Improvement of Dissolution and Suppository Release Characteristics of Flurbiprofen by Inclusion Complexation with Heptakis(2,6-di-O-methyl)- β -cyclodextrin

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Abstract The inclusion behavior of methylated β -cyclodextrins, heptakis(2,6-di-O-methyl)- β -cyclodextrin (2), and heptakis-(2,3,6-tri-O-methyl)- β -cyclodextrin (3) in solution and the solid state was compared with that of natural β -cyclodextrin (1) using an anti-inflammatory drug, flurbiprofen, as a guest molecule. Stability constants were determined by the solubility method at various temperatures, and the thermodynamic parameters were calculated for inclusion complex formation in aqueous solution. The solid complexes were obtained in a molar ratio of 1:1, and their dissolution behavior and release from suppository bases were examined. The data suggest that the inclusion mode of the complex with 3 is somewhat different from that of the complexes with 1 and 2. From a practical point of view, 2 seems to be particularly useful for improving the pharmaceutical properties of flurbiprofen in various dosage forms.

 β -Cyclodextrin (1) is a cyclic oligosaccharide consisting of 7 glucose units, in which 7 primary and 14 secondary hydroxyl groups are located on the smaller entrance side and the larger entrance side of the torus, respectively. The most remarkable property of 1 is its ability to form inclusion complexes with a variety of drug molecules,¹⁻³ in which the drug molecules are included in the relatively hydrophobic cavity of 1 (internal diameter, 6.4 Å).

Although 1 has been used extensively to improve various physicochemical properties of drug molecules, it contains some undesirable characteristics.^{4.5} The main cause of the application limit of 1 in the pharmaceutical field seems to be related to the relatively low aqueous solubility (1.8% at 25°C). Recently, chemically modified cyclodextrins have received considerable attention because their physical properties and inclusion behaviors are different from those of natural cyclodextrins.^{6.7} For example, the methylated cyclodextrins, such as heptakis(2,6-di-O-methyl)- β -cyclodextrin (2) and heptakis(2,3,6-tri-O-methyl)- β -cyclodextrin (3), are much more soluble in both water and organic solvents compared with 1. Thus, it is important to survey the possible utility of methylated cyclodextrins in pharmaceutical formulations.

Flurbiprofen [2-(2-fluoro-4-biphenylyl)propionic acid], an anti-inflammatory drug, was employed as a test compound, since its inclusion complexations with natural cyclodextrins were recently described.^{8,9} The present paper is mainly concerned with the inclusion complexation of flurbiprofen with 2 and 3 in solution and the solid state. Moreover, certain properties of these complexes, such as dissolution rate and release from suppository bases, were examined and compared with those of 1 complex.

Experimental Section

Materials—Flurbiprofen (Mitsubishi Yuka Pharmaceutical Co. Ltd., Ibaraki, Japan), 1 (Nihon Shokuhin Kako Co. Ltd., Tokyo, Japan), and 2 (Toshin Chemical Co. Ltd., Tokyo, Japan) were used as supplied. Compound 3 was prepared according to the method reported previously.¹⁰ All other materials and solvents were of analytical reagent grade. Deionized double-distilled water was used.

0022-3549/85/0800-0841\$01.00/0 © 1985, American Pharmaceutical Association Solubility Studies—Solubility measurements were carried out according to the method of Higuchi and Connors.¹¹ Excess amounts of flurbiprofen were added to an aqueous solution containing various concentrations of cyclodextrins, and the solutions were shaken at constant temperatures (15–45°C). After equilibrium was reached (~10 d), an aliquot was centrifuged and pipetted through a cotton plug. A 0.5-mL aliquot was diluted with water and analyzed spectrophotometrically (model 100-60; Hitachi Ltd., Tokyo, Japan) at 247 nm. The molar absorptivity of flurbiprofen was not affected by cyclodextrins under the present analytical conditions, and no correction of absorbancy change by complexation was made. A 1:1 stability constant, K, was calculated from the initial linear portion of the phase solubility diagram. There was no significant change in the solubility of flurbiprofen in water under these experimental conditions (pH 3–4).

Preparation of Solid Complexes—Flurbiprofen (3 and 0.15 g, respectively) was dissolved in the solutions of 2 (20 g/100 mL) and 3 (1.6 g/100 mL), and the solutions were stirred at 55°C for 24 h. The complexes, which precipitated as microcrystalline powders, were removed by filtration using filter paper (Whatman no. 1) with suction and dried under reduced pressure at 40°C for 48 h. This powder corresponded to a 1:1 complex of flurbiprofen with 2 or 3. The 1:1 complex of flurbiprofen with 1 was prepared according to the method described previously.⁹

X-ray Diffractometry—The powder X-ray diffractometer (Geiger-Flex 2012; Rigaku Denki Co. Ltd., Tokyo, Japan) was operated under the following conditions: X-ray, Ni-filtered Cu-K_{α} radiation; voltage, 30 kV; current, 20 mA; time constant, 2 s; scanning speed, 1°/min.

Differential Thermal Analysis (DTA)—The thermal analyzer (model DT-20B thermal analyzer; Shimadzu Co. Ltd., Kyoto, Japan) was operated at a scanning rate of 10°C/min over the temperature range of 25–350°C. The sample weight was 5 mg.

Infrared Spectrometry—IR spectra (model DS-701; Jasco Ltd., Tokyo, Japan) were measured as potassium bromide pellets.

Dissolution Studies—The dissolution behaviors of flurbiprofen and its inclusion complexes in water were examined according to the dispersed-amount method.¹² An excess amount of the drug (100 mesh, 20 mg) or its complexes (equivalent to 20 mg of flurbiprofen for the 1 and 3 complexes and to 100 mg of flurbiprofen for the 2 complex) beyond the equilibrium solubilities was put into 25 mL of water in a dissolution cell which was kept at constant temperatures (15-45°C), and the dissolution medium was stirred at 150 rpm. At an appropriate interval, 0.5 mL of solution was sampled by a pipet with a cotton plug, diluted with water, and assayed spectrophotometrically. The cumulative dilution caused by sampling was corrected for by replacing the sample by equal volumes of the original medium.

Suppository Release Studies—Witepsol H-15 (Dynamit Nobel Chemicals, Troisdorf-Oberlar, F.R.G.) and a 1:1 (w/w) mixture of polyethylene glycols 1500 and 4000 (Katayama Chemical Co., Osaka, Japan) were used as examples of hydrophobic and hydrophilic bases, respectively. The suppository (2 g) was prepared by suspending flurbiprofen or its inclusion complexes in the melted base to yield a drug concentration of 2.5% (w/w). The melt was then poured into aluminum suppository molds (Erwaka G.m.b.H., Frankfurt, F.R.G.) and allowed to cool at room temperature.

The release rate of the drug from the suppositories was measured using a suppository release apparatus (Toyama Sangyo Co., Osaka, Japan) according to the method of Muranishi et al.¹³ Each suppository was placed in the cylindrical chamber, which was lined from the inside with filter paper (pore size, 3.0 μ m; Millipore Co., Bedford, MA) as a barrier for diffusion of the suppository base and lowered into a flask containing a normal saline solution (300 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. At an appropriate interval, a 3-mL sample was withdrawn from the release phase and assayed spectrophotometrically. A correction was applied for the cumulative dilution.

Results and Discussion

Complexation in Aqueous Solution-The complexing behavior of flurbiprofen with three kinds of β -cyclodextrins in water at various temperatures was studied by the solubility method. Figure 1 shows a typical example of the phase solubility diagrams obtained for flurbiprofen-cyclodextrin systems at 37°C. The solubility plot for 1 shows a B_s type solubility curve.¹¹ The initial rising portion is followed by a plateau region and then decreases in total concentration of flurbiprofen with precipitation of a microcrystalline complex at high 1 concentration. The solubility plot for 3 is qualitatively similar to that for 1, indicating a solid complex formation of limited solubility. The 1:1 stoichiometry in the solid phase was determined for both the flurbiprofen-1 and flurbiprofen-3 systems on the basis of the data in the plateau region of solubility diagrams. The results were in good agreement with those obtained by isolation and analysis of the solid complexes. In the case of 2, the solubility of flurbiprofen increased remarkably in a linear fashion as function of 2 concentration, and the resulting solubility curve can be classified as A_{L} .¹¹ In this system, a highly watersoluble complex may exist in the solution, since no precipitation was observed even at concentrations of 2 as high as 0.3M at 37°C. With a rise in temperature, however, a microcrystalline complex began to precipitate, due to the decrease in solubility of 2.14 A 1:1 stoichiometry was also ascertained for flurbiprofen-2 by isolation and analysis of the solid complex.

The 1:1 stability constants, K, for three complexes were then calculated from the initial straight-line portion of the solubility diagrams obtained at temperatures of $15-45^{\circ}$ C (Table I). The van't Hoff plots were nearly straight lines over the temperature range employed (Fig. 2). The thermodynamic parameters were then calculated from the temperature dependency of K values (Table I). The K value of the 2 complex was found to be the largest among the three complexes, which may be responsible for the greatest solubilization effect of 2 in the wide range of temperatures.

As shown in Table I, all the enthalpy changes (ΔH) were negative, while the entropy changes (ΔS) were considerably different in each case. In the case of the 3 complex, the favorable ΔH could more than compensate for the unfavorable ΔS . This tendency was generally observed for the inclusion complexations of 1 with various drug molecules.^{15,16} On the other hand, 1 and 2 complexes showed a positive ΔS value, indicating that a number of water molecules are set free in the complexation. This is expected because 1 and 2 have hydroxyl groups on the rims of their tori and may contain a number of hydrated water molecules. By the formation of the inclusion complex, the removal of these water molecules from the ordered structure should result in a favorable ΔS change. The unfavorable ΔS change observed for the 3 complex may be due to the smaller disordering of the displaced water molecules which are released from flurbiprofen and 3, since the K value of the 3 complex is rather small compared with other complexes. Since methylated cyclodextrins are highly surface active,¹⁴ other factors, such as micellar dissociation of the host molecules, also should be considered for the remarkable changes in ΔH and ΔS values,¹⁷ particularly for the 3 complex.

The above results indicate that the mode of inclusion of the 3 complex may be somewhat different from that of the 1 and 2 complexes in aqueous solutions. The different inclusion mode was also predicted by various spectroscopic techniques, such as UV, circular dichroism, fluorescence, and 13 C NMR.¹⁴

Complexation in the Solid State—To examine the interaction of flurbiprofen with cyclodextrins in the solid state, IR spectroscopy, X-ray diffractometry, and DTA were employed



Figure 1—Phase solubility diagrams of flurbiprofen–cyclodextrin systems in water at 37 °C. Key: (●) flurbiprofen–1 system; (○) flurbiprofen–2 system; (△) flurbiprofen–3 system.

Figure 2—The van't Hoff plots for stability constants of flurbiprofencyclodextrin complexes. Key: (\bullet) flurbiprofen-1 system; (\bigcirc) flurbiprofen-2 system; (\triangle) flurbiprofen-3 system.

Table I—Stability Cor	nstants and Thermo	dynamic Parameter	s for Complexation	of Flurbiprofen with	β-Cyclodextrin and	Methylated
β-Cyclodextrins		•	-	•		

Compound	Stability Constant, M ⁻¹				Δ <i>G</i> (298 K),	ΔΗ.	Δ <i>S</i> (298 K).
	15°C	25°C	37°C	45°C	kcal`• mol ^{−1}	kcal · mol ⁻¹	$cal \cdot K^{-1} \cdot mol^{-1}$
1 2	5850 12300	4340	3670 9230	3340 8500	-4.96 -5.46	-3.33	5.47
3	2380	1490	480	220	-4.33	-14.60	-34.50

842 / Journal of Pharmaceutical Sciences Vol. 74, No. 8, August 1985 to compare the corresponding physical mixtures in a 1:1 molar ratio. Figure 3 shows the IR spectra in the carbonyl stretching region of the carboxyl group in flurbiprofen. In the case of the complexes, the 1695 cm⁻¹ band was found to shift to 1705 cm⁻¹, 1730 cm⁻¹, and 1735 cm⁻¹ for 1, 2, and 3, respectively; the greatest shift was obtained for the flurbiprofen–3 complex. In contrast, the physical mixtures showed no appreciable spectral changes in all cases. The shifts to the high wave number region observed for the complexes may be due to the dissociation of the intermolecular hydrogen bonds of flurbiprofen through inclusion complexation.¹⁸



Figure 3—IR spectra of flurbiprofen-cyclodextrin systems measured by the KBr disk method. Key: (A) flurbiprofen-1 system; (B) flurbiprofen-2 system; (C) flurbiprofen-3 system; (----) complex of flurbiprofen with cyclodextrin; (-----) physical mixture of flurbiprofen and cyclodextrin.

Figure 4 shows the powder X-ray diffraction patterns of the complexes and physical mixtures. The diffraction patterns of the physical mixtures were simply a superposition of each component, while those of the complexes were apparently different from each constituent and constituted a new solid phase. It is interesting to note the difference in diffraction patterns of the complexes. The diffraction pattern of the 2 complex appeared to be analogous to that of the 1 complex, where the three characteristic peaks were observed around 6° , 11°, and 17° at 2 θ , suggesting a similar crystal packing. On the other hand, the diffraction pattern of the 3 complex is obviously different from that of the 1 and 2 complexes, showing the strong peaks around 8° at 2θ . It is known that the macrocyclic ring of 2 is almost round, as is that of 1, while that of 3 is distorted by the steric hindrance caused by the methyl groups introduced to all the hydroxyl groups of 1.¹⁹ Thus, the data in Fig. 4 may indicate that the methylation affects not only the macrocyclic conformation of the host



Figure 4—Powder X-ray diffraction patterns of flurbiprofen–cyclodextrin systems. Key: (A) physical mixture of flurbiprofen and 1; (B) complex of flurbiprofen with 1; (C) physical mixture of flurbiprofen and 2; (D) complex of flurbiprofen with 2; (E) physical mixture of flurbiprofen and 3; (F) complex of flurbiprofen with 3.

molecule but also the inclusion mode of host-guest interaction. In fact, our preliminary results on the molecular structures of the crystalline complexes indicated that (R)- and (S)isomers of flurbiprofen are separately included in the cavity of 1 to form a head-to-head dimer.²⁰ On the other hand, the (S)-isomer, which has higher physiological activity than racemic flurbiprofen,²¹ is stereoselectively included in the cavity of 3, showing a head-to-tail type channel structure.

Figure 5 shows DTA thermograms of the flurbiprofencyclodextrin systems. In the case of the physical mixtures, an endothermic peak due to the melting of flurbiprofen was observed at ~120°C. In sharp contrast, the complexes showed no appreciable endothermic peak of flurbiprofen within the melting and/or degradation temperatures of cyclodextrins.¹⁴ These thermal behavior changes may result from the breakdown of the hydrogen bonds²² through complexation and then monomolecular dispersion of the guest molecule within the cyclodextrin cavities.²³

Dissolution Behavior—Figure 6 shows a typical example of the dissolution profiles of flurbiprofen and its inclusion complexes in water at 37° C. It was found that all the complexes dissolved much more rapidly than flurbiprofen itself. The observed increase in rate may be due to the increase in solubility, as expected from Fig. 1, although other factors, such as wettability, diffusion coefficient, and the dissociation of the complex in the dissolution medium, should



Figure 5—DTA thermograms of flurbiprofen–cyclodextrin systems. Key: (A) flurbiprofen; (B) 1; (C) physical mixture of flurbiprofen and 1; (D) complex of flurbiprofen with 1; (E) 2; (F) physical mixture of flurbiprofen and 2; (G) complex of flurbiprofen with 2; (H) 3; (I) physical mixture of flurbiprofen and 3; (J) complex of flurbiprofen with 3.



Figure 6—Dissolution profiles of flurbiprofen and its cyclodextrin complexes in water at 37 °C, measured by the dispersed-amount method. Key: (\Box) flurbiprofen alone; (\bullet) **1** complex; (\bigcirc) **2** complex; (\triangle) **3** complex.

Journal of Pharmaceutical Sciences / 843 Vol. 74, No. 8, August 1985 be considered in the rate enhancement. In the case of the 2 complex, which has a larger stability constant, the drug concentration following dissolution exceeded its normal solubility as much as 60-fold. This supersaturation state was quite stable, and no precipitation of the drug occurred. In contrast, the 3 complex, with a small stability constant, elicited a rather small rate enhancement, probably due to the dissociation of the complex after dissolution.

Therefore, it is interesting to examine the temperature effect on the dissolution behavior of the complexes, because the temperature dependency of the stability constant and solubility of the complexes are different in each case. Figure 7 shows the dissolution profiles of the three complexes in water at temperatures from 15°C to 45°C. It is apparent that the dissolution behaviors of the 2 and 3 complexes were different from that of the 1 complex. As a general rule, the dissolution rate of the 1 complex (Fig. 7A) as well as flurbiprofen itself (not shown here) increased with increasing temperature under the experimental conditions used. However, the dissolution rates of the 2 and 3 complexes initially increased, then decreased with increasing temperature, as shown in Fig. 7B and C, respectively. This anomalous dissolution behavior, i.e., the decrease in rate at higher temperature, may be explained by the fact that the solubilities of the complexes are inversely proportional to the temperature increase. In the case of the 3 complex, the dissolution rate is much more sensitive to temperature change, indicating that other factors, such as the dissociation of the complex, may further complicate the situation. The supersaturation observed for the dissolution curves in higher temperature ranges may reflect the dissociation of the complex after dissolution. Thus, the data in Fig. 7 suggest that the 2 complex possesses rather superior dissolution characteristics, maintaining a high drug concentration over a wide temperature range.

Release from Suppository Bases—The release of drug from suppositories is known to be influenced by various factors: drug-vehicle interactions, vehicle composition, solubility, partition coefficient, and particle size of drug in vehicle.²⁴ We have recently reported that the release rate of drugs from a hydrophobic suppository base was significantly improved by inclusion complexation with $1.^{8,25}$ In this study, the release behaviors of the 2 and 3 complexes were examined and compared with that of the 1 complex, using a hydrophobic and a hydrophilic suppository base. The release profiles of flurbiprofen from the fatty base and polyethylene glycol base are shown in Figs. 8 and 9, respectively. The data indicate that the order of initial release rate from the fatty



Figure 7—Dissolution profiles of flurbiprofen–cyclodextrin complexes in water at various temperatures, measured by the dispersed-amount method. Key: (A) 1 complex; (B) 2 complex; (C) 3 complex; (C) 15 °C; (●) 25 °C; (△) 37 °C; (▲) 45 °C.



Figure 8—Release profiles of flurbiprofen and its cyclodextrin complexes from Witepsol H-15 suppositories in normal saline solution at 37°C. Key: (\Box) flurbiprofen alone; (\bullet) 1 complex; (\bigcirc) 2 complex; (\triangle) 3 complex.

Figure 9—Release profiles of flurbiprofen and its cyclodextrin complexes from polyethylene glycol suppositories in normal saline solution at 37 °C. Key: (\Box) flurbiprofen alone; (\bullet) 1 complex; (\bigcirc) 2 complex; (\triangle) 3 complex.

844 / Journal of Pharmaceutical Sciences Vol. 74, No. 8, August 1985 base was 2 complex > 1 complex > 3 complex; from the polyethylene glycol base, the order changed to 2 complex > 3complex > 1 complex. Although the order of release rate was somewhat different for each base, the release patterns closely resembled the dissolution patterns shown in Fig. 6. For methylated cyclodextrin complexes, the release rates from the polyethylene glycol base were much greater than those from the fatty base, reflecting the lesser interaction between the complex and the hydrophilic base. The rather small release rates observed for the 2 and 3 complexes from the fatty base may be due to their high binding affinity to the hydrophobic suppository base, since the methylated cyclodextrins are extremely surface active¹⁴ and oil soluble⁷ compared with natural cyclodextrins.

The above data indicate that 2 is particularly useful for improvement of the release rate of flurbiprofen from both suppository bases. Thus, the present approach of using a rapid-dissolving form of the 2 complex is promising for improvement of the bioavailability of poorly water-soluble drugs.

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