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Spectroscopic characterization of ibuprofen/2-hydroxypropyl-β-cyclodextrin inclusion complex

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Abstract

Ibuprofen has been used widely as an anti-inflammatory and anti-pyretic agent. It is slightly soluble in water. Several investigators have conducted studies to improve the dissolution rate of ibuprofen using cyclodextrin complexation. In this study, the geometry and the structural features of the ibuprofen/hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complex were studied by NMR and fluorescence spectroscopy. The fluorescence intensity of ibuprofen increased as the concentration of HPβCD increased. Continuous variation plots by NMR study suggested that 1:1 stoichiometric complex was formed in solution. The spectral analysis of ^{1}H- and ^{13}C-NMR measurements showed that the signals of the aromatic protons of ibuprofen were shifted upfield, probably resulting from the interaction of HPβCD with aromatic ring of ibuprofen. We also postulated the optimized structure of ibuprofen/HPβCD inclusion complex through a molecular modeling program (SYBYL 6.4) and we found that the features of this postulated structure were in a good agreement with the ^{1}H- and ^{13}C-NMR spectra. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Ibuprofen; 2-Hydroxypropyl-β-cyclodextrin; Inclusion complex; NMR; Fluorescence; Conformation; Stability constant

1. Introduction

Ibuprofen, (±)-2-(*p*-isobutylphenyl)propionic acid (Fig. 1), is a non-steroidal anti-inflammatory drug. The formulation of ibuprofen has been problematic because of poor solubility and wettability of ibuprofen in water (Chow and Karara, 1986; Loftsson et al., 1993; Kagkadis, et al., 1996; Shakthshneider et al., 1996). Cyclodextrins are known to form inclusion complexes with hydrophobic molecules, and it has been used in formulations to improve water solubility (Chen et al., 1996; Roy and Guillory, 1996). The interior cavity

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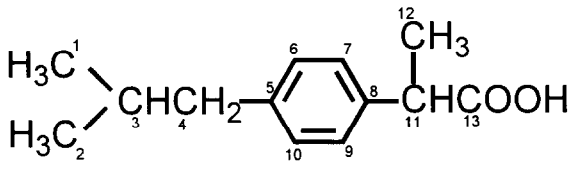

Fig. 1. Chemical structure of ibuprofen.

of cyclodextrin is rather hydrophobic, whereas the exterior is highly hydrophilic. This unique physical property of cyclodextrins allows various guest molecules to be included in hydrophobic cavities.

In recent years hydroxypropyl-β-cyclodextrin (HPβCD) has been extensively used in pharmaceutical formulations owing to its high water solubility (> 50 g/100 ml) and low toxicity (Chen et al., 1996). HPβCD has been used to improve dissolution rate and bioavailability of ibuprofen (Loftsson et al., 1993; Oh et al., 1993; Kagkadis, et al., 1996; Oh et al., 1997). The structural features of cyclodextrin complexes in solution have been studied by NMR (Bettinetti et al., 1991; Mulinacci et al., 1993; Nishijo et al., 1997).

In this study, the structure and geometry of ibuprofen/HPβCD complex in solution were elucidated by NMR and molecular modeling. The stability constant for inclusion complex formation was determined by fluorescence spectroscopy.

2. Materials and methods

2.1. Materials

Ibuprofen was obtained from Dong-A Pharm. (Korea). HPβCD, D_2O, NaOD and tetramethylsilane were purchased from Sigma (St. Louis, USA). All other chemicals were analytical reagent grade.

2.2. Methods

2.2.1. Measurement of NMR spectra

Ibuprofen was dissolved in pD 11 D_2O adjusted with NaOD, and it was mixed with HPβCD solution. ^{1}H-NMR and ^{13}C-NMR spectra were determined with a high-field NMR spectrometer (Varian unity 300 plus) at room temperature. All chemical shifts were assigned relative to tetramethylsilane, an external reference.

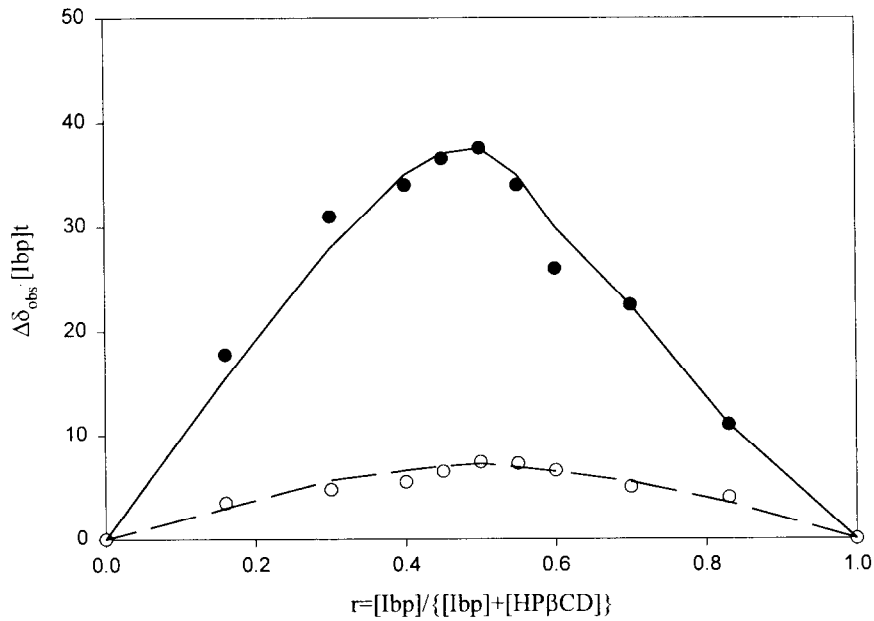

Fig. 2. Continuous variation plot (Job's plot) for protons of ibuprofen [●, H–C(7, 9)]; ○, H–C(6, 10)]. $\Delta\delta_{obs}$ is the difference in ^{1}H-NMR chemical shift with respect to the free ibuprofen.

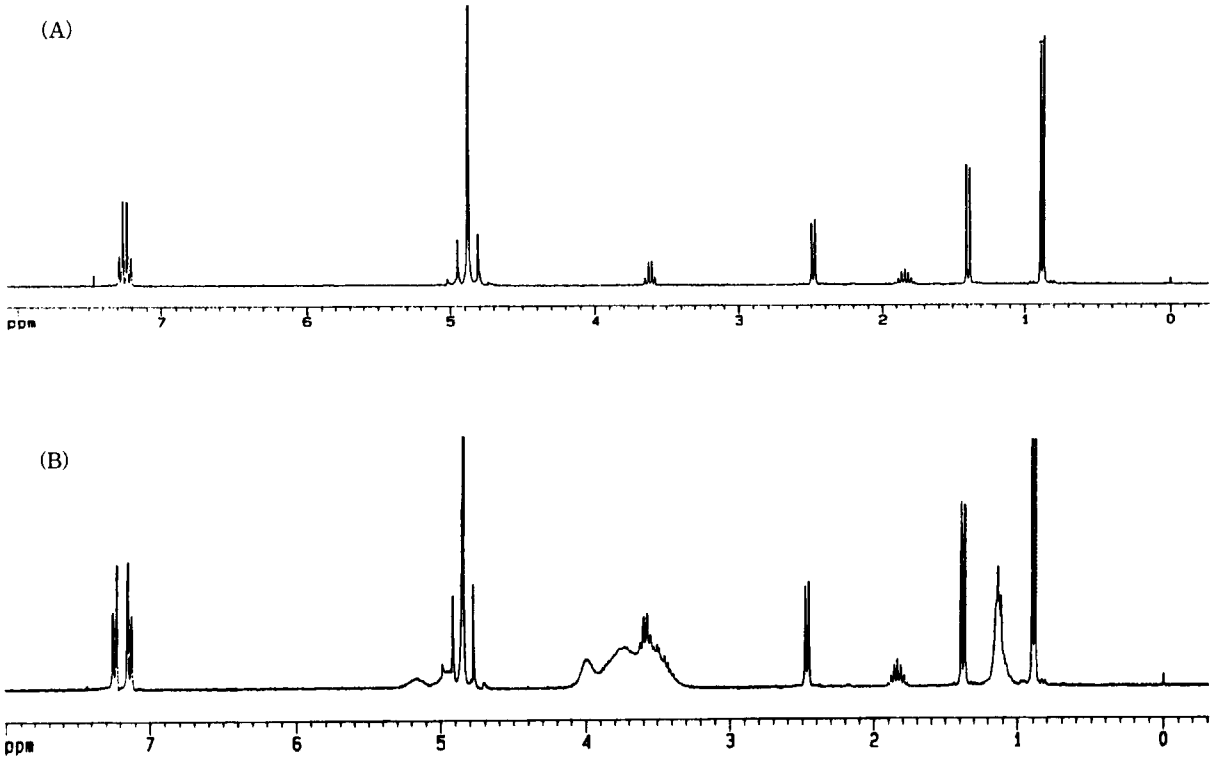

Fig. 3. ^{1}H-NMR spectra (300 MHz, 25°C) of ibuprofen (10^{-2} M) in the absence (A) and in the presence (B) of HPβCD (10^{-2} M).

2.2.2. Determination of stoichiometry of ibuprofen–HPβCD complex

The continuous variation method was adopted to determine the stoichiometry of the complex (Djedani et al., 1990; Bettinetti et al., 1991). The total concentration of the two species, ibuprofen and HPβCD, was kept constant, and the mole ratio was varied from 0 to 1. The differences of chemical shifts of NMR spectra between free ibuprofen and the complex were measured for a given mole ratio.

2.2.3. Docking study

A model of the inclusion complexes was constructed using a molecular graphic program, SYBYL 6.4 (Tong et al., 1991; SYBYL, 1997). The structures of ibuprofen and HPβCD were built separately, and the conformational energies were minimized using Tripos and MM3 force fields. A set of minimum-energy structures was chosen, and the guest molecule (ibuprofen) was introduced into the cavity of the host molecule (HPβCD) by docking procedure. Steric and electrostatic interactions between the host and the guest molecules were calculated by moving the atoms of the molecules back and forth. Because of the limitation in computing time, solvents or any other environmental factors were not considered. The simulation was carried out as the molecules were in vacuum environment. At the final cycle, both of the atoms of guest and host molecules were allowed to move freely, and a most stable docking position of the two compounds was obtained.

2.2.4. Fluorescence spectra

Fluorescence spectra were taken on a spectrofluorometer (Jasco FP-777, Japan) at different temperatures of 4, 20 and 37°C. The temperature was maintained with a circulating water bath. The temperature variations were kept within $\pm$ 0.1°C. The excitation wavelength was 264 nm and emis-

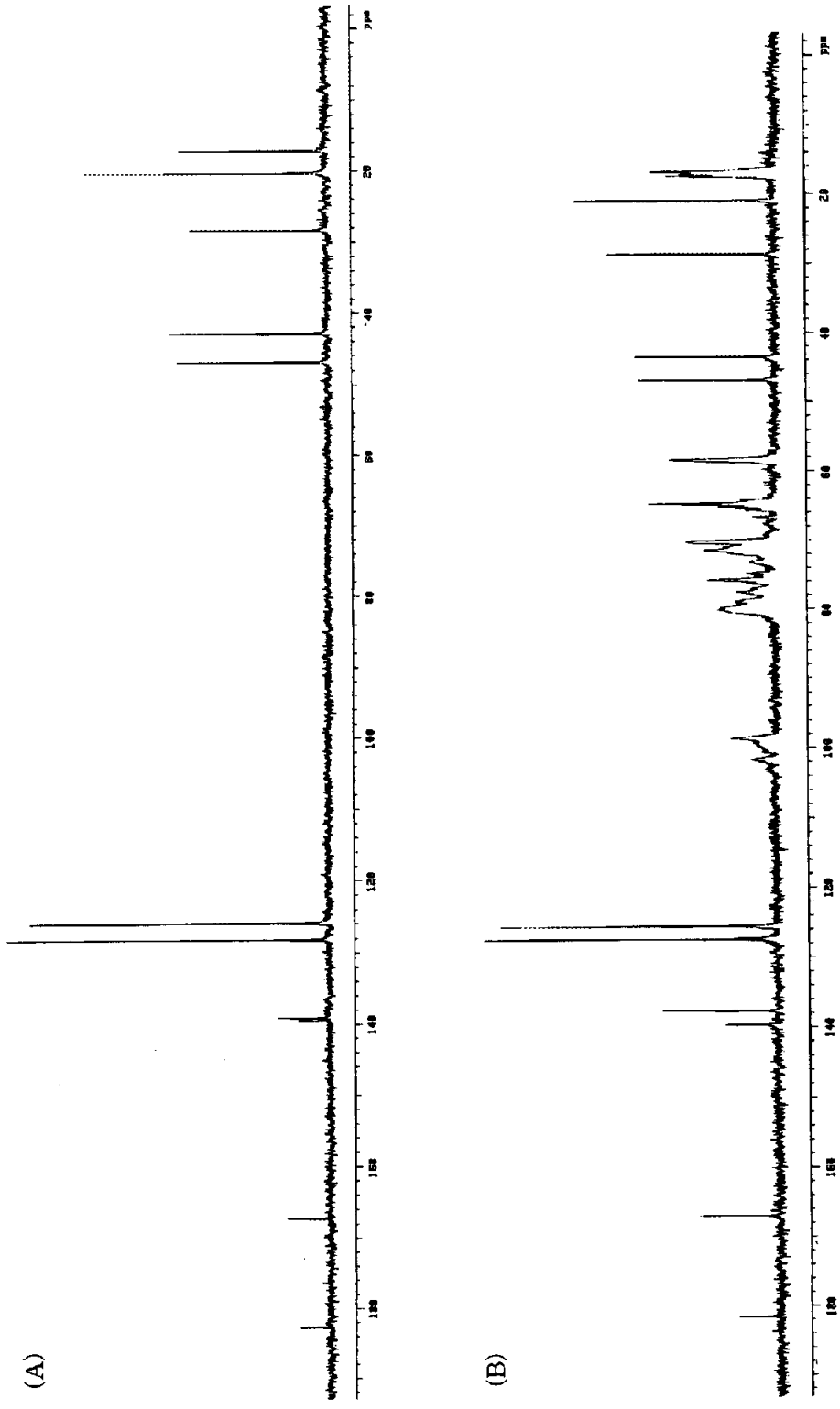

Fig. 4. ^{13}C-NMR spectra (300 MHz, 25°C) of ibuprofen (10^{-2} M) in the absence (A) and in the presence (B) of HPβCD (10^{-2} M).

Table 1
Changes of ^{1}H-NMR chemical shift (300 MHz, 25°C) of ibuprofen (10^{-2} M) at various concentrations of HPβCD

Hydrogen atom of ibuprofen	Concentration of HPβCD ($\times 10^2$ mol/l)				
	0.5	0.7	1	3	5
H–C(12)	−0.023	−0.0363	−0.066	−0.1409	−0.1524
	—	—	—	−0.1339	−0.1445
	−0.0233	−0.0364	−0.0662	−0.1413	−0.1523
	—	—	—	−0.134	−0.1449
H–C(4)	−0.0193	−0.0311	−0.0579	−0.1288	−0.1398
	−0.0196	−0.0313	−0.0581	−0.1297	−0.141
H–C(7, 9)	−0.1027	−0.1464	−0.2171	−0.3451	−0.3606
	−0.1036	−0.1473	−0.2174	−0.3472	−0.3632
H–C(6, 10)	−0.0434	−0.063	−0.1024	−0.1854	−0.196
	−0.0437	−0.0648	−0.1049	−0.1907	−0.2027

sion was scanned over the ranges of 200–350 nm. The fluorescence spectra of ibuprofen (10^{-4} M) were measured in the presence of different concentrations of HPβCD in phosphate buffer solution (pH 7). The stability constant was calculated using the Stern–Volmer equation (shown below).

$$F/F_o - 1 = K_c \times [CyD]_t$$

where F_o and F are fluorescence intensity of ibuprofen without and with HPβCD, respectively. $[CyD]_t$ is the total concentration of HPβCD and K_c is a stability constant of HPβCD. The overall thermodynamic parameters such as enthalpy ($\Delta H°$) and entropy ($\Delta S°$) were obtained from the plot of ln K vs $1/T$ (Uccello-Barretta et al., 1993).

Table 2
Changes of ^{13}C-NMR chemical shifts (300 MHz, 25°C) of the ibuprofen (10^{-2} M) at various concentrations of HPβCD

Carbon atom of ibuprofen	Concentration of HPβCD ($\times 10^2$ mol/l)		
	1	5	10
C(12)	0.306	0.125	0.255
C(4)	−0.065	0.631	0.87
C(6)	−0.25	−0.33	−0.37
C(7)	−0.34	−0.74	−0.91
C(8)	−0.53	−1.49	−1.85
C(5)	−0.17	0.07	0.15

3. Results and discussion

NMR spectroscopy was used to determine the structure and geometry of inclusion complex of ibuprofen and HPβCD. To determine the stoichiometry of the complex, the continuous variation method was employed (Fig. 2). Fig. 2 shows the Job's plot of the $\Delta\delta \cdot$[ibuprofen]$_t$ vs the mole ratio of ibuprofen, r. The Job's plot showed a maximum at $r = 0.5$, indicating 1:1 stoichiometry of complex (Uccello-Barretta et al., 1993).

The ^{1}H-NMR spectra of ibuprofen dissolved in D_2O in the absence and presence of HPβCD are shown in Fig. 3. In the presence of HPβCD, the signals of the aromatic protons (δ: 7.2–7.5) were shifted upfield. Table 1 shows the induced chemical shifts of ibuprofen at various concentrations of HPβCD. The extent of chemical shifts of ibuprofen/HPβCD complex depended on the concentration of HPβCD. The upfield shift for the resonance of aromatic proton indicates that the aromatic portion of ibuprofen is mainly involved in the complex formation with HPβCD. Upon inclusion into the HPβCD cavity, the methyl protons [H–C(12)] attached to the asymmetric carbon atom of ibuprofen showed the multiplicity of NMR signal. Although these phenomena were hardly detectable at low concentrations of HPβCD, it became apparent as the concentration of HPβCD increased. Similar results have been

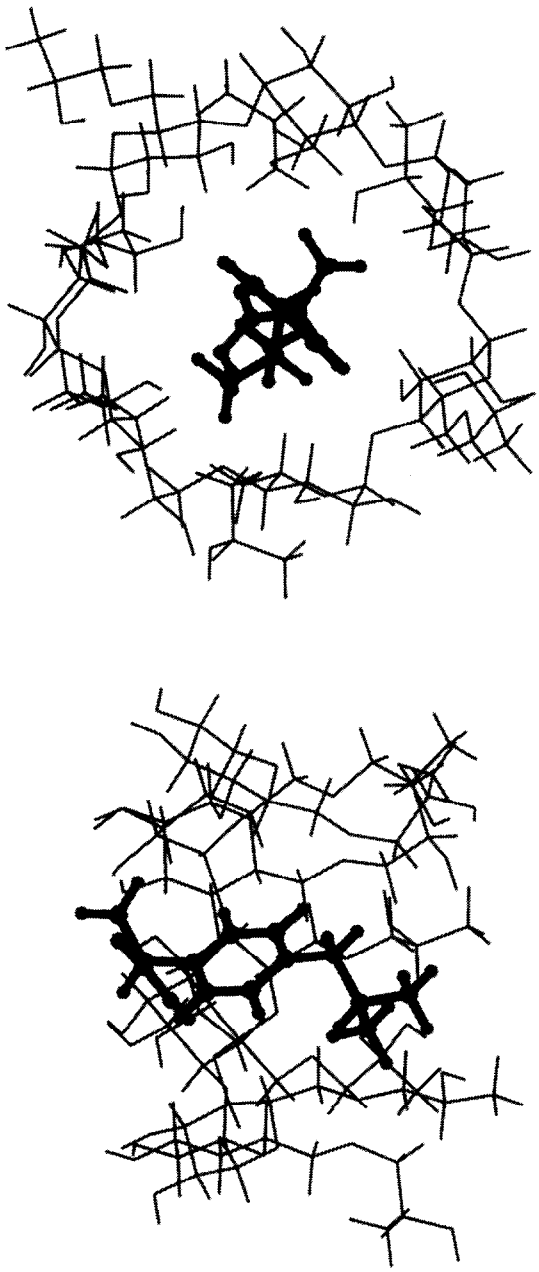

Fig. 5. A proposed model of the inclusion complex by SYBYL. Left, side view; right, top view.

reported by other researchers. Roy and Guillory (1996) reported the similar multiplicity of the NMR signals for the *N,N*-dimethyl group of the cyclopentolate in the presence of the substituted β-cyclodextrins. They interpreted that the tail of the cyclopentolate molecule lied flat on the surface of the cyclodextrin ring, so the two methyl groups were placed in different environments and the peak was split into two singlets. Farkas et al. (1993) reported that the enantiomers of ibuprofen were separated using a β-cyclodextrin silica stationary phase. Amato et al. (1992) also reported that in the presence of the chiral cyclodextrin, some proton signals of racemic guest molecule appeared in duplicate for diastereoisomeric pairs. Our experimental results also suggest that the multiplicity of signal might have arisen from the differences of shielding extent exposed to methyl protons.

^{13}C-NMR spectroscopy is known to be one of the most useful methods in the analysis of the structure and molecular dynamics of cyclodextrin inclusion complex in aqueous solution (Bettinetti et al., 1991). ^{13}C-NMR spectra of ibuprofen alone and its complex with HPβCD are shown in Fig. 4. The aromatic carbons (δ: 126–140) and aliphatic carbons (δ: 20; 43; and 47) of ibuprofen showed upfield and downfield shifts, respectively in the presence of HPβCD. Table 2 shows the changes of ^{13}C chemical shift at various concentrations of HPβCD. As the concentration of HPβCD increased, the chemical shifts for the aliphatic C-4 and C-12 atoms of ibuprofen slightly moved downfield, indicating that the aliphatic carbon atoms of ibuprofen experience diminished rotational freedom on complexation (Bettinetti et al., 1991). Meanwhile, the upfield shift of the resonance of the aromatic carbons could be attributed to the interaction with HPβCD. These results suggest that the aromatic ring of ibuprofen may be completely included within HPβCD cavity. This postulation is also supported by other report. Matsubara et al. (1997) reported that the carbon atoms included within the hydrophobic cavity of cyclodextrins are largely shielded compared with the carbon atoms located around the wider secondary hydroxyl side of the cavity. Moreover, these upfield shifts have been attributed to the changes of the electric environment of carbon atom, that is, from aqueous solvent (high dielectric constant) to non-

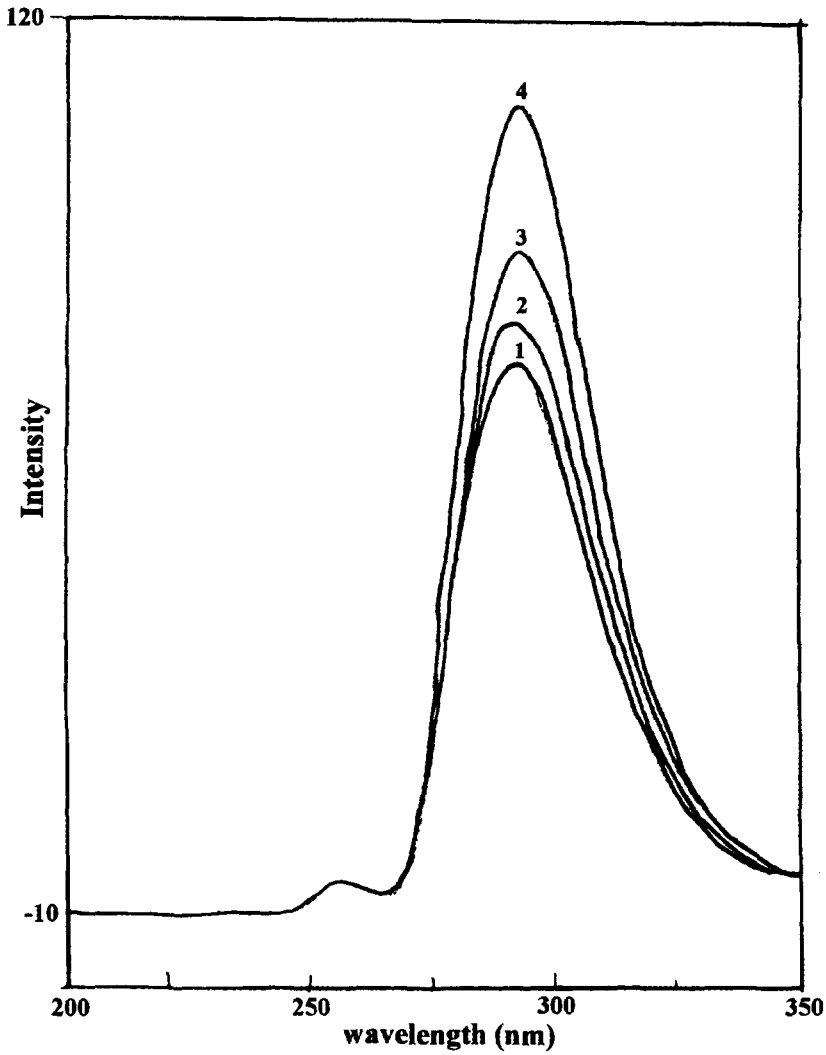

Fig. 6. Fluorescence spectra of ibuprofen (1.0×10^{-4} M) at pH 7, at 20°C in the presence of HPβCD. The concentration of HPβCD is (1) 0; (2) 0.4×10^{-4}; (3) 1×10^{-4}; (4) 3×10^{-4} mol/l.

polar cavity of HPβCD (low dielectric constant) (Choi, 1992).

Based on the ^{13}C- and ^{1}H-NMR data, the structure and geometry of the host and guest molecules were built using a molecular graphic program. A minimum-energy ensemble obtained by the docking study showed that ibuprofen molecule was set at the center of HPβCD cavity (Fig. 5). This model showed that the phenyl group of ibuprofen was surrounded by the pyranose rings of cyclodextrin, and the isopropyl group of ibuprofen was wrapped in hydroxypropyl groups of HPβCD molecule. The proposed model is quite in accordance with the experimental results from NMR studies.

Fig. 6 shows the fluorescence spectra of ibuprofen measured at pH 7 in the absence and presence of HPβCD at room temperature, respectively. Fluorescence intensities of ibuprofen increased with the increase of the HPβCD concentration. It is generally accepted that the fluorescence intensity increases as the co-planarity of aromatic ring

Table 3
Thermodynamic parameters for inclusion complex formation of ibuprofen with HPβCD

Temperature (°C)	K_c (M^{-1})	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (e.u.)
4	703	−3.61	—	—
20	533	−3.66	−2.7	3.06
37	412	−3.71	—	—

increases in non-aqueous solvent or as the movements of the molecules are more restricted in a viscous solution (Nishijo et al., 1991). It is believed the increase in fluorescence intensity in our experiment can be attributable to both cases.

Assuming 1:1 stoichiometry, we applied Stern–Volmer equation to calculate the stability constant (K_c). The K_c values determined at 4, 20, and 37°C are shown in Table 3. These values are well in accordance with our previous results obtained from phase-solubility or UV absorption difference method (Oh et al., 1993). Loftsson et al. (1993) also determined the stability constant to be 210 and 50 M^{-1} at pH 6.24 and 7.54, respectively, at 30°C. These values are within the range of our experimental results considering the differences in experimental conditions. However, Kagkadis et al. (1996) reported the stability constant to be 2480 M^{-1} at 25°C using the solubility method. The large difference in K_c values may be caused by the differences in experimental conditions such as pH and temperature.

The van't Hoff plot was obtained by plotting log K_c against the reciprocal of absolute temperature (Fig. 7). The enthalpy ($\Delta H°$) for the complexation was determined from the slope of the plot. Table 3 shows the thermodynamic parameters for inclusion complex formation of ibuprofen and HPβCD. The signs of apparent enthalpy and entropy of complexation were negative and positive, respectively. Negative enthalpy value means that the system is releasing energy upon complexation accompanying dipoles and van der Waals interaction. In addition, this loss of heat upon

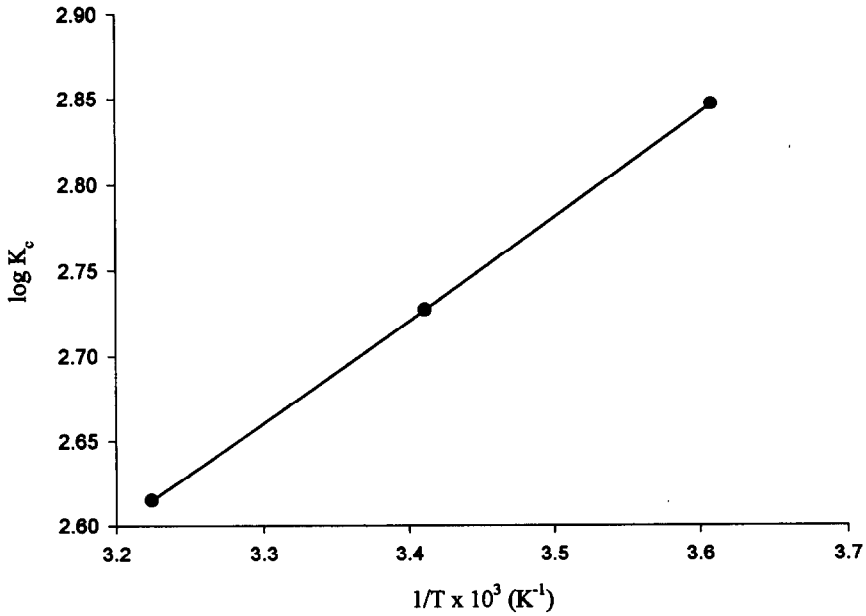

Fig. 7. The van't Hoff plot for ibuprofen.

complexation could correspond to the energy released by the enthalpy rich water molecules trapped within the cyclodextrin cavity (Menard et al., 1990). Positive entropy value means that the randomness of the overall system increases upon complexation. As the inclusion complex was formed, the water molecules surrounding the cyclodextrin in orderly fashion were disrupted and squeezed out into a more randomized structure, leading to the increase of the entropy of the overall system. According to Nishijo et al. (1997), the negative changes in enthalpy and positive changes in entropy on complexation are mainly caused by the van der Waals–London dispersion force and hydrophobic interaction.

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