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Isothermal titration calorimetry and ^{1}H NMR studies on host–guest interaction of paeonol and two of its isomers with β-cyclodextrin

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Abstract

Thermodynamic parameters of inclusion complex of β-cyclodextrin (β-CD) with paeonol and two of its isomers in aqueous solution have been determined with nano-watt-order isothermal titration calorimetry (ITC) and the host–guest inclusion structure has been investigated by using ^{1}H NMR spectra at 298.2 K. The analysis of thermodynamic data reveals that stoichiometry of β-CD complex with paeonol (Pae) or acetovanillone (Ace) is 1:1 whereas the inclusion complex of β-CD with 2-hydroxyl-5-methoxyacetophone (Hma) is in 1:1 coexistence with 2:1 stoichiometry. Further analysis indicates that formation of all the complexes is simultaneously driven by enthalpy and entropy, the inclusion complexation of Pae·β-CD, Ace·β-CD and Ham·β-CD$_2$ is predominantly driven by entropy while Ham·β-CD by enthalpy. The ^{1}H NMR spectra data provide clear evidence of the inclusion phenomena, which shows that the aromatic ring of the guest molecule insert itself into the torus from the narrow side of the cavity.

Keywords: β-Cyclodextrin; Paeonol; Molecular recognition; ^{1}H NMR; Isothermal titration calorimetry

1. Introduction

Paeonol (or peonol, ab. Pae), 2-hydroxyl-4-methoxyacetophone, is one of the major components of Moutan cortex, which has been used as a tranquillizer and an antihypertensive (Kim et al., 2004). It has analgesic, antipyretic and antibacterial properties and may find use in the treatment of arthritis and suppress ADP- or collagen-induced human blood platelet aggregation in a dose-dependent manner (Wu et al., 2003). Paeonol has also been shown to possess anti-inflammatory properties and to have a diuretic action (Chou, 2003). As one isomer of paeonol, 4-hydroxyl-3-methoxyacetophone (acetovanillone, trivial name: apocynin, ab. Ace) is a kind of active component extracted from the roots of *Picrorrhiza kurroa*, a small perennial herb that grows in the Himalays (Vejrazka et al., 2005), which is an NADPH oxidase inhibitor and has significant anti-inflammation properties (Peters et al., 2001; Van den Worm et al., 2001). Another isomer of paeonol, 2-hydroxyl-5-methoxyacetophone (ab. Hma), has little bioactivity and is often used as flavor. To understand the difference in bioactivity of the natural isomers, we need a bio-model molecule to be utilized to interact with them.

Cyclodextrins (CDs), a homologous of cyclic oligosaccharides whose molecules have hydrophilic out surfaces and a hydrophobic cavity in the centers are often used as imitation of enzyme (Hoskin et al., 1999; Loftsson and Brewster, 1996). They have been widely studied because of their ability to form inclusion compounds with a large variety of molecules (Ali et al., 2004) and to discern various types of guest molecules by selectively incorporating such molecules through size and polarity consideration (Szejtli, 1982). Up to now, several driving forces have been proposed for the inclusion of CDs with substrates (Connors, 1997): hydrogen binding, Van der Waals force, hydrophobic interaction and the release of 'high energy water' molecules from the cavity. In aqueous solutions, molecules bound within the inclusion complex are in a dynamic equilibrium with free molecules. Thus, cyclodextrins are able to enhance the aqueous solubility, chemical reactivity and spectral properties of many lipophilic drugs without changing their intrinsic ability to permeate lipophilic membranes (Duan et al., 2005). This makes cyclodextrins attractive as enabling pharmaceutical excipients (Loftsson and Masson, 2001). Among this class of "host" molecule, β-cyclodextrin (β-CD) with seven glucose

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units in its molecule is broadly used to enhance the solubility, stability and bioavailability of drugs (Uekama et al., 1983; Kang et al., 2002) and is the favorite encapsulation of drugs in the pharmaceutical industry, for its lower price and higher productive rate (Li et al., 2005). Inclusion complex of paeonol binding to β-CD has been gotten and identified by UV–vis Absorption spectrometry (Li and Ren, 2004), but to our best knowledge, there have been no report on direct investigating the complexation thermodynamic parameters of the inclusion interaction between β-CD and Pae; Ace and Hma. Isothermal titration calorimetry (ITC) is an extremely powerful and highly sensitive technique that is capable of measuring the interaction of reacting species in solution and has hitherto been used with great success in the study of interaction between biomolecules in dilute aqueous solutions both from thermodynamic and kinetics points of view (Jelesarov and Bosshard, 1999). Recent development in the instrumentation for calorimetry allow for detection of very weak interactions involving low heats of binding on the order of micro-joules, thus making it suitable to evaluate weaker interaction associated with cyclodextrin complexation (Buckton and Beezer, 1991). The purpose of this presentation is to investigate complexation thermodynamics of β-CD with Pae and two of its isomers utilizing ITC. In order to relate the thermodynamic parameters with the microcosmic structures, we have also determined ^{1}H NMR spectra of the inclusion complexes.

2. Materials and methods

2.1. Materials

Paeonol, 2-hydroxyl-4-methoxyacetophone (Pae), acetovanillone, 4-hydroxyl-3-methoxyacetophone (Ace), 2-hydroxyl-5-methoxyacetophone (Hma) were purchased from Aldrich Chemical Co. and were ≥99% pure. They were used without further purification. The molecular structures for Pae, Ace and Hma are provided in Scheme 1. β-Cyclodextrin (β-CD, reagent grade) from Shanghai Chemical Reagent Company (Shanghai China) was purified twice by recrystallization in redistilled water and dried under reduced pressure at 353 K for 48 h prior to use. The reagents were stored over P_2O_5 in a vacuum desiccator at the room temperature.

2.2. Solutions preparing

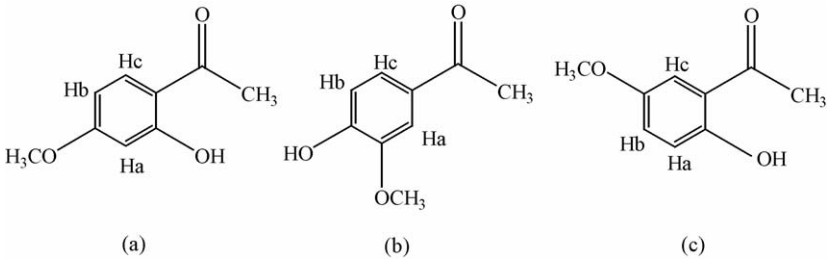
All solutions were prepared with triply distilled water (no buffer was used). 10 mM β-CD was prepared by dissolving 0.11352 g of β-CD in 8 mL of triply distilled water, and then the volume of the solution was made up to 10 mL. 1.00 mM Pae, 3.00 mM Ace and 2.00 mM Hma were prepared by dissolving 0.04154 g Pae, 0.12462 g Ace and 0.08308 g Hma, respectively, in 240 mL of triply distilled water. The solutions were placed in 35–40 °C water bath so as to make the solid reagents dissolved, and then the volume of the solutions were made up to 250 mL at 25 °C. Mass of each sample was weighed by using a Mattler Toledo AG 135 balance precise to ±0.00001 g.

2.3. Isothermal titration calorimetry (ITC)

The nano-watt isothermal titration microcalorimeter was a calorimeter supported by Thermometric 2277, Thermal Activity Monitor (Thermometric, Sweden), controlled by Digitam 4.1 software. The instrument had an electrical calibration with a precision better than ±1% and the accuracy was regularly determined by measuring the dilution enthalpy of a concentrated sucrose solution (Bai et al., 2002). For the measurement of host (β-CD)–guest (Pae, Ace and Hma) solutions, The 1 mL sample cell and reference cell of the calorimeter made from stainless steel were initially loaded with 500 μL 1.00 mM Pae (2.00 mM Hma or 3.00 mM Ace) solution and 620 μL pure water, respectively. A 10.00 mM β-CD solution was injected into the stirred sample cell in 30 portions of 8 μL using a 250 μL Hamilton syringe controlled by a Thermometric 612 Lund Pump. The interval between two injections was 45 min, which was sufficiently long for the signal to return to the baseline. The system was stirred at 50 rpm with a gold propeller. To deduct the dilution heats of guest (Pae, Ace and Hma) and the β-CD solutions, titration experiments were also performed for β-CD solution dropped into pure water and pure water into the guest solutions, respectively. The dilution heat of the two experiments was found to be negligible. All experiments were performed at a fixed temperature of (298.2 ± 0.1) K and repeated thrice. The reproducibility was within 4%. A representative titration curve was given in Fig. 1.

2.4. ^{1}H NMR measurements

^{1}H NMR spectra were recorded on a FT-NMR 1500A model 400M superconductive Nuclear Magnetic Resonance Instrument (American Varian Co.). All the experiments were recorded using D_2O (99.9% isotopic purity, Beijing Chemical Reagents company, Beijing China) as solvent. The solutions were transferred in 5 mm NMR tubes, giving a sample total volume of



Scheme 1. Molecular structure of the three chemicals, (a): paeonol (Pae); (b): acetovanillone (Ace); (c): 2-hydroxyl-5-methoxyacetophone (Hma).

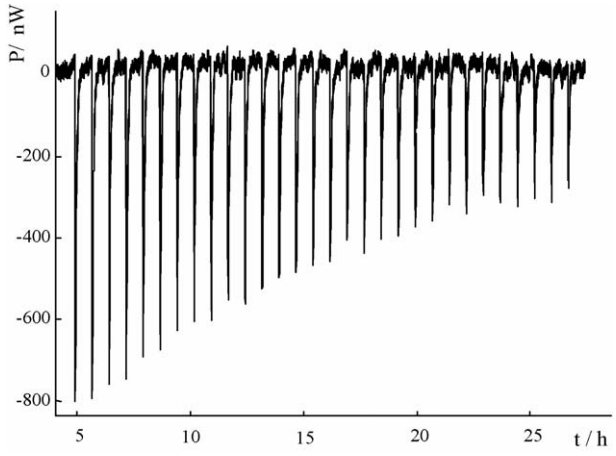


Fig. 1. Variation of heat-flow/electrical power P as a function of time t, titrant: 10.00 mM β-CD; titrand: 3.00 mM Ace.

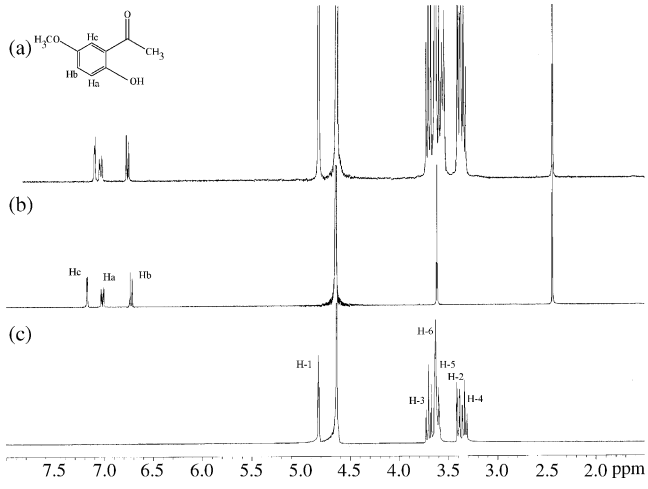


Fig. 2. ^{1}H NMR spectra of β-CD, Hma, β-CD–Hma–water system, (a): [β-CD] = 13.3 mM, [Hma] = 4.5 mM; (b): [Hma] = 4.5 mM; (c): [β-CD] = 13.3 mM.

500 μL. The probe temperature was regulated to 298.2 K. The resonance at 4.64 ppm due to residual solvents, presented as impurities (H_2O and HDO) was used as internal reference. ^{1}H NMR, the representative spectra of the β-CD in the presence as well as absence of the guest molecule and the guest molecule without β-CD were given in Fig. 2.

3. Results and discussion

3.1. Thermodynamic data analysis

3.1.1. Analysis process

To evaluate the standard enthalpy of formation for inclusion complex of drug with cyclodextrin in aqueous ΔH_i°, apparent equilibrium constants β_i for overall reactions have been defined as follow:

$$\mathrm{M} + i\mathrm{L} = \mathrm{ML}_i \qquad \beta_i = \frac{[\mathrm{ML}_i]}{[\mathrm{M}][\mathrm{L}]^i} \qquad \Delta H_i^\circ \tag{1}$$

where $i = 1$ or 2 or 3. In Eq. (1), M or L can represent either guest (Pae, Ace and Hma) or host (β-CD), so there are five possible reaction models. The total concentration of M and L can be obtained from the expressions thereinafter

$$[\mathrm{M}]_0 = [\mathrm{M}]\left(1 + \sum \beta_i [\mathrm{L}]^i\right) \tag{2}$$

$$[\mathrm{L}]_0 = [\mathrm{L}]\left(1 + [\mathrm{M}]\sum i\beta_i [\mathrm{L}]^{(i-1)}\right) \tag{3}$$

The relationship between the overall equilibrium constants, β_i, and the steptwise equilibrium constants, K_i, is given by

$$\beta_i = \prod K_i \tag{4}$$

Thermodynamic parameters, ΔH_i° and β_i (or K_i), can be obtained by regression analysis (Isabel and Hallén, 1993). According to the thermodynamic formula:

$$\Delta G^\circ = -RT \ln K^\circ \qquad \Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{5}$$

standard changes of Gibbs free energy (ΔG°) and entropy effect ($T\Delta S^\circ$) of the formation of the complexes can be derived. We use the Ligand Binding Analysis process available within the software Digitam 4.1. After performing calculations for the five reaction models with Pae β-CD, we confirmed the formation of the inclusion complex of β-CD with Paeonol in 1:1 stoichiometry, as shown in Fig. 3. The best fit for the other two pairs of (host + guest) inclusion complex systems are shown in Figs. 4 and 5, and the corresponding ΔH°, ΔG°, and $T\Delta S^\circ$ for the three guest molecules + β-CD complex are listed in Table 1 with an uncertainty for ΔH° which is the mean deviation of three independent experiments.

3.1.2. Thermodynamic parameters

From Table 1, it can be seen that the stoichiometry of β-CD complex with Pae and Ace is 1:1 whereas the stoichiometry of complex of Hma with β-CD is 1:1 and 1:2. This discrepancy of stoichiometry can be attributed to their different structure. An explanation for this observation is that the different molecular structure of the guest makes the different length of the molecule diameter, so the different interaction leads to different stoichiometry.

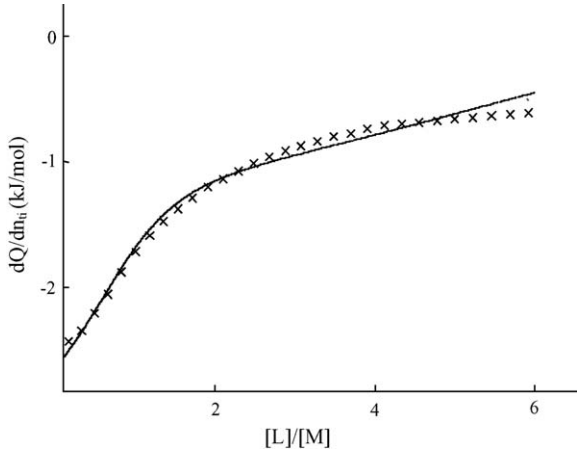


Fig. 3. Change rate of experimental thermal effect for inclusion process vs. the concentration ratio of host to guest, where points are gotten from experiments and the line is the result of calculated. L (host): β-CD; M (guest): Pae.

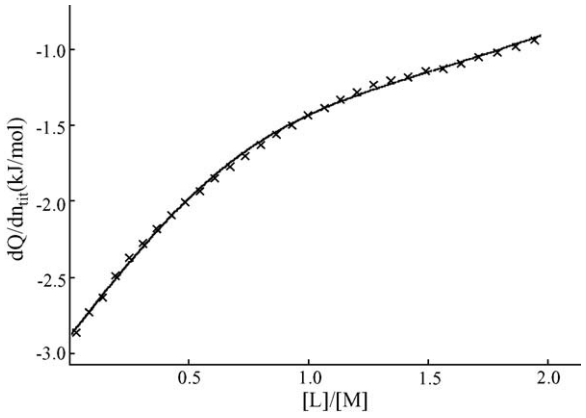


Fig. 4. Change rate of experimental thermal effect for inclusion process vs. the concentration ratio of host to guest, where points are gotten from experiments and the line is the result of calculated. L (host): β-CD; M (guest): Ace.

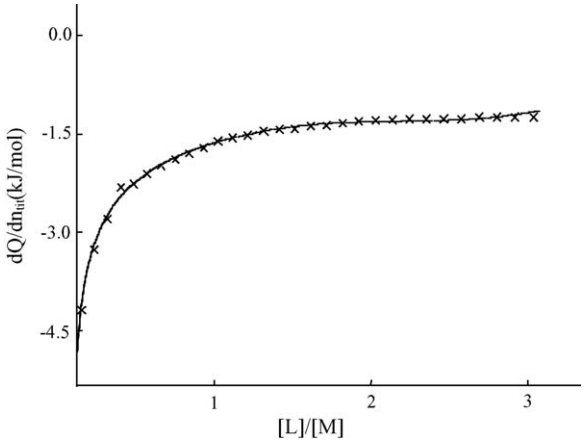


Fig. 5. Change rate of experimental thermal effect for inclusion process vs. the concentration ratio of host to guest, where points are gotten from experiments and the line is the result of calculated. L (host): β-CD; M (guest): Hma.

Standard formation enthalpies (ΔH°) of the host–guest inclusion compounds, listed in Table 1, are −3.35, −3.92, −9.35 and −3.06 kJ mol^{-1} for the inclusion complexes, Pae·β-CD, Ace·β-CD, Ham·β-CD and Ham·β-CD$_2$, respectively. These values are all negative, which indicate that the formation of host–guest inclusion complexes is weak exothermic process. Because the water molecules in the hole of a completely hydrated β-CD molecule will be released from the hydrophobic hole to the bulk aqueous phase, which involves an exothermic process, hydrophobic interaction between the host and the guest must be also quite weak.

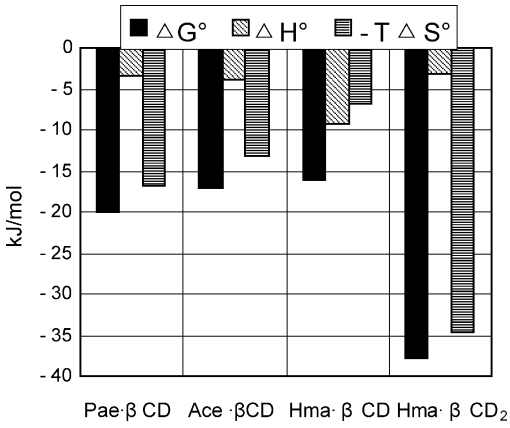


Fig. 6. Proportion by which the binding entropy contributes to the Gibbs energy of binding for each of the host–guest complexes that was studied.

The entropy effect ($T\Delta S^\circ$) of the three host–guest complexes in 1:1 stoichiometry, and one complex in 2:1 stoichiometry, Pae·β-CD, Ace·β-CD, Ham·β-CD and Ham·β-CD$_2$, are 16.8, 13.1, 6.8 and 34.7 kJ mol^{-1}, respectively, which are all positive and make evidently larger contribution to the negative change of standard Gibbs energy (ΔG°) than the heat effects do. The positive entropy effect may be due to the total result of the host–guest combination, which has negative contribution to entropy, and the releasing water molecules from the cavity, which has positive contribution to entropy. The differences in the proportion by which the binding entropy and enthalpy contribute to the Gibbs energy, which is illustrated in Fig. 6, reflect the differences in the interaction established between the various drug molecules with β-CD. The inclusion complexation of Pae·β-CD, Ace·β-CD and Ham·β-CD$_2$ is predominantly driven by entropy while the complexation of Ham·β-CD is predominantly driven by enthalpy.

The negative values of standard Gibbs energy change (ΔG°), which decided by enthalpy changes and entropy changes, indicate that formation of host–guest inclusion complexes in aqueous solution is spontaneous process. The stability of inclusion complexes (1:1 stoichiometry) decreased in the order Pae·β-CD > Ace·β-CD > Ham·β-CD obtained from the Gibbs energy changes of formation complexes of β-CD with three guests (Pae·β-CD: −20.1 kJ mol^{-1}, Ace·β-CD: −17.0 kJ mol^{-1} and Ham·β-CD: −16.1 kJ mol^{-1}, respectively). From the analysis hereinbefore it can be concluded that the different entropy change result in this different stability of the inclusion complexes. So the molecular recognition ability of the host (β-CD) for the three guests is mainly controlled by the difference in

Table 1
Stability constants (β), standard changes of enthalpy (ΔH°), Gibbs function (ΔG°) and products of temperature and entropy ($T\Delta S^\circ$) for the formation of complexes from the host (β-CD) with three guests (Pae, Ace and Hma) in aqueous solution at the temperature 298.2 K

Guest	Reaction model	ΔH° (kJ mol^{-1})	β_i (dm^3 mol^{-1})^i	ΔG° (kJ mol^{-1})	$T\Delta S^\circ$ (kJ mol^{-1})
Pae	M + L = ML[a]	−3.35 ± 0.49[b]	3.33×10^3	−20.1	16.8
Ace	M + L = ML[a]	−3.92 ± 0.13[b]	937.7	−17.0	13.1
Hma	M + L = ML[a]	−9.35 ± 0.76[b]	661.8	−16.1	6.8
	M + 2L = ML$_2$[a]	−3.06 ± 0.19[b]	4.06×10^6	−37.7	34.7

[a] L: host (β-CD); M: guest (Pae/Ace/Hma).
[b] The error of each ΔH° is from calculation according to results of three replicates.

Table 2
^{1}H chemical shifts corresponding to β-CD in the presence and absence of paeonol (Pae)

β-CD	H-1	H-2	H-3	H-4	H-5	H-6
$\delta_{(free)}$	4.936	3.516	3.815	3.451	3.725	3.744
$\delta_{(complex)}$	4.909	3.489	3.774	3.435	3.621	3.683
$\Delta\delta$[a]	−0.027	−0.027	−0.039	−0.017	−0.104	−0.061

[a] $\Delta\delta = \delta_{(complex)} - \delta_{(free)}$.

Table 3
^{1}H chemical shifts corresponding to β-CD in the presence and absence of acetovanillone (Ace)

β-CD	H-1	H-2	H-3	H-4	H-5	H-6
$\delta_{(free)}$	4.936	3.516	3.815	3.451	3.725	3.744
$\delta_{(complex)}$	4.915	3.498	3.763	3.439	3.622	3.696
$\Delta\delta$[a]	−0.021	−0.018	−0.052	−0.012	−0.103	−0.048

[a] $\Delta\delta = \delta_{(complex)} - \delta_{(free)}$.

entropy. It might be imagined that if there be some accepting site on bio-tissue similar to the CD molecule in structure, Pae must be most affinity to it.

3.2. ^{1}H NMR spectrophotometry

^{1}H NMR spectroscopy was used to obtain further information on β-CD aggregate formation. The induced shift, $\Delta\delta$, is defined as the difference in chemical shifts in the presence and absence of the other reactants. In the present case, the induced shifts were calculated by the following equation: $\Delta\delta = \Delta\delta_{(complex)} - \Delta\delta_{(free)}$. In this convention, positive and negative signs show a downfield and upfield shifts, respectively (Fernandes et al., 2003). Chemical shift variations of specific host or guest nucleus could provide evidence for the formation of inclusion complexes in solution, since significant changes in microenvironment are known to occur between the free and bound states. Information about the interaction of the three guests with β-CD from NMR was primarily inferred from the changes in chemical shifts. The ^{1}H chemical shifts of β-CD in the absence and presence of the three drugs are shown in Tables 2–4.

From Tables 2 and 3 it can be seen that the H-3 and H-5 protons, located inside the cavity, and the H-6 proton, located on the cavity rim at the narrow end of the molecule, are evidently shifted upfield, however, the upfield shifts of the signals due to H-1, H-2 and H-4 are appreciably. The upfield shifts of the protons located within or near the β-CD cavity (i.e. H-3, H-5, and H-6), as well as the minor modification observed for those at the exterior of the torus (e.g. H-1, H-2, and H-4) can be regarded as evidence of the existence of an interaction between the guest molecule and the interior of the host cavity, with a partial or complete inclusion on the torus and, hence, complexation (Fernandes et al., 2003).

Table 4
^{1}H chemical shifts corresponding to β-CD in the presence and absence of 2-hydroxyl-5-methoxyacetophone (Hma)

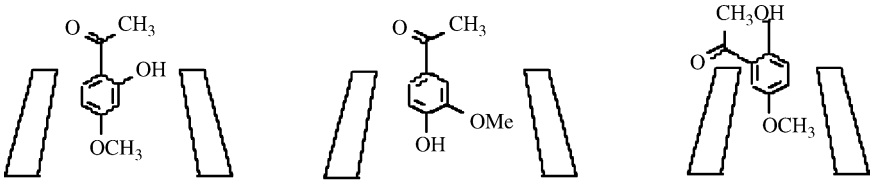
β-CD	H-1	H-2	H-3	H-4	H-5	H-6
$\delta_{(free)}$	4.936	3.516	3.815	3.451	3.725	3.744
$\delta_{(complex)}$	4.933	3.504	3.815	3.450	3.658	3.715
$\Delta\delta$[a]	−0.003	−0.012	0.000	−0.001	−0.067	−0.029

[a] $\Delta\delta = \delta_{(complex)} - \delta_{(free)}$.

It is well known that the magnetic anisotropy of an aromatic ring results in an upfield ^{1}H-chemical shift of protons located above (or below) the Π-electron cloud. Thus, the upfield shifts that observed for H-3, H-5 and H-6 protons of β-CD can be explained with the replacement of water molecules by the hydrophobic aromatic benzene ring of the three drug molecules inside the cavity. In fact, these shift, could be the result of anisotropic shielding induced by the 'ring-current' effect produced by the aromatic ring inside the β-CD macrocycle (Otagiri et al., 1975). Shift to higher fields of the protons located within the β-CD cavity suggested that a hydrophobic interaction was predominant between the drug and the CD (Fathy and Sheha, 2000). The upfield shift of the H-5 proton located on the inner surface at the primary hydroxyl group side was the most prominent ($\Delta\delta = -0.104$ to Pae·β-CD and $\Delta\delta = -0.103$ to Ace·β-CD, respectively.), followed by the H-3 proton lying on the inner surface of the cavity of the secondary hydroxyl group side ($\Delta\delta = -0.039$ to Pae·β-CD and $\Delta\delta = -0.052$ to Ace·β-CD, respectively.) and by the H-6 located at the rim of the narrow side of the β-CD molecule ($\Delta\delta = -0.061$ to Pae·β-CD and $\Delta\delta = -0.048$ to Ace·β-CD, respectively.) Because of the higher shielding effect on the H-5 proton with respect to H-3 and H-6, it can be hypothesized that Pae or Ace molecule preferentially insert its phenol group into the torus from the more inaccessible narrow side of the cavity, where the primary hydroxyl groups are located (Zhu et al., 2001).

By a similar analysis to Table 4, it can be concluded that the interaction between Hma molecule and the interior of the β-CD cavity is a partial inclusion on the torus for there is no change for the upfield shift of the H-3 proton lying on the inner surface of the cavity of the secondary hydroxyl group side (i.e. $\Delta\delta = 0.000$), and that Hma also penetrate the torus from the more inaccessible narrow side of the cavity because of the higher shielding effect on H-5 ($\Delta\delta = -0.067$) with respect to H-6 ($\Delta\delta = -0.029$). The difference is that the depth of aromatic benzene ring of Hma penetrating into the torus of β-CD is not as good as that of Pae (or Ace). So Hma has chance to associate with another β-CD molecule but the binding ability is weak, which accords with the conclusion obtained from the thermodynamics parameters (i.e. the stoichiometry of β-CD complex with Pae and Ace is 1:1 whereas the stoichiometry of complex of Hma with β-CD is 1:1 or 1:2). If we only observe the stability of the complexes in 1:1 stoichiometry by comparing the values of ΔG°, Hma·β-CD is the most unstable one, which is in good accordance with the NMR analysis. So we can give rough schemes of the inclusion complex listed in Scheme 2.

Furthermore, the proton chemical shift changes ($\Delta\delta$) of Pae, Ace and Hma summarized in Table 5 also are favor anterior conclusion. From Table 5 it can be seen that chemical shift changes of the aromatic protons and one branch chain (–OCH_3) of the three guest molecules (e.g. Pae: β-CD system: $\Delta\delta_a = 0.015$,



Scheme 2. The model structure complexes of host–guest in 1:1 stoichiometry. (a): Pae·β-CD; (b): Ace·β-CD; (c): Hma·β-CD.

Table 5
Variation in ^{1}H chemical shift ($\Delta\delta = \delta_{(complex)} - \delta_{(free)}$)of guest molecular during complexation

	Ha	Hb	Hc	H(–$COCH_3$)	H(–OCH_3)
Pae	0.015	−0.013	−0.081	−0.005	−0.016
Ace	−0.009	0.027	0.016	0.001	−0.005
Hma	0.047	0.028	−0.065	0.005	−0.014

$\Delta\delta_b = -0.013$, $\Delta\delta_a = -0.081$, $\Delta\delta_{-OCH_3} = -0.016$) are more obvious than that of protons in another branch chain (–$COCH_3$) (e.g. Pae: β-CD system: $\Delta\delta_{-COCH_3} = -0.005$), which indicate that the aromatic benzene ring of the three drug molecules is embedded into the torus of β-CD. Thus, the aromatic ring penetrates into the cavity of β-CD with hydrophobic interaction and results in the release of 'high energy water' molecules from the cavity, which provides negative ΔH° and positive ΔS° contribution (Manzoori and Amjadi, 2003). The entropy change is the main driving force for the complexation of β-CD with Pae, Ace and Hma, in the viewpoint of thermodynamics.

4. Conclusions

The inclusion interactions between three drug (Pae, Ace, Hma) chemicals and β-CD have been investigated systematically by isothermal titration calorimetry and ^{1}H NMR in aqueous solution at 298.2 K. The microcalorimetry allowed us obtained the standard enthalpy changes, stoichiometry, equilibrium constants, standard Gibbs energy changes and the entropy effect of the inclusion processes based on the directly calorimetric data utilizing non-linear simulation method. ^{1}H NMR spectra provide directly microcosmic evidence and showed that the interactions belong to an inclusion phenomenon since the modifications obtained for the signals have involved hydrogens that were oriented toward the cavity. The driving force for complexation is mainly hydrophobic interactions and the release of 'high energy water' molecules from the cavity. The stoichiometry of inclusion complex of β-CD with Pae and Ace is 1:1, and that of β-CD with Hma is 1:1 coexistence with 1:2, which exhibits the recognition function of the host molecules to the guest molecules. The different location of substitute groups on aromatic benzene ring make the difference size of guest molecule diameter, which influence the hydrophobic interaction between the host and guest molecules and causes different changes of thermodynamic functions. The result might be helpful to understand the different bioactivity of the chemicals.

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