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Two types of inclusion realized in the complexation between phenobarbital and 2-hydroxypropyl-ß-cyclodextrin in aqueous solution[1]

Hatsumi Aki*, Tokihiro Niiya, Yukiko Iwase, Magobei Yamamoto

Faculty of Pharmaceutical Sciences, Fukuoka University, 8-19-1 Nanakuma, Fukuoka 814-80, Japan

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Abstract

The inclusion complexation of phenobarbital (PHB) with 2-hydroxypropyl-ß-cyclodextrin (HPCyD) in aqueous solution has been investigated by microcalorimetry, ^{13}C-NMR spectroscopy and molecular dynamics simulations. Two different types of PHB-HPCyD inclusion complexes at 1 : 1 stoichiometry were realized by un-ionized PHB. In the first type of inclusion with higher affinity with HPCyD, the phenyl ring of PHB was included within the HPCyD cavity, whereas in the second type, the barbituric acid ring seemed to penetrate into the cavity. The ethyl side-chain remained outside of the cavity in both types. Complexation was independent of the concentrations of both PHB and HPCyD. The first type of inclusion was characterized by an entropy-driven reaction associated with constant values of ΔG_1 (-2.69 ± 1.0 kJ mol^{-1}), ΔH_1 (-3.73 ± 0.86 kJ mol^{-1}), and ΔS_1 (77.5 ± 1.5 J mol^{-1} K^{-1}) at various pH, and the hydrophobic interaction dominated the stabilization of the complex. The second type was characterized by large negative values of ΔH_2 (-19.2 ± 0.7 kJ mol^{-1}) and small ΔS_2 (8.6 ± 2.5 J mol^{-1} K^{-1}) at pH below 7.0, reflecting van der Waals' and/or electrostatic interactions, and all the thermodynamic parameters markedly decreased at pH>8.0. ^{13}C-NMR chemical shifts of barbituric acid ring and of a phenyl ring substituted at C5 on barbituric acid ring were significantly moved upfield upon penetrating into HPCyD cavity. © 1998 Elsevier Science B.V.

Keywords: 2-Hydroxypropyl-ß-cyclodextrin; Inclusion complex; Microcalorimetry; Molecular dynamics simulations; Phenobarbital; ^{13}C-NMR spectrometry

1. Introduction

Cyclodextrins (CyDs) are a well-known class of water-soluble hosts that form stable inclusion complexes with a variety of guests [1–3]. The inclusion of a guest in a CyD cavity is essentially a substitution of the included water molecules by the less polar guest. This process is an energetically favoured interaction of the relatively non-polar guest molecule with an imperfectly solvated hydrophobic cavity. Both entropy and enthalpy changes play a role in this process [3].

Barbiturates used widely as sedatives and anticonvulsants are attractive targets of molecular recognition to form inclusion complexes with CyDs, as they possess hydrophobic moieties of appropriate size, shape and polarity to fit in the corresponding CyD cavity. Furthermore, the improved aqueous solubility and the dissolution rates enhance their absorption and bioavailability [4–7]. The mechanism of the complex

*Corresponding author. Tel.: 0081 92871 6631; fax: 0081 92863 0389; e-mail: pp030168@psat.fukuoka-u.ac.jp

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formation in aqueous solution, however, remains under dispute [8–12] and the structures and driving forces based on their complexation and the stabilization in aqueous solution has not yet been clarified.

We reported the results of binding of some barbituric acid derivatives to 2-hydroxypropyl-ß-cyclodextrin (HPCyD) in aqueous solution at 25°C [13]. We examined the structural effects on the stability constants and thermodynamics, and explored models of inclusion complexes. In the solid state, a 1 : 1 stoichiometric complex was obtained by lyophilization. However, depending on the concentrations, 1 : 2, 2 : 1 and/or 2 : 2 complexes may also coexist in solution. To further detail the mechanism of the inclusion complexation, we examined the complexation between HPCyD and phenobarbital in aqueous solution by microcalorimetry and ^{13}C-NMR spectrometry. The structure geometries of the stable inclusion complex were determined by computer modeling using molecular dynamics simulations taking water molecules into account.

2. Materials and methods

2.1. Materials

Phenobarbital and phenobarbital sodium (PHB) were purchased from Wako (Osaka, Japan). 2-Hydroxypropyl-ß-cyclodextrin (HPCyD) was supplied by Toshin (Tokyo, Japan). The average values of molecular weight and substitution degree were determined to be 1500.4 and 6.3, respectively, by FAB mass spectrometry [14]. All other materials were of analytical reagent grade.

2.2. Flow microcalorimetry

Measurements were taken using a multichannel isothermal microcalorimeter (Thermal Activity Monitor 2277, Järfälla, Sweden) [15] and a differential flow microcalorimeter with a twin-cell flow system at 25.0±0.005°C [16]. The instruments were calibrated both electrically and chemically. PHB and HPCyD dissolved in buffer solutions. HPCyD at different concentrations (10^{-5}–10^{-3} M) were titrated sequentially with a constant concentration of PHB solution at equal flow rates (0.10–0.12 ml min^{-1}).

Assuming that PHB and HPCyD form several types of inclusion complexes in aqueous solution as follows,

$$\mathrm{PHB} + \mathrm{HPCyD} \leftrightarrow \sum_{i=1}^{n} [\mathrm{PHB} - \mathrm{HPCyD}]_i \quad (1)$$

the heat effect (Q_r) is proportional to the quantity of the complexes formed with PHB:

$$Q_r = \Delta H F_r \sum_{i=1}^{n} [\mathrm{PHB} - \mathrm{HPCyD}]_i \quad (2)$$

where ΔH is an apparent enthalpic change of the complexation and F_r is the constant flow rate of the calorimetric solutions. In the case of i=1, PHB forms only one type of inclusion complex with HPCyD at a molar ratio of 1 : 1. In the case of i is over 2, it is assumed that several types of inclusion complex are independently formed. Thus, Q_r in Eq. (2) can be expressed in the form.

$$Q_r = [\mathrm{PHB_t}] F_r \sum_{i=1}^{n} \frac{\Delta H_i K_i}{1 + K_i[\mathrm{HPCyD}]} \quad (3)$$

where [HPCyD] is free concentration of HPCyD, [PHB$_t$] the total concentration of PHB, K_i and ΔH_i are the association constant and the enthalpy change for the ith type of inclusion complex, respectively. The values of these parameters can be computed from the actual calorimetric data with an iterative non-linear least-square method using a FACOM M 380R computer program [17]. The initial values of ΔH_1 were experimentally estimated from the slope of the initial part of the titration curve.

2.3. ^{13}C-NMR spectrometry

^{13}C-NMR spectra were taken on a JEOL GX-400 interfaced with a DEC RSX-11 M computer operating at 100.5 MHz at 35.0°C. PHB and HPCyD were mixed in D_2O for 12 h at various ratios of HPCyD/PHB. The ^{13}C-Chemical shifts for free and complexed PHB were measured in the absence and presence of HPCyD, respectively and assigned based on the external standard sodium 2,2,3,3-d_4-3-trimethylsilylpropionate. Chemical shift changes were calculated according to the equation: $\Delta\delta = \delta_{(\mathrm{complex})} - \delta_{(\mathrm{free})}$. Measuring conditions were as follows: spectral width 25 kHz, pulse width 5.2 µs, acquisition time 0.655 s, pulse delay

0.500 s, data points 32.8 k. Spinning tubes of 5 mm o.d. containing 0.8 ml of solution were used. The apparent association constants (K_{nmr}) of complexes between PHB and HPCyD were estimated from the values of HPCyD-induced ^{13}C-NMR shifts, according to the following equation.

$$\Delta\delta = \frac{\Delta\delta_{max}}{2m[PHB_t]}\left(A - \sqrt{A^2 - 4m[PHB_t][HPCyD_t]}\right) \quad (4)$$

where

$$A = m[PHB_t] + [HPCyD_t] + 1/K_{nmr}$$

and m is the molar ratio of $[HPCyD_t]$ to $[PHB_t]$ and $\Delta\delta_{max}$ is the limiting value of this change for infinite complex concentration.

2.4. Computational details of molecular dynamics simulations

Molecular dynamics (MD) simulations of PHB-ß-cyclodextrin (PHB-ß-CyD) inclusion complexes in aqueous solution were performed using the AMBER program (version 4.0) running on an NEC UP4800/650 computer [18]. The geometries of ß-cyclodextrin (ß-CyD) and PHB were optimized using the MM3 program [19]. An inclusion complex was placed in the center of 6 Å-cubic box filled with TIP3P water molecules [20], and subjected to energy minimization to obtain more realistic, low-energy starting structures for MD simulations using the Monte Carlo technique. The MD simulations were equilibrated by 30 ps (Δt=0.001 ps and 30,000 time steps) at a 293 K constant temperature. Intermediate structures were saved in a file after every 50 steps to obtain a representative, sequential set of structures generated during the simulation.

3. Results and discussion

3.1. Heat of reaction of PHB-HPCyD inclusion complexation

The calorimetric titrations of HPCyD to PHB with the concentrations of 0.05, 0.10, 0.15, and 0.2 mM at pH 5.5 in 1/30 M phosphate buffer solution are shown in Fig. 1. In addition to these experiments, the reaction heat of HPCyD with PHB was measured at pH 5.5 in 1/15 and 1/60 M phosphate buffer. The calorimetric data were directly fitted to Eq. (3), where i was varied from 1 to 3 assuming 1 : 1 complex formation with one to three types of inclusion, respectively. In all the titrations, the data did not fit the complex formation with only one type of inclusion. Although the association constants of each type of inclusion, K_1, K_2 and K_3, were computed for the complex formation with three types of inclusion, the values of K_3 were much smaller than those of K_1 and K_2 and could not stabilize the inclusion complexes. The fit was better between the calorimetric titration data and the curves generated from the complex formed with two types of inclusion. The estimated values of the association and the thermodynamic parameters of the first and the second types in PHB-HPCyD inclusion complexation are listed in Table 1. The heat effect (Q_r) increased exothermically with increasing both concentrations of PHB and HPCyD (Fig. 1). However, the values of the parameters were essentially constant at all calorimetric titrations for PHB-HPCyD complexation in pH 5.5 phosphate buffer solutions: K_1=6.96±1.58×10^4 M^{-1}, ΔG_1=−27.6±0.13 kJ mol^{-1}, ΔH_1=−4.49±0.5 kJ mol^{-1}, and ΔS_1=77.7±0.3 J mol^{-1} K^{-1} for the first type of inclusion complexation, and K_2=5.93±1.20×10^3 M^{-1}, ΔG_2=−21.5±0.2 kJ mol^{-1}, ΔH_2=−18.3±1.5 kJ mol^{-1}, and ΔS_2=10.9±1.2 J mol^{-1} K^{-1} for the second type of inclusion

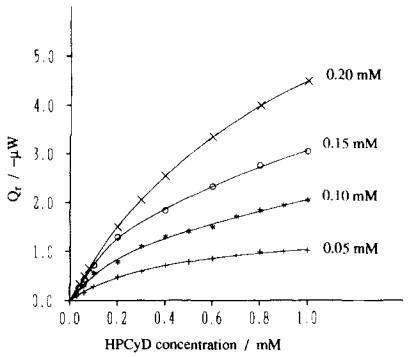

Fig. 1. Heat effect of the reaction between phenobarbital (PHB) and 2-hydroxypropyl-ß-cyclodextrin (HPCyD) at pH 5.5 in 1/30 M phosphate buffer and 25°C. The concentrations of PHB were 0.05, 0.1, 0.15, and 0.2 mM. Points show the means of experimental data from triplicate measurements and solid lines represent the computer-generated best fit curves assuming complex formation

Table 1
Thermodynamic and association parameters of PHB-HPCyD complexation in phosphate buffer pH 5.5 at 25°C

Concentration of PHB (mM)	First type of inclusion complex				Second type of inclusion complex			
	K_1/ (10^4 M^{-1})	$-\Delta H_1$/ (kJ/mol)	$-\Delta G_1$/ (kJ/mol)	ΔS_1/ (J/mol K)	K_2/ (10^3 M^{-1})	$-\Delta H_2$/ (kJ/mol)	$-\Delta G_2$/ (kJ/mol)	ΔS_2/ (J/mol K)
0.05 [a]	6.61±2.12	4.24±0.28	27.5	78.1	4.96±2.59	17.2±1.9	21.1	13.1
0.10 [a]	6.27±1.72	4.28±0.35	27.4	77.6	5.73±1.23	17.9±1.3	21.4	11.7
0.15 [a]	7.05±2.20	4.53±0.43	27.7	77.8	6.33±1.36	17.8±1.7	21.7	13.1
0.20 [a]	7.25±1.42	4.52±0.14	27.7	77.8	6.34±0.87	18.5±1.1	21.7	10.7
0.20 [b]	7.00±2.66	4.60±0.92	27.6	77.2	6.22±1.88	18.2±1.9	21.6	11.4
0.20 [c]	7.19±1.31	4.63±0.71	27.7	77.4	5.87±0.91	18.7±1.6	21.5	9.4
0.50 [a]	7.34±1.29	4.66±0.80	27.8	77.7	6.04±1.44	19.6±1.6	21.6	6.7

[a] 1/30 M Phosphate buffer,
[b] 1/15 M Phosphate buffer,
[c] 1/60 M Phosphate buffer.

complexation. The results suggest that there is no significant dependence of the PHB concentration or the variation of the ionic species in the reaction system on the obtained parameters and the stoichiometry.

3.2. *Influence of pH on PHB-HPCyD inclusion complexation*

PHB is essentially un-ionized at pH 5.5 because of the pK_a (pK_a=7.2). The phenyl ring and the barbituric acid ring of PHB are hydrophobic. Generally, the interaction with HPCyD is more powerful with an un-ionized, than an ionized guest. To evaluate the inclusion type contributing most to the stability of the complexation in aqueous solution, the heat of reaction between PHB and HPCyD was measured at various pH. Fig. 2 shows the calorimetric titration curves of HPCyD with PHB at pH 5.1, 6.6, 7.0, 7.4, 8.0, 9.2, and 10.2. The heat of reaction decreased at higher pH values (≥7.4), where PHB is ionized. The association constants and the enthalpic changes computed from the titration curves using a model of complex formation with two types of inclusion are listed in Table 2. The thermodynamic parameters at various pH values in the first and the second types of inclusion were summarized in Fig. 3. The profiles showed an increasing tendency to the thermodynamic behaviors of PHB-ß-CyD complex.

In the first type of inclusion complexation with a higher affinity of K_1, the thermodynamic parameters were not affected by the pH change (Fig. 3a): ΔG_1= -26.9 ± 1.0 kJ mol^{-1}, $\Delta H_1=-3.73\pm0.86$ kJ mol^{-t},

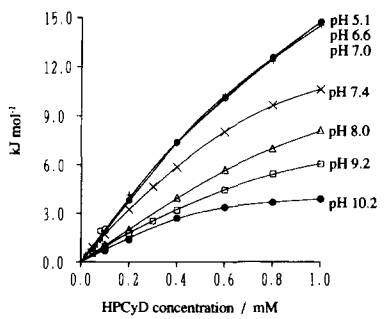

Fig. 2. Calorimetric titration curves for PHB-HPCyD complexation at various pH. In all the experiments, the concentration of PHB was 0.2 mM. Solid lines represent computer-generated gest fit curves.

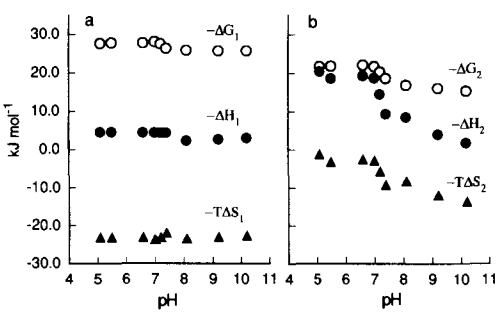

Fig. 3. The pH profiles of thermodynamic parameters for the first (a) and second types (b) of PHB-HPCyD inclusion complex in aqueous solution.

and ΔS_1=77.5±1.5 J mol^{-1} K^{-1}. The smaller negative values of ΔH_1 and the large positive ΔS_1 indicate hydrophobic interaction, reflecting complexation

Table 2
Association parameters and enthalpic changes computed from the calorimetric titration curves for PHB-HPCyD complexation with two types of inclusion

pH	$K_1/(10^4\ M^{-1})$	$K_2/(10^3\ M^{-1})$	$-\Delta H_1$/(kJ/mol)	$-\Delta H_2$/(kJ/mol)
5.1	8.971	5.115	5.816	21.32
5.5	8.834	5.805	5.328	22.51
6.1	7.341	6.338	5.594	19.47
6.6	7.160	7.206	5.309	19.21
7.0	8.995	7.443	4.293	16.99
7.2	5.801	3.402	4.251	14.39
7.4	4.056	0.7053	4.262	9.190
8.0	3.352	0.8464	2.199	9.343
9.2	3.630	0.6050	2.595	8.817
10.2	5.622	0.4806	2.832	3.104

between the phenyl ring of PHB and the hydrophobic cavity of HPCyD. In the second type of inclusion complexation with lower affinity of K_2, there was a marked decrease in the values of $-\Delta G_2$, $-\Delta H_2$ and ΔS_2 at pH values above 8 (Fig. 3b). The large negative values of ΔH_2 (-19.2 ± 0.7 kJ mol^{-1}) and the small values of ΔS_2 (8.6 ± 2.5 J mol^{-1} K^{-1}) at lower pH than 7.0 were owing to the van der Waals and/or electrostatic interaction. The barbituric acid ring of PHB are ionized, making the molecule more hydrophilic, as a result of which hydrophobic interactions decrease, adding to the repulsive effect and the electrostatic interactions.

3.3. NMR evidence of inclusions

The results of the calorimetric experiments revealed the coexistence of two inclusion types of PHB-HPCyD complexes in aqueous solution. To identify these structures, ^{13}C-NMR spectra of PHB-HPCyD complexes were measured in D_2O. Fig. 4 shows the induced changes ($\Delta\delta$) in ^{13}C-chemical shifts for PHB as a function of the HPCyD/PHB molar ratio. Negative and positive $\Delta\delta$ show upfield and downfield shifts, respectively. The parameters, K_{nmr}, $\Delta\delta_{max}$ and m, were calculated from the chemical shift changes using Eq. (4). The values of m for all carbons were obtained to be about 1.0 ($m=0.97\pm0.3$), indicating that a chemical shift change of every carbon was induced by PHB-HPCyD inclusion complexation at a ratio of 1 : 1. The chemical shifts of C2, C4 and C6 signals in the barbituric acid ring were shifted upfield by adding HPCyD. The maximum upfield shifts ($\Delta\delta_{max}$) were

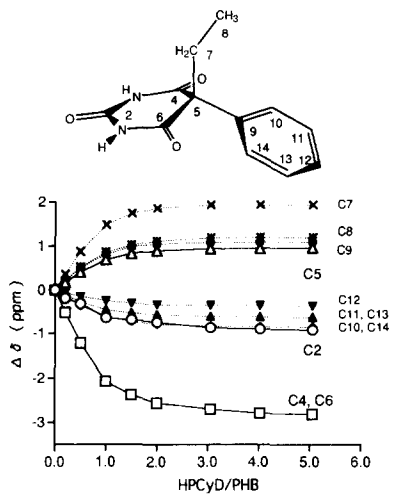

Fig. 4. Changes in ^{13}C-NMR chemical shifts of PHB in D_2O as a function of the molar ratio of HPCyD/PHB.

-3.03 ppm for C4 and C6 signals and -1.14 ppm for C2. The chemical shifts of carbonyl groups are easily affected by the electrical environment change around the carbonyl oxygen [21]. High upfield shifts have generally been noted for complexes in which the carbonyl group is preferentially bound in the HPCyD cavity [22]. The chemical shift changes of the carbons C10 to C14 in the phenyl ring also moved upfield although the increase was less significant than those of the barbituric acid ring, and reached the plateau around an HPCyD/PHB ratio of 1.0. The shielding effect to aromatic carbons of the phenyl ring was due to interaction with the ring current as a consequence of complete inclusion within the HPCyD cavity. This

could be considered as the result of both hydrophobic interactions and a ring current effect caused by inclusion of the phenyl ring moiety. The highest values of K_{nmr} computed from the chemical shift changes of C10 to C14 in the phenyl ring of PHB, $K_{nmr}=7.24\pm1.01\times10^4\ M^{-1}$, indicate that the phenyl side-chain easily penetrates into the HPCyD cavity. The signals of the ethyl side-chain (C7 and C8) moved downfield due to the hindrance of the rotation freedom in the cavity of HPCyD. Thus, two types of complexes were formed by the inclusion of either a barbituric acid ring or a phenyl ring of PHB within the HPCyD cavity.

3.4. Molecular dynamics simulations of PHB-ß-cyclodextrin inclusion complexes

The results showed that HPCyD probably includes either a phenyl ring or a barbituric acid ring of PHB. Both ring systems would be suitable as inclusion groups according to a preliminary inspection of the structure of PHB (Fig. 4) and the void volume of the HPCyD cavity. To study the inclusion behavior of PHB, the unsubstituted ß-cyclodextrin (ß-CyD) was adequate as a host molecule because HPCyD is an intrinsically amorphous mixture of many chemical individuals with various degrees of substitution. ß-Cyclodextrin should be regarded as a truncated cone rather than a cylinder, where all the primary hydroxy groups are on the narrower base and the secondary hydroxy groups are on the wider base of the toroid, when involved in inclusion complex formation. The pertinent structural features for four modes of inclusion complexes may be summarized as follows: Modes I and I′ with the phenyl ring of PHB inside the cavity whereas other groups are located at the rim of the secondary and primary hydroxy sides of ß-CyD, respectively; Modes II and II′ with the barbituric acid ring of PHB inside the cavity whereas other groups are located at the rim of the secondary and primary hydroxy sides, respectively.

The molecular dynamics simulation suggested that the PHB-ß-cyclodextrin inclusion complexes of Modes I and II shown in Fig. 5, are stable in aqueous solution, at least through the 30 ps simulation time. The calculated total energies at the equilibrated states were −12,088 and −12,041 kJ mol^{-1} for Modes I and II, respectively, indicating that the former was energetically more stable than the latter. On the other hand, PHB in Modes I′ and II′ gradually slipped out of the grip of the cyclodextrin ring after about 10 ps. These results are in agreement with observations by ^{1}H-NMR [8] and circular dichroism spectrometry [9] regarding complexation between PHB and ß-CyD.

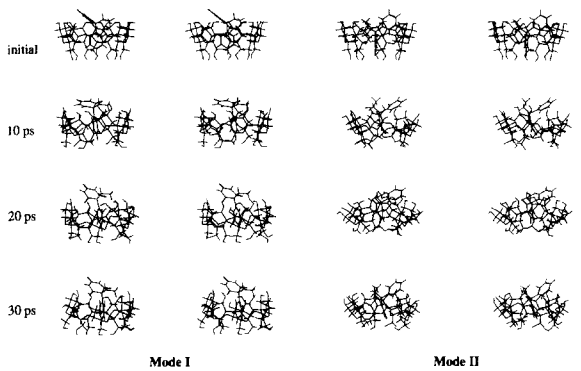

Fig. 5. Stereoscopic views of the stable inclusion types for PHB-ß-cyclodextrin complexation in aqueous solution. The geometries were calculated by molecular dynamics simulations (0–3 ps).

In the chemical structure of HPCyD, 2-hydroxypropyl groups are linked to the primary side-chains of ß-CyD. The phenyl ring of PHB easily penetrated into the cavity from the secondary hydroxy side of HPCyD, and the first type of inclusion (Mode I), which provides rigidity and stability to the complex, was formed in aqueous solution. The second type of inclusion through insertion of the barbituric acid ring into the cavity of PHCyD (Mode II), was affected by the pH of the solution, showing clear-cut inflections of all the thermodynamic parameters around the pK_a value. The hydrophobic and van der Waals' interactions contributed in establishing the first and the second types of inclusion complex, respectively.

References

[1] D. Duchêne, ed., Cyclodextrins and Their Industrial Uses; Editions de Santé, Paris (1987).
[2] J. Szetjli, Cyclodextrin Technology; Kluwer Academic Publishers, Dordrecht (1988).
[3] K.H. Frömming, J. Szetjli, Cyclodextrins in pharmacy; Kluwer Academic Publishers, Dordrecht (1993).
[4] K. Koizumi, K. Mitsui, K. Higuchi, Yakugaku Zasshi (J. Pharm. Soc. Japan) 94 (1974) 1515.

[5] K. Koizumi, H. Miki, Y. Kubota, Chem. Pharm. Bull. 28 (1980) 319.
[6] R. Iwaoku, K. Arimori, M. Nakano, K. Uekama, Chem. Pharm. Bull. 30 (1982) 1416.
[7] O. Shirakura, N. Nambu, T. Nagai, J. Incls. Phen. 2 (1984) 613.
[8] A.L. Thakker, P.V. Demarco, J. Pharm., Sci. 60 (1971) 652.
[9] A.L. Thakker, P.B. Kuehn, J.H. Perrin, W.L. Wilham, J. Pharm. Sci. 61 (1972) 1841.
[10] M. Otagiri, T. Miyaji, K. Uekama, K. Ikeda, Chem. Pharm. Bull. 24 (1976) 1146.
[11] A. Lopata, F. Darvas, A. Stadler-Szóke, J. Szejtli, J. Pharm. Sci. 74 (1985) 211.
[12] M. Suzuki, K. Ito, C. Fushimi, T. Kondo, Chem. Pharm. Bull. 41 (1993) 1444.
[13] H. Aki, M. Yamamoto, M. Ichimaru, Proc. Int. Cyclodextrins Symp., 7, 1994, p. 154.
[14] J. Pitha, T. Irie, P.B. Sklar, J.S. Nye, Life Sci. 43 (1988) 493.
[15] J. Suurkuusk, I. Wadsö, Chemica Scripta 20 (1982) 155.
[16] M. Yamamoto, H. Aki, J. Biochem. Biophys. Methods. 16 (1988) 271.
[17] H. Aki, M. Yamamoto, Biophys. Chem. 46 (1993) 91.
[18] D.A. Pearlman, D.A. Case, J.C. Caldwell, G.L. Seibel, C. Singh, P. Weiner, P.A. Kollman, AMBER 4.0 (1991), University of California, San Francisco.; P.K. Weiner, P.A. Kollman, J. Comp. Chem. 2 (1981) 287.
[19] N.L. Allinger, Y.H. Yuh, J.H. Lii, J. Am. Chem. Soc. 111 (1989) 8551.
[20] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, J. Chem. Phys. 79 (1983) 926.
[21] Y. Inoue, H. Hoshi, M. Sakurai, R. Chujo, J. Am. Chem. Soc. 107 (1985) 2319.
[22] R.I. Gelb, L.M. Schwartz, R.F. Johnson, D.A. Laufer, J. Am. Chem. Soc. 101 (1979) 1869.