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Experimental and Theoretical Studies on the Inclusion Complexation of β-Cyclodextrin with Phenothiazine Derivatives

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Abstract. Inclusion complexation of β-cyclodextrin (β-CD) with *N*-phenylphenothiazine (**1**), *N*-benzylphenothiazine (**2**) and *N*-phenethylphenothiazine (**3**) has been studied by means of UV-vis spectroscopy and molecular dynamics simulations. The association constants (K_a) were determined to be 126, 312 and 211 dm^3/mol for inclusion of β-CD with **1**, **2** and **3**, respectively. It shows that the K_a values are affected by the substituents of the guest compounds. The structures of the complexes and the conformation of the guest compounds bound by β-CD in the complex have been discussed.

Key words: β-cyclodextrin, inclusion complexation, molecular dynamics, phenothiazine derivatives.

1. Introduction

It is well known that the cyclodextrins (CDs) are water-soluble host compounds mostly composed of 6, 7 or 8 glucose units attached by α (1→4) linkages in α-, β- or γ-CD. One of the most important properties of the cyclodextrins is their ability to form inclusion complexes with many organic and inorganic substrates [1–4]. Among them, β-CD has been widely used as a receptor for many aromatic compounds, such as benzene, naphthalene, anthracene and their analogs and derivatives [5–8].

Phenothiazine derivatives have been the subject of study for decades because of their significant applications to drugs, dyestuffs and solar energy conversion materials [9–12]. The inclusion complex of β-CD with phenothiazine has been reported [13]. Recently, we studied the electron transfer reactions of *N*-substituted phenothiazines with 2,2,6,6-tetramethyl-4-acetyloxypiperidine oxoammonium ion in aqueous solution in the presence of β-CD. The electron transfer reactions were markedly influenced by the inclusion complexation of β-CD with phenothiazine derivatives [14, 15]. This finding led us to explore the structure of the complexes,

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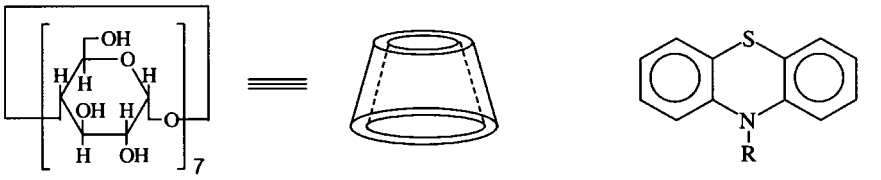
Table I. Some of the potential energy function parameters used in the calculations

Atoms	GROMOS87 atom types	Charges (e)
S	22	−0.1
N	31	−0.2
C-1, C-1′	17	0.05
C-6, C-6′	17	0.1
Bonds	Standard bond length (Å)	Force constants (kJ mol^{-1} Å^{-2})
–S–C	1.76	2510
>N–C	1.40	3347
>N–CH_2	1.48	3347
Bond angles	Standard bond angle (degree)	Force constants (kJ mol^{-1} Å^{-2})
S–C–C	120	418
C–S–C	100	460
C–N–C	120	418

particularly the conformation of the *N*-substituted phenothiazines bound by β-CD in the complex. In this paper, we report the inclusion complexation of β-CD with *N*-phenylphenothiazine (**1**), *N*-benzylphenothiazine (**2**) and *N*-phenethylphenothiazine (**3**) by a spectroscopic method and molecular dynamics simulation.



β–CD

R = phenyl (**1**), benzyl (**2**), phenethyl (**3**)

2. Experimental

2.1. INSTRUMENTATION

The absorption spectra of *N*-substituted phenothiazines in water in the presence of β-cyclodextrin were measured with a Shimadzu UV-2100 spectrophotometer. Elemental analyses were carried out with an Italian 1106 analyzer. ^{1}HNMR spectra were recorded on a Bruker AM 400 NMR spectrometer using $CDCl_3$ as solvent and TMS as reference.

2.2. MATERIALS

β-Cyclodextrin was obtained commercially and purified by recrystallization from distilled water three times and dried at 90 °C in vacuum for 24 hrs before use. Triply distilled water was used in the experiments. *N*-Substituted phenothiazines were synthesized according to the literature [16], the analytical data are as follows:

1, m.p. 92–93.5 °C. *Elemental anal.: Calc* for $C_{18}H_{13}NS$, C, 78.54, H, 4.73, N, 5.09; found: C, 77.64, H, 4.94, N, 4.92. ^{1}HNMR: δ_H: 6.61–6.80 (8H, m, ArH), 7.45 (5H, m, phenyl).

2, m.p. 90–91 °C. *Elemental anal.: Calc*. for $C_{19}H_{15}NS$, C, 78.89, H, 5.19, N, 4.84; found: C, 78.81, H, 5.28, N, 4.98. ^{1}HNMR: δ_H: 5.06 (2H, s, CH_2), 6.40–7.12 (8H, m, ArH), 7.30 (5H, m, phenyl).

3, m.p. 71–71.5 °C. *Elemental anal.: Calc*. for $C_{20}H_{17}NS$, C, 79.21, H, 5.61, N, 4.62; found: C, 79.41, H, 5.83, N, 4.27. ^{1}HNMR: δ_H: 3.14 (2H, t, CH_2Ph), 4.12 (2H, t, N-CH_2), 6.96–7.26 (8H, m, ArH), 7.36 (5H, m, phenyl).

2.3. PREPARATION OF SAMPLES

Aqueous solutions of *N*-substituted phenothiazine (5.0×10^{-6} to 1.8×10^{-5} mol/dm^3) containing β-CD in the range from 6.5×10^{-4} to 1.6×10^{-3} mol/dm^3 were prepared. The solutions were stirred for 3 hrs and then allowed to stand for several hrs at room temperature. The absorption spectra were recorded at 25 °C.

3. Molecular Dynamics Calculation

The structural data of β-CD were taken from the neutron diffraction crystallographic study [17]. Energy was minimized by 100 steepest descent steps and then followed by 200 conjugate gradient steps. Starting from the energy minimized structure, 15 ps molecular dynamics simulation was carried out. The final structure was used to build the initial structure of the complexes.

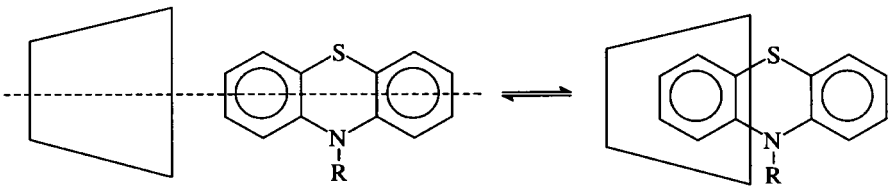
The initial structures of the phenothiazine derivatives were built by standard bond lengths and bond angles given in the GROMOS87 potential energy function [18].

The complete initial structure of β-CD·*N*-substituted phenothiazine complexes were obtained by putting the phenothiazine framework in the β-CD cavity along the axis and the substituent pointing away from the axis. In the resulting structures, approximately a half of the phenothiazine (one aromatic ring and a half of the heterocyclic ring) is embedded in the cavity of β-CD, and the other half as well as the substituent is located outside the β-CD cavity. The energy was minimized and then equilibrated using 30 ps molecular dynamics simulation. After that, 120 ps molecular dynamics running was performed.

Table II. Various non-bonded interaction energies (kJ/mol) of the β-CD substituted phenothiazine complexes averaged over 120 ps sampling simulations, each broken into three 40 ps sections

Guest compound	Time range (ps)	$V_{h,ele}$	$V_{h,vdw}$	V_{h-g}	V_g
1	0–40	5954	−711	−799	1.3
	40–80	5941	−711	−803	1.7
	80–120	5895	−715	−795	0.8
2	0–40	5950	−728	−766	−75
	40–80	5937	−715	−799	−75
	80–120	5121	−724	−799	−75
3	0–40	5958	−720	−841	−42
	40–80	5966	−728	−799	−100
	80–120	5954	−728	−795	−100

$V_{h,ele}$, $V_{h,vdw}$, V_{h-g} and V_g correspond to the intramolecular electrostatic interaction of the host, intramolecular van der Waals interaction of the host, intermolecular interaction between the guest and host, and intramolecular interaction of the guest, respectively.



The GROMOS87 force field [18] has been used in the molecular dynamics simulation. The potential energy function parameters not in the standard GROMOS87 force field are listed in Table I. Bond lengths of the bonds containing hydrogen atoms have been constrained using the SHKE algorithm [19]. The length of the integration time steps is 0.0005 ps. Each system is coupled to a heat bath at 300K using the weak coupling method [20] with a relaxation time of 0.1 ps.

4. Results and Discussion

4.1. INCLUSION COMPLEX FORMATION

The absorption intensity of the phenothiazine derivatives in water varied upon addition of β-CD. It showed that the inclusion complexes of β-CD with *N*-substituted phenothiazines were formed (Figure 1). The changes of absorbance (ΔA) were observed as a function of the concentration of β-CD added. The association constant

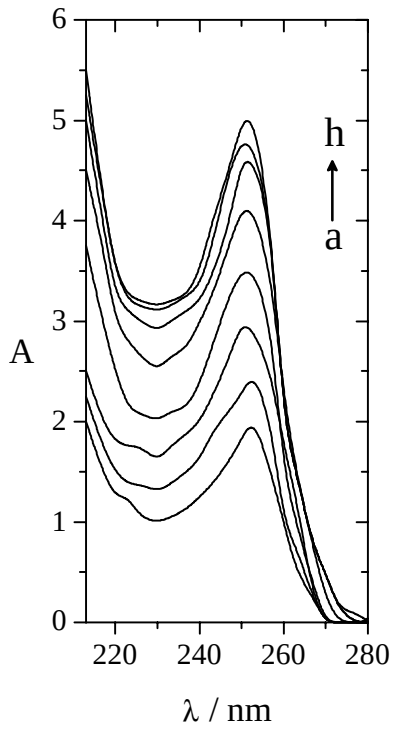


Figure 1. Absorption spectra of *N*-benzylphenothiazine (1.8×10^{-5} mol/dm^3) in aqueous solution alone (a), and in the presence of β-CD with the concentration of 1.60×10^{-3} (b), 2.03×10^{-3} (c), 2.43×10^{-3} (d), 3.24×10^{-3} (e), 4.05×10^{-3} (f), 5.27×10^{-3} (g) and 6.48×10^{-3} mol/dm^3 (h).

values K_a can therefore be evaluated from the Benesi–Hildebrand equation [21] for the 1:1 inclusion complexes of β-CD with phenothiazine derivatives **1**, **2** and **3**.

$$1/\Delta A = 1/\Delta\epsilon[\mathrm{S}] + 1/\Delta\epsilon K_a[\mathrm{S}][\mathrm{C}]$$

Where [S] and [C] represent the concentrations (mol/dm^3) of substrate and β-CD, respectively, ΔA is the change in the absorbance of the substrates before and after addition of β-CD, and $\Delta\epsilon$ is the difference in the molar absorptivities between complexed and free substrate. Plotting $1/\Delta$A against 1/[C] gives a straight line with slope equal to $1/\Delta\epsilon K_a[\mathrm{S}]$. The association constant K_a was directly obtained from the intercept/slope ratio (Figure 2).

The K_a values were determined to be 126 ($\pm$10), 312 ($\pm$31) and 211($\pm$19) dm^3/mol for the inclusion complexation of β-CD with phenothiazines **1**, **2** and **3**, respectively. It is clear that the stability of the inclusion complexes is in the order: β-CD $\cdot$ **2**$>$ β-CD $\cdot$ **3** $>$ β-CD $\cdot$ **1**. It indicates that the stability of β-CD inclusion complexes with *N*-substituted phenothiazine derivatives is dependent on the substituents of the guests.

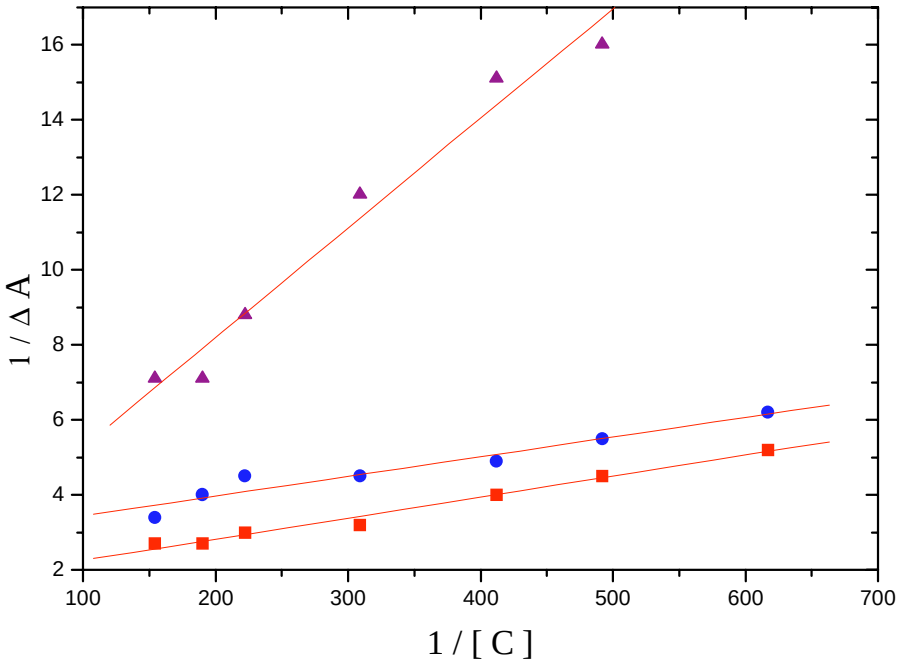
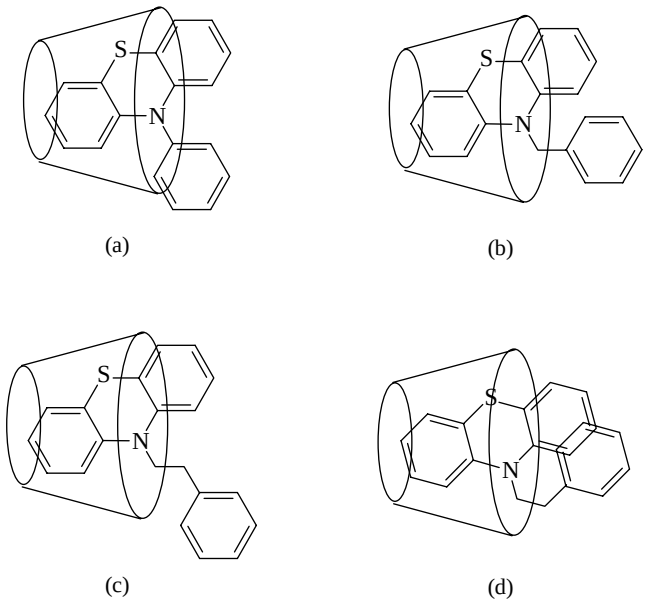


Figure 2. Plots of 1/ΔA *vs* 1/[C] for inclusion complexation of *β*-CD with *N*-phenylphenothiazine (▲), *N*-benzylphenothiazine (●) and *N*-phenethylphenothiazine (■) in aqueous solution.

4.2. CONFORMATIONAL ANALYSIS

For *N*-phenylphenothiazine in the *β*-CD · **1** complex, it is expected that a half of the phenothiazine moiety is included in the *β*-CD cavity, the substituent phenyl which is perpendicular to the heterocycle and the other half of the phenothiazine moiety is located outside the *β*-CD cavity. The rotation of the N–C bond in *N*-phenyl must be constrained, since there is steric hindrance between the phenyl and the secondary hydroxyl groups of *β*-CD. Therefore, the guest compound penetrates the *β*-CD cavity loosely (Scheme 1a). This is the reason why the association constant for *β*-CD · **1** is small (126 dm^3/mol). For *N*-benzylphenothiazine in the *β*-CD · **2** complex, on the other hand, the rotation of the N–CH_2 bond is strongly constrained by the *β*-CD cavity. A preferential conformation shows the CH_2 group is shielded by the *β*-CD cavity, the phenyl group of the benzyl is located outside the *β*-CD cavity and parallel to the aromatic ring of the phenothiazine moiety. This conformation, illustrated in Scheme 1b, allows the guest compound to penetrate into the *β*-CD cavity snugly, thus the complex is more stable (K_a = 312 dm^3/mol). In the *β*-CD · **3** complex, similarly, the rotation of the N–CH_2 bond is constrained, however, the rotation of the CH_2–CH_2 bond gives a preferential conformation as shown in Scheme 1c. Compared with *β*-CD · **2**, the decrease in the association constant (211 dm^3/mol) for the inclusion complex of *β*-CD with **3** is probably due

to loss of entropy by formation of the water cluster [22–24] surrounding the phenyl group. By rotation of the CH_2–CH_2 bond, another possible conformation in which the phenyl group is located over one of the aromatic rings of the phenothiazine may be formed. Although favorable aromatic/aromatic interactions exist, the steric interaction between the β-CD and the phenyl ring is unfavorable (Scheme 1d).



4.3. STRUCTURE OF THE INCLUSION COMPLEXES

To check the convergence of the simulations, each of the 120 ps sampling runs was broken into three 40 ps sections. The potential energies are listed in Table II. From Table II it can be seen that the potential energies converge well, different 40 ps sections give approximately the same averaged energies for each system. The energies for the intramolecular electrostatic ($V_{h,ele}$) and van der Waals ($V_{h,vdw}$) interactions of β-CD, and the intermolecular interactions between β-CD and phenothiazines (V_{h-g}) in the complexes are almost unchanged with different guest compounds **1**, **2**, and **3**. However, the energies for intramolecular interactions of the guests (V_g) are obviously different. Interestingly, it shows no intramolecular interaction in *N*-phenylphenothiazine (V_g = 0.84 kJ/mol for time range 80–120 ps), whereas there are considerable intramolecular interactions in *N*-benzylphenothiazine (V_g = −75.3 kJ/mol) and *N*-phenethylphenothiazine (V_g = −100.4 kJ/mol). The favorable energy changes may be due to the aromatic/aromatic interactions between the aromatic ring of the phenothiazine framework and the substituent benzyl and phenethyl groups.

On the complexation of β-CD with different guest compounds, the intramolecular interaction energy of β-CD itself is approximately the same as that of the empty

β-CD molecule. This is in agreement with the β-CD molecule being rather rigid and its conformation is not obviously influenced by the presence of the guest compounds. This property of the β-CD molecule, together with the facts that it is a cyclic compound and consists of identical glucose units, can explain the relatively rapid convergence of the simulations.

Although the solvent effect was not considered in the simulations, we believe that the water molecules as solvent will further favor the binding process. There is a strong tendency for the hydrophobic guest *N*-substituted phenothiazine breaking the water shell to penetrate an apolar β-CD cavity, and thus to form a stable inclusion complex, which process is favorable in energy. A common feature of the host-guest interaction is that the phenothiazine framework enters the hydrophobic cavity along the central axis of β-CD. The rotation of the guest molecule around the central axis of the β-CD is allowed and the transition along the same axis is not observed.

The molecular dynamics simulations show that the substituent phenyl in β-CD · **1** is perpendicular to the phenothiazine framework and blocked by the wider rim of the β-CD cavity (Figure 3a). For *N*-benzylphenothiazine in β-CD · **2**, the rotation around the N–CH_2 bond is highly constrained by the internal wall of β-CD, the phenyl group and one of the aromatic rings of phenothiazine located outside the β-CD cavity, giving favorable interactions between the guest and the internal surface of the β-CD wall by van der Waals and hydrophobic forces [25, 26], as well as the aromatic/aromatic interaction in the guest compound (Figure 3b). For the β-CD · **3** complex, the conformational changes in the guest molecule have been observed during the 120 ps sampling simulation. Owing to the rotation around the CH_2–CH_2 bond of the substituent group in **3**, one of the conformations shows the phenyl ring is packed on top of the phenothiazine and located outside the β-CD cavity with aromatic/aromatic interaction (Figure 3c). In the other conformation, the preferential form, the phenyl group rotates away from the top of the phenothiazine exposed to the solvent (Figure 3d).

As Figure 3 shows, one end of the phenothiazine is included in the β-CD cavity, the other end and the substituent are located outside the cavity. The results are in agreement with that discussed in the conformational analysis as well as in the previous studies by NMR [13]. Another possibility of the inclusion complexation, i.e. the substituent phenyl ring of the phenothiazine derivatives **1**, **2**, and **3** is located in the β-CD cavity and the phenothiazine moiety outside the cavity, can be ruled out by a further study with the molecular dynamics calculation. When the substituent phenyl ring is considered to be included in the β-CD cavity, the stabilization energy, e.g. for the complexation of β-CD with **1**, decreased by *ca*. 100 kJ/mol.

5. Conclusions

In summary, the inclusion complexes of β-CD with *N*-phenylphenothiazine, *N*-benzylphenothiazine, and *N*-phenethylphenothiazine were prepared in aqueous

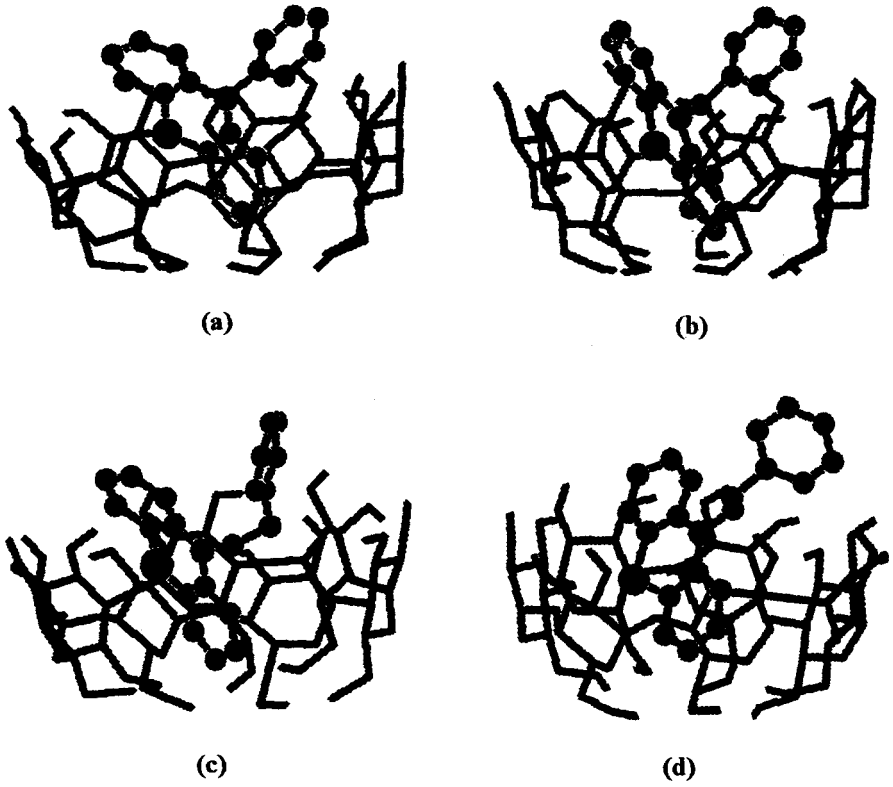


Figure 3. Structures of the inclusion complexes of β-CD with *N*-phenylphenothiazine (a), *N*-benzylphenothiazine (b) and *N*-phenethylphenothiazine (c and d) generated from the molecular dynamics simulations.

solution. The association constants determined by UV-vis spectroscopy show that the stability order is: β-CD · **2** > β-CD · **3** > β.CD · **1**. The stability of the inclusion complexes is influenced by the conformation of the guest compounds, on the other hand, the conformation is controlled by the complexation of β-CD. Molecular dynamics simulations indicate that one aromatic ring and a half of the heterocyclic ring of phenothiazine are embedded in the β-CD cavity, and the remaining part of the phenothiazine and the substituent are located outside the β-CD cavity.

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