its methylation was confirmed by elemental analysis and ^{1}H-NMR. Table I lists some physical properties of three kinds of β-CyDs. All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

TABLE I. Some Physical Properties of β-CyD and Methylated β-CyDs

Host molecule	Melting point (°C)	$[\alpha]_D^{25}$	Surface[a] tension (mN/m)
β-CyD	280	+163	71
DM-β-CyD	295-300	+160	62
TM-β-CyD	157	+158	56

a) Concentrations of β-CyDs: 0.1 mM.

2.2. Physicochemical Studies

To determine the solubility, excess amounts of β-CyDs were added to water and shaken at 25, 40, 55, and 70°C. After equilibrium was attained, the concentrations were measured by polarimeter (Jasco DIT-4). In the moisture sorption studies, approximately 2g of powder was placed in the desicator maintaining the various relative humidities (R.H.) at 25°C for 2 days. Reported values (in Figure 2) represent the average moisture content of the 3 measurements, on a dry weight basis. The surface tension of test solutions (0.1 mM of β-CyDs) were measured using a Shimadzu Du Noüy Surface & Interfacial Tensionmeter.

2.3. Spectroscopic Studies

The UV (Hitachi 556S), CD (Jasco J-50A) and fluorescence (Hitachi MPF-3) spectra were measured at 25°C. ^{13}C-NMR experiments (JEOL JNM-FX 200) were made at 20°C. The concentrations of FP and CyDs in 0.05 M NaOD solvent were 0.02 M. ^{13}C-chemical shifts were recorded with an accuracy of ±0.024 ppm using tetramethylsilane as an external reference.

2.4. Dissolution and Suppository Release Studies

The dissolution behaviors of FP and its complexes in water were examined according to the dispersed amount method. The equivalent amount (30 mg) of FP as a 100 mesh powder was put into a dissolution

cell (25 ml of water) at 37°C and stirred at 57 rpm. A Witepsol H-15 (Dynamit Nobel Chemicals) was used as an example of hydrophobic base. The suppositories were prepared to give 50 mg of FP in each 2 g suppository. Full details of dissolution and suppository release studies were given elsewhere (8, 11).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Methylated β-CyDs and β-CyD

Figure 1 shows solubility curves of three kinds of β-CyDs, where the different temperature dependency is clearly noted. With the rise in temperature, the solubility of β-CyD increases as a general rule. On the other hand, the solubilities of DM-β-CyD and TM-β-CyD decrease with increasing temperature, showing a feature of nonionic surfactant. In fact, the methylated β-CyDs are highly surface active, as can be seen in Table I. The anomalous solution properties of methylated β-CyDs may be responsible for various factors such as lattice energy, hydration and association.

Figure 2 shows the moisture adsorption curves of three β-CyDs. In the case of β-CyD, the adsorption isotherm appears stepwise. That is, the moisture content is found to be about 12%(w/w) at 30% R.H. and is finally reached to about 20%(w/w). It is interesting to note that the 20%(w/w) moisture content corresponds to twelve water molecules on the crystallization of one β-CyD molecule as expected from a X-ray

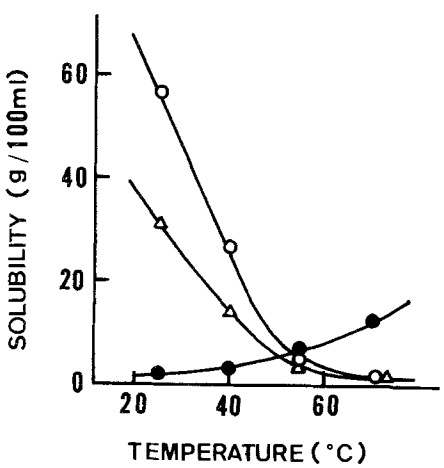


Figure 1. Solubility Curves of β-CyD and Methylated β-CyDs in Water as a Function of Temperature
●: β-CyD, ○: DM-β-CyD,
△: TM-β-CyD.

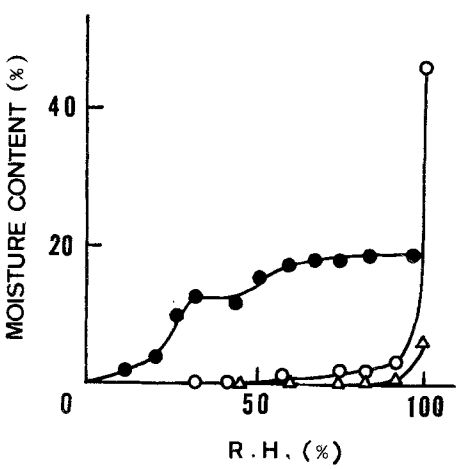


Figure 2. Moisture Sorption Curves of β-CyD and Methylated β-CyDs at 25°C under Various R.H.
●: β-CyD, ○: DM-β-CyD,
△: TM-β-CyD.

analysis of β-CyD hydrate (13). On the other hand, DM-β-CyD and TM-β-CyD adsorbed water hardly even at 95% R.H., indicating a lesser hygroscopicity.

3.2. Inclusion Complexations of FP with Methylated β-CyDs and β-CyD

The 1:1 stability constants of inclusion complexes determined by solubility and spectroscopic (UV and CD) methods are listed in Table II. The stability constants obtained by the three methods are in good agreement with each other, and the magnitude of stability constants increases in the order of DM-β- > β- > TM-β-CyD. These suggest that the 3-O-methyl groups in TM-β-CyD apparently reduce the necessary interaction between the FP and host molecules.

TABLE II. Stability Constants of FP Complexes with β- and Methylated β-CyDs Obtained by Various Methods at 25°C

Method	Stability constant, $K(M^{-1})$		
	β-CyD complex	DM-β-CyD complex	TM-β-CyD complex
Solubility[a]	4340	10060	1490
UV[b]	5350	9250	1530
CD[b]	4780	7860	1630

a) Determined in water.
b) Determined in 0.1 M phosphate buffer of pH 7.0.

The interactions between FP and β-CyDs in aqueous solution were further examined by UV, CD and fluorescence spectroscopies. Figure 3 shows the UV, CD and fluorescence spectra of FP in the absence and presence of three kinds of β-CyDs. The optical activities of FP were induced positively by the binding to β- and DM-β-CyD but negatively by TM-β-CyD, where significant decreases in UV absorbance were observed. In contrast to UV changes, the fluorescence intensity of FP was enhanced by the addition of three β-CyDs. These spectral changes may be caused by the different geometry and position of FP in β-CyD cavities as well as the different conformation of the host molecules.

The ^{13}C-NMR technique was employed to gain insight into the inclusion modes of FP—β-CyD complexes in aqueous solution. The effects of FP on ^{13}C-chemical shifts of three β-CyDs are summarized in Table III. The values in Table III and IV were calculated using eq. (1) (13) along with the stability constants of inclusion complexes.

$$\delta_{obs} = \alpha \cdot \delta_{complex} + (1-\alpha) \cdot \delta_0 \qquad (1)$$

α: the mole fraction of substrate bound.

The carbons of TM-β-CyD, in particular, C1, C3 and C3' were found to be remarkably deshielded by complexation, differing from β-CyD and DM-β-CyD. The significant displacement observed for TM-β-CyD may be a reflection of the distorted structure of TM-β-CyD ring.

Table IV summarizes the effects of three β-CyDs on the ^{13}C-chemical shifts of FP. All the carbon signals of the FP molecule were

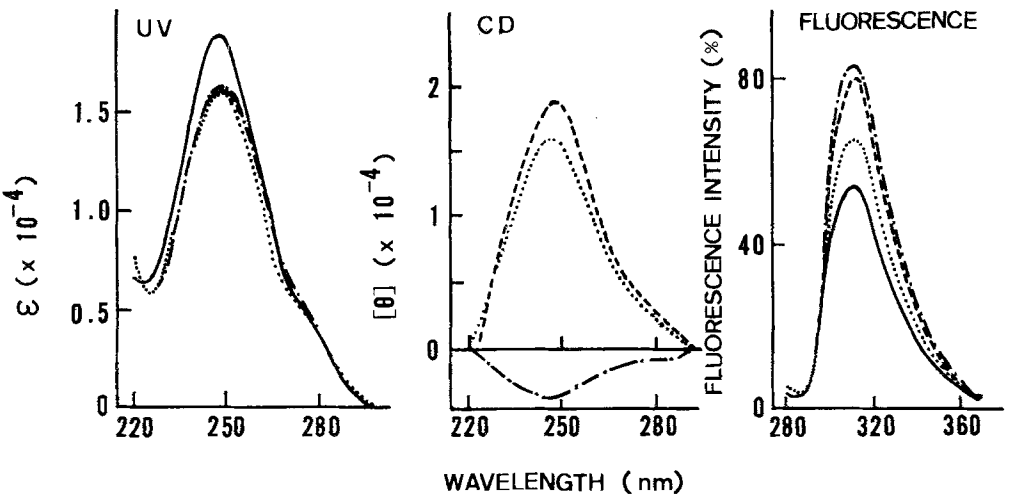
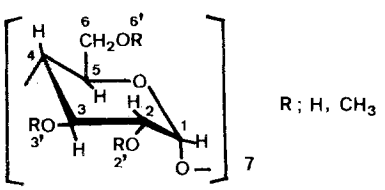


Figure 3. UV, CD and Fluorescence Spectra of FP in the Absence and Presence of β-CyDs in 0.1 M Phosphate Buffer (pH 7.0) at 25°C

———: FP, ·····: FP + β-CyD, - - - - - -: FP + DM-β-CyD, —·—: FP + TM-β-CyD.

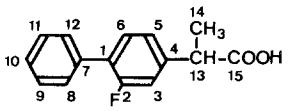
TABLE III. Effect of FP on ^{13}C-Chemical Shifts of β- and Methylated β-CyDs



Carbon	β-CyD		DM-β-CyD		TM-β-CyD	
	without FP, δ_0	$\Delta\delta$ a)	without FP, δ_0	$\Delta\delta$	without FP, δ_0	$\Delta\delta$
1	102.335	-0.094	99.635	0.210	97.105	1.377
2	72.707	-0.162	81.488	0.026	80.223	0.582
3	73.558	-0.040	72.512	-0.052	77.110	2.250
4	81.513	-0.525	82.048	0.131	81.148	0.052
5	71.977	-0.081	70.056	0.131	70.566	0.078
6	60.545	-0.687	70.834	-0.707	71.029	-0.146
2'			58.331	0.157	58.234	-0.116
3'					59.766	0.970
6'			59.523	0.026	58.550	0.029

a) $\Delta\delta = \delta_{complex} - \delta_0$.
Negative signs indicate upfield displacements.

TABLE IV. ^{13}C-Chemical Shifts of FP in the Absence and Presence of β-CyD and Methylated β-CyDs



Carbon[a)]	without β-CyDs	with β-CyDs, Δδ[b)]		
		β-CyD	DM-β-CyD	TM-β-CyD
1	126.410	-0.074	-0.609	-0.424
2	159.450	-0.242	-0.233	-0.142
3	114.935	-0.101	0.236	0.400
4	145.609	0.633	1.073	1.011
5	123.826	-0.310	0.170	0.365
6	130.637	-0.714	-1.086	-0.451
7	135.599	-0.323	-0.301	0.248
8	128.873	-0.391	-0.681	-0.276
9	128.995	-0.242	-0.340	-0.175
10	127.998	0.175	0.262	0.116
11	128.995	-0.242	-0.340	-0.175
12	128.873	-0.391	-0.681	-0.276
13	48.273	0.242	0.406	0.349
14	18.317	0.721	1.126	0.907
15	183.069	-0.943	-1.597	-1.542

a) Assigned according to ref. 9.
b) $\Delta\delta = \delta_{complex} - \delta_0$
Negative signs indicate upfield displacement.

affected by the addition of β-CyDs. It is noteworthy that the ortho carbons (C6, C8, C12) of FP showed significant upfield shifts compared with other carbons, where the magnitude of increase is in the order DM-β- > β- > TM-β-CyD. These phenomena can be explained on the basis of the hydrophobic interaction of host and guest molecules (13), and the ring current effects caused by the torsion of biphenyl ring through inclusion complexation (14). From the total inspection of the data in Table III and IV, it is reasonable to assume that the biphenyl moiety of FP molecule predominantly interacts with three kinds of β-CyDs and that FP molecule could penetrate further into the β- and DM-β-CyD cavities. These inclusion modes of FP to β- and TM-β-CyD in aqueous solution are also supported by the results of X-ray crystallographic analysis (15, 16).

Furthermore, interactions of FP with three kinds of β-CyDs in the solid state were assessed by infrared (IR), differential thermal analysis (DTA) and powder X-ray diffractometry. In IR spectra of β-CyD complexes, carbonyl-stretching band of FP was shifted to higher wave number, suggesting a dissociation of the intermolecular hydrogen bonds of FP (17) through inclusion complexation. The interactions of FP with β-CyDs were accompanied by the disappearance of the endothermic

peak around 120°C corresponding to the melting of FP. Moreover, the powder X-ray diffraction patterns of the complexes differ significantly from those of the physical mixtures, indicating a constitution of new solid phase. From the above observations, it is apparent that FP forms 1:1 complex with β-, DM-β-, TM-β-CyDs in water and in solid phase.

3.3. Some Pharmaceutical Properties of Inclusion Complexes

Figure 4 shows the dissolution profiles of FP and its complexes in water at 37°C. It is evident that the dissolution rate of FP was significantly improved by inclusion complexation, particularly by DM-β-CyD. The observed increase in dissolution rate may be due to the increase in FP solubility. It is noted that the dissolution profile of TM-β-CyD complex exhibited more negative curvature than the β-CyD complex with the passage of time, although the initial rate of the former was greater than that of the latter. This may be due to rather quick dissociation of the TM-β-CyD complex to its components during the dissolution process, because of its smaller stability constant.

The release of drug from suppository bases is known to be influenced by various factors such as drug-vehicle interactions, vehicle composition, solubility and particle size of drug in vehicle (18).

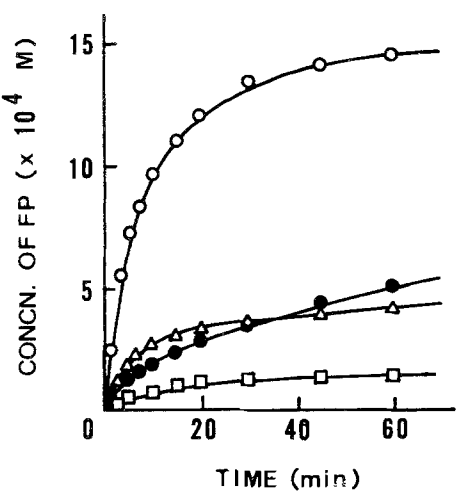


Figure 4. Dissolution Profiles of FP and Its Complexes in Water at 37°C
□: FP alone, ●: β-CyD complex,
○: DM-β-CyD complex,
△: TM-β-CyD complex.

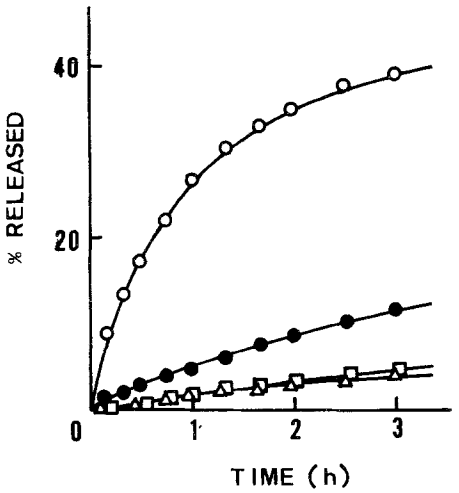


Figure 5. Release Profiles of FP and Its Complexes from Witepsol H-15 in Normal Saline Solution at 37°C
□: FP alone, ●: β-CyD complex,
○: DM-β-CyD complex,
△: TM-β-CyD complex.

Figure 5 shows the release profiles of FP and its complexes from Witepsol H-15 suppositories. It is evident that the release rate of FP was significantly improved by the inclusion complexations with β- and DM-β-CyDs, while that from TM-β-CyD complex was almost the same as FP alone. The different release behavior between the three complexes may be attributed to the difference in dissolution rate and binding affinity of the complexes to the hydrophobic suppository base. In fact, the release rate of FP from hydrophilic bases such as macrogol suppositories containing the DM-β-CyD and TM-β-CyD complexes, was greater than that from hydrophobic bases such as Witepsol H-15 (11).

Furthermore, the serum levels of FP following oral administration to rabbits were found to be significantly increased by methylated β-CyDs. All the data suggest that DM-β-CyD is particularly useful for improving the bioavailability of poorly water soluble drugs in various dosage forms.

REFERENCES

1. M.L. Bender and M. Komiyama: *Cyclodextrin Chemistry,* Springer-Verlag, Berlin (1978).
2. J. Szejtli: *Cyclodextrins and Their Inclusion Complexes,* Académiai Kiadó, Budapest (1982).
3. Y. Nakai, K. Yamamoto, K. Terada and H. Horibe: *Chem. Pharm. Bull.* 30, 1796-1802(1982).
4. M. Otagiri, T. Imai and K. Uekama: *J. Pharm. Dyn.* 5, 1027-1029 (1982).
5. A.P. Croft and R.A. Bartsch: *Tetrahedron* **39**, 1417-1474(1983).
6. J. Szejtli: *J. Incl. Phenom.* 1, 135-150(1983).
7. K. Harata, K. Uekama, M. Otagiri and F. Hirayama:*Bull. Chem. Soc. Jpn.* **56**, 1732-1736(1983).
8. M. Otagiri, T. Imai, N. Matsuo, K. Uekama: *Acta Pharm. Suec.* **20**, 1-10 (1983).
9. M. Otagiri, T. Imai, F. Hirayama, K. Uekama and M. Yamasaki: *Acta Pharm. Suec.* **20**, 11-20(1983).
10. S. Hakomori: *J. Biochem.* **55**, 205-207(1964).
11. K. Uekama, T. Imai, T. Maeda, T. Irie, F. Hirayama and M. Otagiri: submitted.
12. K. Lindner and W. Seanger: *Carbohydr. Res.* **99**, 103-115(1982).
13. R.I. Gelb, L.M. Schwartz, B. Cardelino, H.S. Fuhrman, R.F. Johnson and D.A. Laufer: *J. Am. Chem. Soc.* **103**, 1750-1757 (1981).
14. K. Fujii, T. Yamada and E. Fujita: *Org. Magn. Reson.* **17**, 250-256 (1981).
15. K. Uekama, F. Hirayama, T. Imai, M. Otagiri and K. Harata: *Chem. Pharm. Bull.* **31**, 3363-3365(1983).
16. K. Harata, F. Hirayama, T. Imai, K. Uekama and M. Otagiri: *Chem. Lett.* **1984**,1549-1552.
17. J.L. Flippen and R.D. Gilardi: *Acta Cryst.* **B31**, 926-928(1975).
18. T.J. Roseman, G.R. Derr, K.G. Nelson, B.L. Lieberman and S.S. Butler: *J. Pharm. Sci.* **70**, 646-651(1981).