

Cyclodextrins as carriers for cinchona alkaloids: a pH-responsive selective binding system†

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A series of cyclodextrin–cinchona alkaloid inclusion complexes were prepared from β -cyclodextrin, heptakis(2,6-di-*O*-methyl)- β -cyclodextrin and heptakis(2,3,6-tri-*O*-methyl)- β -cyclodextrin and four cinchona alkaloids in *ca.* 90% yields, and their inclusion complexation behavior was investigated at pH 7.2 and 1.5 by means of fluorescence, UV/Vis and 2D NMR spectroscopy. The results showed that the cinchona alkaloids can be efficiently encapsulated in the cyclodextrin cavity in an acidic environment and sufficiently released in a neutral environment, which makes these cyclodextrin derivatives the potential carriers for cinchona alkaloids. The binding ability and molecular selectivity of cyclodextrins toward cinchona alkaloids were discussed from the viewpoint of the size–fit concept and multiple recognition mechanism between host and guest.

Introduction

Malaria, the third most infectious cause of mortality, is prolific in more than 40% of the world's population and causes more than one million deaths each year, especially in Africa.^{1–3} Although malaria has been widely eradicated in many parts of the world, the global number of cases continues to rise. This alarming situation has led to great efforts being made to contribute to the design and synthesis of various kinds of new antimalarial drugs,^{4–6} and research on their mechanisms of action.⁷ Among the numerous clinical antimalarial drugs, cinchona alkaloids (cinchonine, cinchonidine, quinine and quinidine) have an ancient therapeutic heritage and are commercially the most important drugs among the alkaloid family.⁸ For example, quinine was the first drug in the medical pharmacopoeia to cure a specific illness and revolutionized the study and treatment of the disease, leading to the foundation of chemotherapy.⁹ Even today, it is still regarded as one of the most efficient antimalarial drugs. Moreover, quinidine can be used as a sodium channel blocker in the prevention and treatment of a wide variety of cardiac arrhythmias.¹⁰ However, the poor water solubilities of these cinchona alkaloids greatly limit their applications, resulting in poor and erratic absorption upon oral administration. Therefore, seeking an efficient and nontoxic carrier for cinchona alkaloids has become an important approach to further their clinical applications.

It is well known that cyclodextrins (CyDs) are a class of cyclic oligosaccharides containing 6–8 D-glucose units. Possessing the torus-shaped structure with hydrophilic external faces and hydrophobic inner surface, CyDs are able to include various organic and biological guests within their hydrophobic cavities to afford host–guest complexes or supramolecular species in aqueous solution.^{11–14} Furthermore, because they are well-known to be nontoxic macrocyclic sugars of natural origin, CyDs are considered as a successful family of pharmaceutical excipients and drug carriers to solve the low bioavailability of insoluble and unstable drugs.^{15–16} For example, CyDs can form inclusion complexes with lipophilic drugs and thus obviously improve their water solubilities.^{17–19} Recently, we reported that oligoethylenediamine bridged bis(β -CyD) can form a 2 : 1 inclusion complex with paclitaxel, which significantly enhances

the water solubility of paclitaxel from *ca.* 0 to 2 mg mL⁻¹.²⁰ Herein, we wish to report our investigation on the selective binding behaviors of native β -CyD and methylated β -CyDs with four cinchona alkaloids (cinchonine, cinchonidine, quinine and quinidine) at different pH values. These studies will help us to a deeper insight into the association and release process of CyD–alkaloid complexes in different biological environments, such as serum (pH *ca.* 7.2) or gastric acid (pH *ca.* 1.5), and consequently explore their potential application in drug delivery.

Experimental

Materials and instruments

All guest cinchona alkaloids, *i.e.*, cinchonine (CIN), cinchonidine (CID), quinine (QUN) and quinidine (QUD) (Chart 1),

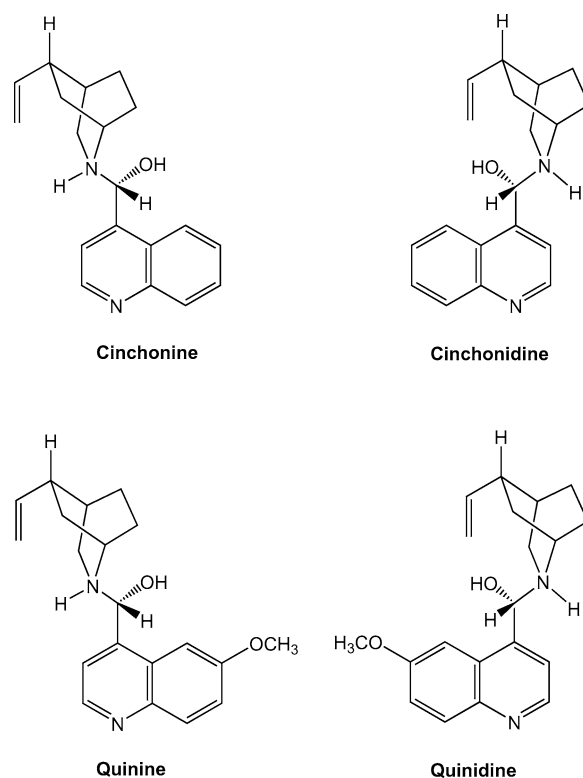


Chart 1

† Electronic supplementary information (ESI) available: Method for the calculation of K_s values and method for the inclusion complexation stoichiometry. See <http://dx.doi.org/10.1039/b506053b>

were commercially available and used without further purification. β -CyD of reagent grade was recrystallized twice from water and dried *in vacuo* at 95 °C for 24 h prior to use. Heptakis(2,6-di-*O*-methyl)- β -CyD (DM β CyD) and heptakis(2,3,6-tri-*O*-methyl)- β -CyD (TM β CyD) (Chart 2) were synthesized according to the reported procedures.²¹

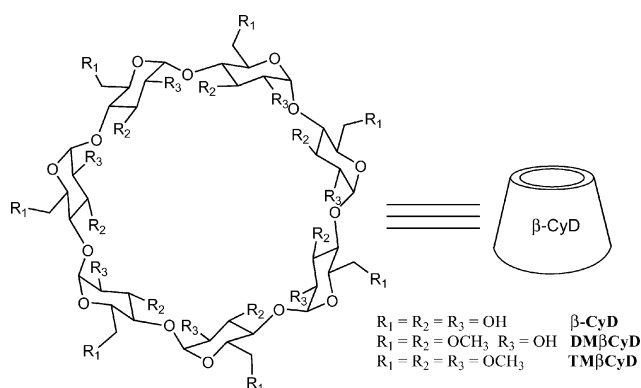


Chart 2

Elemental analyses were performed on a Perkin-Elmer-2400C instrument. NMR spectra were recorded on a Varian Mercury VX300 instrument. UV spectra were recorded on a Shimadzu UV2401 spectrophotometer. Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) at 25 °C on a JASCO FP-750 spectrometer equipped with a constant-temperature water bath, with the excitation and emission slits width of 10 nm. In the spectral measurements, disodium hydrogen phosphate dodecahydrate (25.79 g) and sodium dihydrogen phosphate dihydrate (4.37 g) were dissolved in 1000 mL of deionized water to make a 0.10 M aqueous phosphate buffer solution of pH 7.2, whereas 0.02 M potassium chloride in deionized water was adjusted to pH 1.5 with 1 M hydrochloric acid to give an acidic buffer solution, which were used as solvents for all measurements.

Preparation of the alkaloid–cyclodextrin inclusion complexes

To prepare the alkaloid–cyclodextrin complexes, alkaloid drugs (0.015 mM) and β -CyD derivatives (0.01 mM) were completely dissolved in an ethanol–water solution ($v : v = 1 : 5$), which was adjusted to pH 5.0 with diluted hydrochloric acid. Then, the mixture was stirred for 12 h at room temperature. After evaporating the ethanol from the reaction mixture under the reduced pressure, the unreacted alkaloids were removed by filtration. The filtrate was evaporated under the reduced pressure to dryness, and the residue was dried *in vacuo* to give the CyD–alkaloid inclusion complex with a yield of *ca.* 90%.

Results

Inclusion complexation stoichiometry

The stoichiometry for the inclusion complexation of CyDs with cinchona alkaloids was determined by Job's experiments. Fig. 1 illustrates the Job's plot for the β -CyD–QUN system examined by fluorescence spectra. In the concentration range, the plot for β -CyD showed a maximum at a molar fraction of 0.5, indicating the 1 : 1 inclusion complexation between host and guest. The same results were obtained in other cases of the inclusion complexation of CyDs with cinchona alkaloids.

Spectral titration

Quantitative investigation of the inclusion complexation behavior of host CyDs with guest cinchona alkaloids were respectively examined at pH 7.2 and 1.5 by means of fluorescence titrations. As can be seen in Fig. 2, the fluorescence intensity of QID gradually increased with the stepwise addition of TM β CyD.

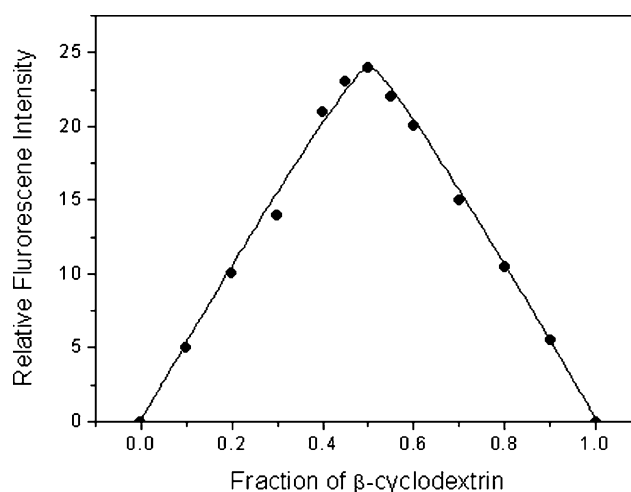


Fig. 1 Job's plot of the β -CyD–QUN system ($[\beta\text{-CyD}] + [\text{QUN}] = 5.0 \times 10^{-6}$ M) in a pH 1.5 buffer.

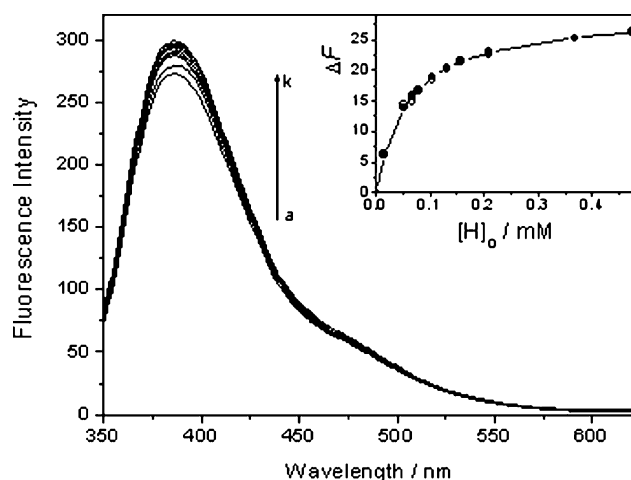


Fig. 2 Fluorescence spectral changes of CID (0.007 mM) upon addition of DM β CyD (0–0.49 mM from *a* to *k*) at pH 1.5 and the nonlinear least-squares analysis (inset) of the differential intensity (ΔF) to calculate the complex stability constant (K_s). The excitation wavelength is 329 nm.

Using a nonlinear least squares curve-fitting method,²² we obtained the complex stability constant (K_s) for each host–guest combination from the analysis of the sequential changes of fluorescence intensity (ΔF) at various CyD concentrations. Fig. 2 (inset) illustrated a typical curve-fitting plot for the titration of QID with TM β CyD, which showed excellent fits between the experimental and calculated data obtained. This good correlation between the experimental and calculated result supported the reliability of the stability constants obtained. The stability constant (K_s) and Gibbs free energy change ($-\Delta G^\circ$) for the inclusion complexation of host CyDs with guest alkaloids were listed in Table 1.

Discussion

Preparation

The CyD–alkaloid complexes were prepared in high yields (*ca.* 90%) by stirring the mixture of CyD and cinchona alkaloid in a dilute EtOH–HCl solution (pH 5). There were two reasons for using a mild acidic solvent in the preparation. One reason was that the inclusion complexation of CyD with cinchona alkaloid preferred an acidic environment rather than a neutral one, as described below. The other was that CyDs tended to be hydrolytically cleaved to linear oligosaccharides if they were exposed to strong acid for a long time.²⁴ It is of note that, in our experiment, CyDs were able to maintain stability at pH 1.5

Table 1 Complex stability constant (K_s) and Gibbs free energy change ($-\Delta G^\circ$) for 1 : 1 inclusion complexation of host CyDs with guest alkaloids in aqueous buffer solution (pH 7.2 and 1.5) at 25 °C

Host	pH	Guest	K_s/M^{-1}	$\log K_s$	$-\Delta G^\circ/kJ\ mol^{-1}$	Ref.	
β -CyD	7.2	Cinchonine	108	2.04	11.6	^a	
	1.5	Cinchonine	3220 \pm 40	3.51	20.0	^b	
	7.2	Cinchonidine	117	2.07	11.8	^a	
	1.5	Cinchonidine	3880 \pm 60	3.59	20.5	^b	
	7.2	Quinine	no inclusion	—	—	^b	
	1.5	Quinine	15300 \pm 300	4.18	23.9	^b	
	7.2	Quinidine	no inclusion	—	—	^b	
	1.5	Quinidine	201 \pm 10	2.30	13.1	^b	
	DM β CyD	7.2	Cinchonine	no inclusion	—	—	^b
		1.5	Cinchonine	4520 \pm 50	3.65	20.9	^b
7.2		Cinchonidine	143 \pm 7	2.15	12.3	^b	
1.5		Cinchonidine	6560 \pm 30	3.82	21.8	^b	
7.2		Quinine	421 \pm 17	2.62	15.0	^b	
1.5		Quinine	1283 \pm 32	3.11	17.7	^b	
7.2		Quinidine	40100 \pm 300	4.60	26.3	^b	
1.5		Quinidine	114800 \pm 700	5.06	28.9	^b	
TM β CyD		7.2	Cinchonine	574 \pm 16	2.76	15.7	^b
		1.5	Cinchonine	4620 \pm 40	3.67	20.9	^b
	7.2	Cinchonidine	907 \pm 34	2.95	16.9	^b	
	1.5	Cinchonidine	1730 \pm 30	3.24	18.5	^b	
	7.2	Quinine	no inclusion	—	—	^b	
	1.5	Quinine	7440 \pm 70	3.87	22.1	^b	
	7.2	Quinidine	15800 \pm 700	4.20	24.0	^b	
	1.5	Quinidine	16300 \pm 500	4.21	24.0	^b	

^a ref. 23; ^b this work.

for at least 4 h. This observation not only ensured the accuracy of experimental results obtained at pH 1.5, but also enabled the possibility for the application of CyDs as drug carriers in an acidic physiological environment.

Solubilization

The water solubility of the CyD–alkaloid complex was assessed by the preparation of its saturated solution. An excess amount of complex was put into 5 mL of water (pH *ca.* 6) and the mixture was stirred for 1 h. The solution's pH value showed no significant changes in the experiment procedure. After removing the insoluble substance by filtration, the filtrate was evaporated under reduced pressure to dryness and the residue was dosed by the weighing method. The results showed that the rather low water solubilities of cinchona alkaloids could be obviously improved after inclusion complexation with CyDs. For example, the water solubilities of CIN–TM β CyD and QUN–TM β CyD complexes, compared with those of CIN (0.5 mg mL⁻¹) and QUN (1 mg mL⁻¹), were increased to 12 mg mL⁻¹ (5.6 mM) and 4 mg mL⁻¹ (1.8 mM) (calculated as alkaloid residue), respectively. In the control experiment, a clear solution was obtained after dissolving CIN–TM β CyD (37 mg) or QUN–TM β CyD (11 mg) complex, which is equivalent to 12 or 4 mg of alkaloid, in 1 mL of water at room temperature. This subsequently confirmed the reliability of the obtained satisfactory water solubility of CyD–alkaloid complexes. In addition, similar experiments were performed in both pH 7.2 and pH 1.5 buffers. The result showed that the water solubilities of CyD–alkaloid complexes at different pH values were similar to those without pH control. For example, the water solubilities of CIN–TM β CyD and QUN–TM β CyD complexes were found to be 14 mg mL⁻¹ and 5 mg mL⁻¹ for QUN–TM β CyD (calculated as alkaloid residue) at pH 1.5 respectively, while these values at pH 7.2 were 11 mg mL⁻¹ and 4 mg mL⁻¹ (calculated as alkaloid residue).

Inclusion mode

2D NMR spectroscopy has recently become an important method to obtain information about the spatial proximity between the atoms of host and guest by observing the inter-

molecular dipolar cross-correlations.²⁵ Two protons, which are closely located in space, can produce a NOE cross-correlation between the relevant protons in a NOESY or ROESY spectrum. In a previous report, we have demonstrated that the CyD cavity could include the 1-azabicyclo[2,2,2]octane unit of cinchona alkaloid from the narrow side in a neutral environment.²⁶ Herein, a similar inclusion mode was also observed. As can be seen from the ROESY spectrum of the CID–DM β CyD complex recorded at pD 7.2 (Fig. 3), no NOE cross-correlations were observed between the interior protons (H-3 and H-5) of DM β CyD and the aromatic protons of CID, indicating that the quinoline ring of CID did not reside in the CyD cavity. However, Fig. 3 exhibited clear NOE cross-correlations (peaks a) between the 1-azabicyclo[2,2,2]octane unit and the H-3 and H-5 protons of DM β CyD. These cross-correlations demonstrated that the 1-azabicyclo[2,2,2]octane unit was, at least, partly accommodated in the CyD cavity.

When changing the pD value to 1.5, the CID–DM β CyD complex gave a more complicated ROESY spectrum. As illustrated in Fig. 4, there existed clear cross-correlations between the 1-azabicyclo[2,2,2]octane protons (H-9', H-10', H-11') of CID and H-5, H-6 as well as H-6(OCH₃) of the CyD cavity (peaks a), the cross-correlations between the allyl protons (H-14') of CID and the H-5 as well as H-6(OCH₃) of CyD cavity (peaks b), the cross-correlations between the ethenyl protons (H-16') of CID and H-5, H-6 as well as H-6(OCH₃) of the CyD cavity (peaks c), and the cross-correlations between the aromatic protons of CID (H-1') and the H-5 protons of CyD cavity (peaks d). Based on these observations, along with the structural feature of CyDs that all of the H-5, H-6 and H-6(OCH₃) were located near the narrow side of CyD cavity, we deduced that the CyD cavity might include the 1-azabicyclo[2,2,2]octane unit, the ethenyl group or the quinoline ring of cinchona alkaloid from the narrow side to form the inclusion complex, and these three modes indeed co-existed as an equilibrium in an acidic environment, as illustrated in Fig. 5.

Enhanced binding ability and molecular selectivity

Extensive studies have revealed that the size/shape–fit concept plays a crucial role in the inclusion complexation of CyD with guest molecules of various structures. On the basis of

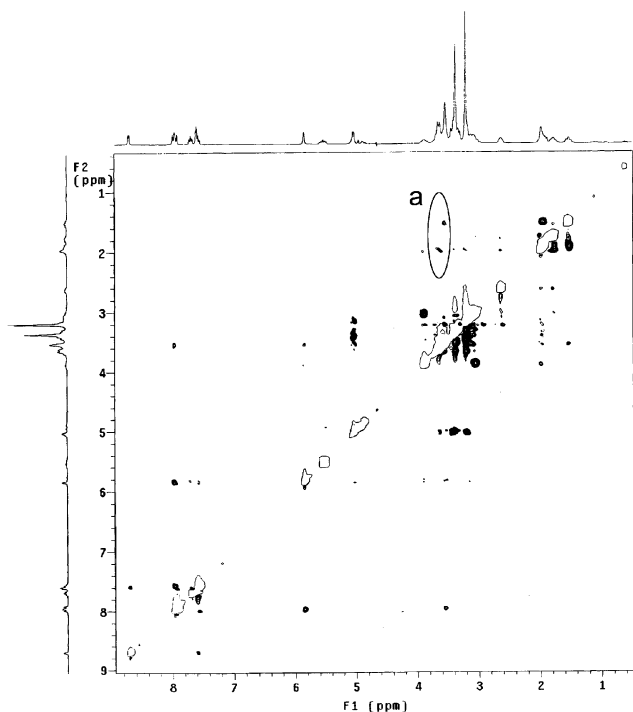
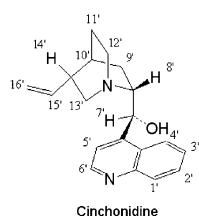


Fig. 3 2D ROESY spectrum of the CID-DM β CyD complex at pH 7.2.

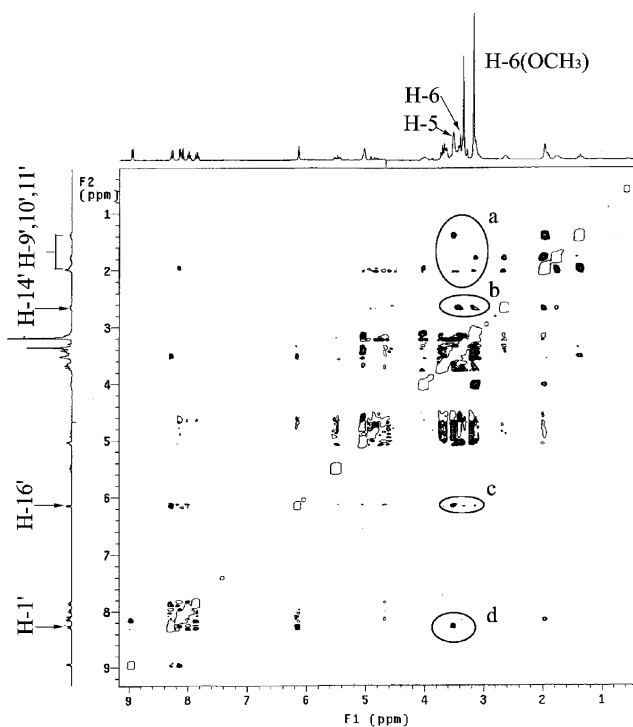


Fig. 4 2D ROESY spectrum of CID-DM β CyD complex at pH 1.5.

the size/shape-fit concept, weak intermolecular forces such as ion-dipole, dipole-dipole, van der Waals, electrostatic, hydrogen bonding and hydrophobic interactions are known to cooperatively contribute to the inclusion complexation. It was demonstrated that, possessing a number of methoxyl groups

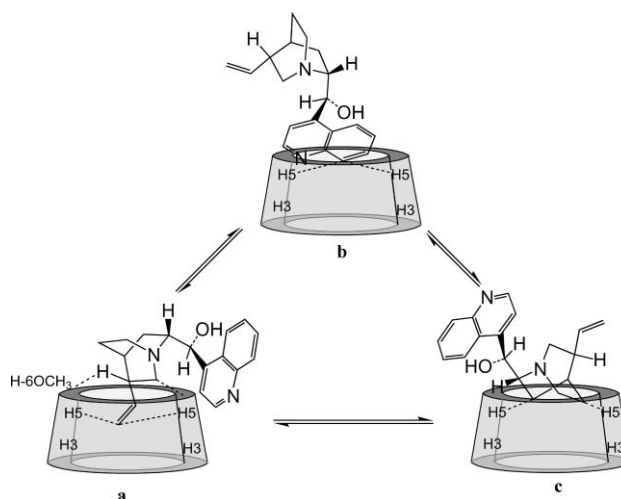


Fig. 5 Possible inclusion modes of CID-DM β CyD complex at pH 1.5.

instead of the hydroxyl groups at the exterior of CyD cavity, the methylated β -CyD, such as DM β CyD or TM β CyD, had a larger opening and a deeper cavity than native β -CyD.^{27,28} This structural feature of methylated β -CyDs would favor their inclusion complexations with relatively big guest molecules, such as cinchona alkaloids, due to a stricter size-fit between host and guest. By comparing the enhancement effect of β -CyD and methylated β -CyDs for each cinchona alkaloid, we could see that the methylated β -CyD which gave the highest K_s enhancement for cinchona alkaloid (with the observed K_s enhancement factors shown in the parentheses) was: in a pH 7.2 buffer, TM β CyD ($\times 5.3$) for CIN, TM β CyD ($\times 7.8$) for CID, DM β CyD (K_s enhanced from zero to 40100 M⁻¹) for QUD; in a pH 1.5 buffer, TM β CyD ($\times 1.4$) for CIN, DM β CyD ($\times 1.7$) for CID, DM β CyD ($\times 571$) for QUD. From these enhancement factors, we may conclude that the guest cinchona alkaloids were better bound by the methylated β -CyDs rather than native β -CyD. Considering the structural features of the hosts and guests, we deduce reasonably that these enhanced binding abilities may be attributed to the host-guest size-fit concept. That is, the bulky 1-azabicyclo[2,2,2]octane unit of cinchona alkaloid was fitter to the bigger cavity of methylated β -CyDs, giving the stronger hydrophobic interactions between host and guest. Moreover, among the four alkaloid guests, QUD tended to fully exploit the inclusion complexation of the methylated β -CyDs, showing the more significantly enhanced effect of K_s value than CIN and CID. One possible explanation is that, possessing a methoxy fragment as a denoted electron group, the quinoline ring of QUD has the higher electron intensity than that of CIN and CID, which would strengthen the C-H... π interactions between the C6-OCH₃ groups of methylated CyDs and the quinoline ring of cinchona alkaloids. As a subsequent result of these enhanced effects on the host-guest binding abilities, the methylated β -CyDs displayed the obviously enhanced molecular selectivity for guest cinchona alkaloids. That is, even the highest molecular selectivity among the four guests employed was $K_{s, \text{CID}}/K_{s, \text{QUN}} = K_{s, \text{CID}}/K_{s, \text{QUN}} = 117/\text{zero}$ toward CID-QUN and CID-QUD pairs by native β -CyD at pH 7.2, but the selectivity was much enhanced to $K_{s, \text{QUD}}/K_{s, \text{CIN}} = 40100/\text{zero}$ toward QUD-CIN pair by DM β CyD and $K_{s, \text{QUD}}/K_{s, \text{QUN}} = 15800/\text{zero}$ toward QUD-QUN pair by TM β CyD.

It was also interesting to compare the host-guest binding abilities at the different pH values. As can be seen in Table 1, most of the host-guest inclusion complexations showed higher K_s values in a pH 1.5 buffer than in a pH 7.2 buffer. For example, β -CyD gave a K_s value as high as 15300 M⁻¹ upon inclusion complexation with QUN at pH 1.5, but showed no inclusion complexation phenomenon at pH 7.2. This indicated that, for an equimolar CyD-QUN mixture at a relatively low concentration ($[\beta\text{-CyD}] = [\text{QUN}] \leq 10^{-2}$ M), more than 92% of the QUN would

be encapsulated in the β -CyD cavity at an acidic pH value like that of the gastric acid (pH 1.5). However, when the pH value of the environment changed to a neutral one like that of serum (pH 7.2), 100% of the QUN that was encapsulated in the β -CyD cavity would be released. The corresponding data for other cinchona alkaloids were: for CIN (DM β CyD as capsule) more than 86% encapsulated at pH 1.5 and 100% released at pH 7.2; for CID (DM β CyD as capsule) more than 88% encapsulated at pH 1.5 and more than 56% released at pH 7.2; for QUD (β -CyD as capsule) more than 50% encapsulated at pH 1.5 and 100% released at pH 7.2. These results strongly validated the potential of CyDs as carriers for cinchona alkaloids. One possible explanation for the different host-guest binding abilities at various pH values is the hydrogen bond interactions. In an acidic environment, the nitrogen atoms in the cinchona alkaloids would be protonated and thus gave the strong hydrogen bond interactions with the numerous oxygen atoms of host CyDs. However, in a neutral environment, these N-H...O hydrogen bond interactions would be significantly weakened because of the deprotonation effect, which subsequently led to the decreased binding abilities between host and guest.

Release process

In order to further evaluate the capability of CyDs as carriers for cinchona alkaloids, we tracked the release process of the encapsulated alkaloids. The solid CyD-alkaloid complex was quickly dissolved in the buffer solution, and the absorbance maximum of the resultant solution was continuously recorded with an interval of 25 s. Fig. 6 illustrates the typical release process of the TM β CyD-QUN complex. At pH 1.5, the absorbance of TM β CyD-QUN complex was nearly unchanged during the time course, indicating that only a little amount of the encapsulated QUN was released. However, at pH 7.2, the absorbance of TM β CyD-QUN complex obviously increased at the initial stage and then reached a constant state after 1100 s. This increased absorbance of TM β CyD-QUN complex might indicate the release of the encapsulated QUN, because the control experiments demonstrated that the inclusion complexation of TM β CyD with QUN would give a decreased absorbance as compared with that of the free QUN. In addition, through a simple calculation based on the K_s value (7440 M⁻¹) and the concentration (0.026 mM) of TM β CyD-QUN complex employed, we could find that only 7.4% of the encapsulated QUN would be released at pH 1.5, but this value would increase to 100% at pH 7.2. This calculated result consequently supported the conclusion drawn from the spectral experiments. That is, most of the alkaloid would be encapsulated in the CyD cavity in an acidic environment but thoroughly released in a neutral one, which was important to the application of CyDs as carriers for these antimalarial drugs.

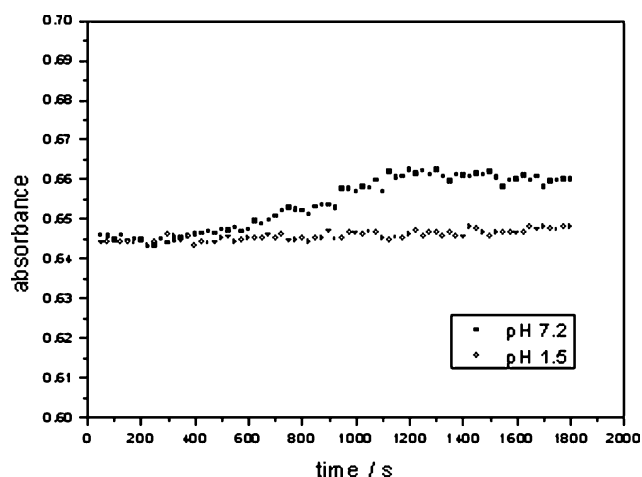


Fig. 6 Absorbance of TM β CyD-QUN complex at pH 7.2 and 1.5 with an interval of 25 s. Observed wavelength: 234 nm.

Conclusion

In summary, we investigated the inclusion complexation behavior of some β -CyD derivatives with four cinchona alkaloids at pH 7.2 and 1.5. The results showed that CyDs could not only enhance the water-solubilities of these alkaloids but also effectively protect them in an acidic environment. Considering that all of the CyDs employed in this work were commercial, non-toxic and easily prepared, they should be regarded as an important choice in the design of the carriers for the biological and medicinal substrates.

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