Dyes and Pigments 82 (2009) 40–46

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

The interaction of C.I. Basic Red 5 with complementary negatively charged hosts: 4-Sulfonatocalix[4]arene and carboxymethyl-β-cyclodextrin

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ARTICLE INFO

Article history:
Received 22 July 2008
Received in revised form
30 October 2008
Accepted 31 October 2008
Available online 12 November 2008

Keywords:
C.I. Basic Red 5
Neutral red
Carboxymethyl-β-cyclodextrin
4-Sulfonatocalix[4]arene
Supramolecule
Complexation
Spectroscopy

ABSTRACT

The formation of the inclusion complexation between C.I. Basic Red 5 and two types of negatively charged host molecules, namely 4-sulfonatocalix[4]arene and carboxymethyl-β-cyclodextrin, was studied using fluorescence, UV–visible absorption and nuclear magnetic resonance spectroscopy. Different fluorescence and absorption behavior were observed upon complexation of the dye with host molecules that possess a hydrophobic cavity. 4-Sulfonatocalix[4]arene showed strong binding ability for the acidic form of the dye, which reduced the fluorescence emission of the dye. In contrast, carboxymethyl-β-cyclodextrin was more suitable for complexation of the neutral form of the dye for which an increase in fluorescence emission was seen. The difference in the complexation behavior of dye towards the two hosts is attributed to the specific effects of the dominant binding modes, namely electrostatic attraction in the case of 4-sulfonatocalix[4]arene and hydrophobic interaction in the case of carboxymethyl-β-cyclodextrin. The thermodynamic parameters for inclusion complexation, which were determined using van't Hoff analysis, supported the different binding modes of the dye with the two host molecules; ^{1}H NMR further confirmed the conclusion.

1. Introduction

The formation of non-covalently bound inclusion complexes and molecular recognition are of current interest in supramolecular chemistry [1]. Crown ethers, cyclodextrins and calixarenes are the first, second and third generation typical host compounds, respectively. They possess a hydrophobic cavity although their structural features and properties are distinctly different in nature [2]. Therefore, it would be interesting to compare the inclusion complexation behavior of the host compounds because of the many contrasting and complementary features exhibited by these hosts. However, the low solubility of calixarene in aqueous medium will limit its comparison with other host and guest systems. The necessity to understand the host–guest interactions and to control their binding has stimulated the design of various synthetic soluble host molecules for the binging of relatively small guest molecules.

Calixarenes as the third generation of supramolecular host compound possess the merits of crown ethers and cyclodextrins [3]. They are widely used as a platform for artificial receptors [4]. Their potential to function as receptors depends on introducing various functional groups either to the upper or to the lower rim of calixarene [5–8]. Sulfonated calixarenes represent a particularly important class of the host molecules because these versatile macrocyclic phenolic compounds are less toxic than cyclodextrins [9], highly soluble in water. Studies performed in water, where most of the biological processes take place, are of particular relevance. Thus, investigation of the complexation properties of sulfonated calixarenes in water towards small neutral organic molecules, drug molecules [10] and biomolecules including amino acids, nucleic acid bases [11] and protein [12] have generated interest.

In addition to calixarenes, cyclodextrins (CDs) are another most important class of host molecules in supramolecular chemistry. Natural CDs, composed of six, seven, or eight D-glucopyranose units are called α-, β-, γ-CD, respectively [13]. The most important property of CDs is the ability to form inclusion complexes with many appropriately sized organic, inorganic, and biological molecules due to CDs' peculiar "interior hydrophobic, exterior hydrophilic" structure [14–18]. However, parent CDs have relatively low molecular binding abilities which limit their further use [19]. On this basis, the modification of CDs has become one of the hot topics in host–guest chemistry recently [20–23]. Native CDs may be derivatized by adding substituents to the hydroxyl groups on the cavity rim in order to change their aqueous solubility, binding properties and charges [24]. Especially for the negatively charged carboxymethyl-β-cyclodextrin (CM-β-CD) [25,26] and sulfurbutylether-β-cyclodextrin (SBE-β-CD) [27], sulfate and carboxymethylate moieties also provide binding sites for SBE-β-CD and CM-β-CD for a variety of guest molecules.

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doi:10.1016/j.dyepig.2008.10.021

C.I. Basic Red 5 (neutral red; NR) is a representative phenazine dye, which exists in two molecular forms in aqueous solution, namely acidic and neutral forms [28]. NR has been used in studies of biological systems [29,30], especially as an intracellular pH indicator within the pH range of 6.0–8.0 [31,32], and a non-toxic stain [33]. NR can also be functioned as a fluorescence probe in acidic medium to investigate the structure of DNA molecules and to construct a sensitive assay for DNA [34–37]. Moreover, NR is an efficient anticancer drug of parent compound targeted to DNA [34]. So, there is no doubt that the interaction between NR with CDs or calixarenes depends on pH of the aqueous medium. As such, it would be interesting to understand the complexation behavior of soluble sulfonatocalixarene and cyclodextrin with NR in water.

Although there are many reports that have focused on the inclusion complexes of modified CDs or water-soluble calixarene derivatives [38,39], the comparison and study of the complexation behavior of these two types of hosts are still limited owing to the poor solubility of calixarenes in aqueous medium. Lang [40] reported the photophysical properties of host–guest complexes of porphyrin with water-soluble negatively calix[4,6,8]arene sulfonates and neutral nature α-, β-, and γ-CDs. Liu [2,41,42] investigated the molecular recognition of dye guest by modified neutral or positively CDs, calixarene sulfonates and cucurbit[7]uril by a fluorescence method. Wang [43] studied the redox control of host–guest recognition by calixarene[6]sulfonates and β-CDs by NMR and an electrochemical method. Mohanty [44] reported the host–guest complexation of neutral red with cucurbit[7]uril and α-cyclodextrin and contrasted pK_a shifts and binding affinities for two host molecules. All these investigations focus only on the complexation of a guest molecule with two host compounds. To our knowledge, the comparison study on the complexation of the different forms of a guest molecule with two host compounds, especially the two negatively hosts, has not been reported.

In this work, NR was chosen as the guest molecule to interact with two kinds of negatively charged host molecules, i.e. 4-sulfonatocalix[4]arene (SCX4) and carboxymethyl-β-cyclodextrin (CM-β-CD) (Scheme 1). As all these guest and hosts are solubility in aqueous solution, this will allow us to investigate their complexation behavior in aqueous system. The anion character and conformational rigidity of SCX4 and CM-β-CD should make them an ideal host for the positively charged guest such as the acidic form of NR.

2. Experimental section

2.1. Apparatus

The UV–visible absorption and fluorescence measurements were performed with a Shimadzu UV-265 absorption spectrophotometer (Tokyo, Japan), and a Hitachi F-4500 spectrofluorometer (Tokyo, Japan), respectively. Excitation and emission bandwidths were both set at 5 nm. All pH measurements were done on a pHS-2 pH meter (The 2nd Instrument Factory of Shanghai, China). ^{1}H NMR spectra were recorded in D_2O on a Bruker – DKX – 300 MHz spectrometer (Switzerland).

2.2. Reagents

C.I. Basic Red 5 (Third Reagent Factory of Shanghai) was recrystallized twice from double distilled water before use. The stock solutions of 4.0×10^{-4} mol L^{-1} NR and 0.010 mol L^{-1} SCX4 (Junsei Chemical Co., Ltd., China) were prepared by dissolving NR and SCX4 into double distilled water, respectively. CM-β-CD (average MW = 1413) was synthesized based on a literature method [25] and its average degree of substitution is 4.8. Phosphate buffer solutions were used to control the pH of the working media. All other reagents were of analytical-reagent or above without purification. Double distilled water was used throughout for all solutions.

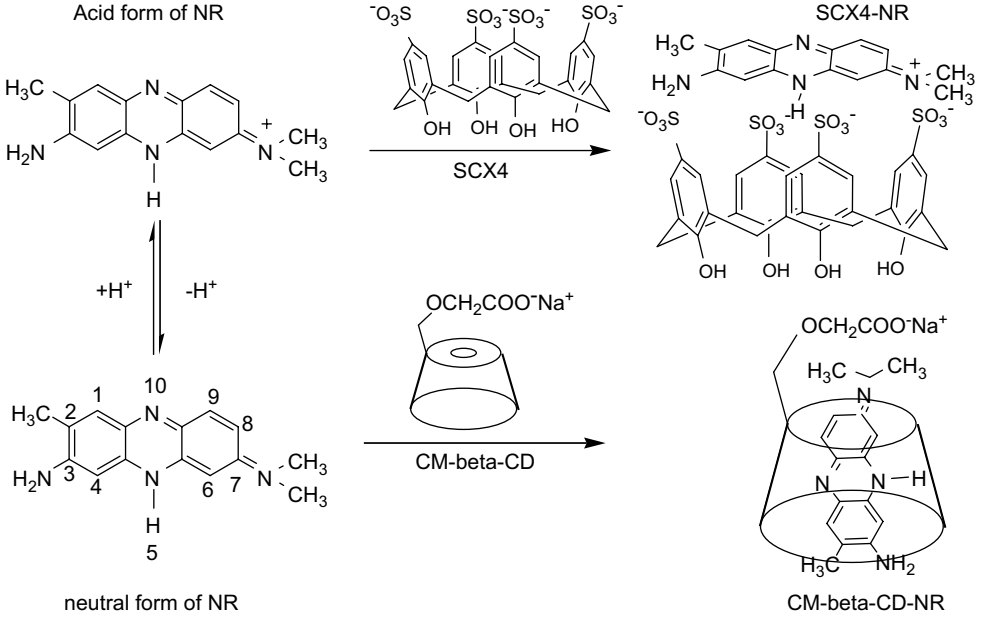
2.3. Procedure

2.3.1. Absorption and fluorescence spectroscopy

A 1.0-mL aliquot of the stock solution of NR (4.0×10^{-4} mol L^{-1}) was transferred into a 10.0 mL volumetric flask and an appropriate amount of 0.010 mol L^{-1} SCX4 or 0.010 mol L^{-1} CM-β-CD was added. The pH was controlled by a 0.5-mol L^{-1} phosphate buffer. The mixed solution was diluted to the final volume with the double distilled water and shaken thoroughly, then equilibrated for 30 min at room temperature.

2.3.2. ^{1}H NMR spectroscopy

In the ^{1}H NMR titration experiments, which were conducted, changes in chemical shift, $\Delta\delta$, of signals in the ^{1}H NMR spectra of the NR. SCX4 or CM-β-CD was added directly into the solutions of



Scheme 1. Proposed complexation of NR with SCX4 and CM-β-CD.

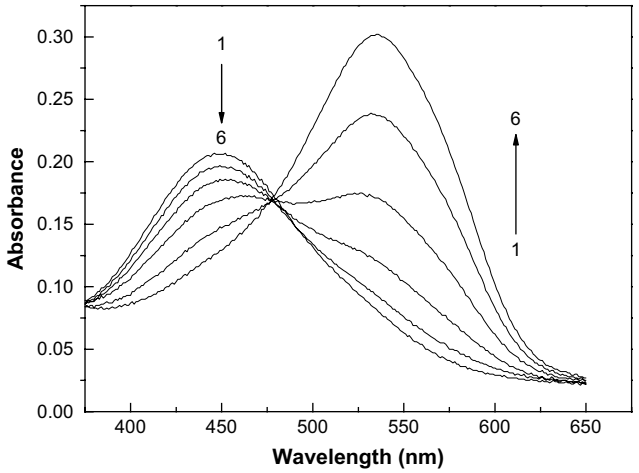


Fig. 1. Absorption spectra of 4.0×10^{-5} mol L^{-1} NR in the presence of various concentrations of SCX4 at pH 9.0. (1) 0.0, (2) 5×10^{-6}, (3) 1.3×10^{-4}, (4) 2.8×10^{-4}, (5) 4.8×10^{-4}, and (6) 7.3×10^{-4} mol L^{-1}.

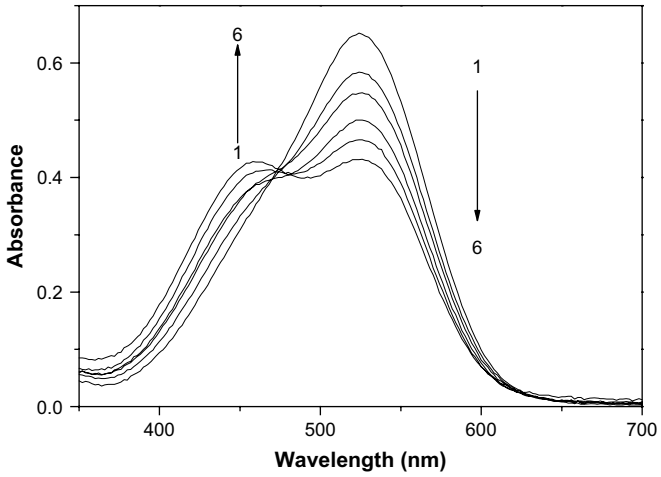


Fig. 2. Absorption spectra of 4.0×10^{-5} mol L^{-1} NR in the presence of various concentrations of CM-β-CD at pH 5.5. (1) 0.0, (2) 1.0×10^{-3}, (3) 2.0×10^{-3}, (4) 3.0×10^{-3}, (5) 4.0×10^{-3}, and (6) 7×10^{-3} mol L^{-1}.

NR in the NMR tubes and the resulting solutions sonicated before recoding the spectra. All the spectra were recorded in 99.96% D_2O at 298 ± 0.1 °C.

3. Results and discussion

3.1. UV–visible absorption of the inclusion complexes of SCX4 and CM-β-CD with C.I. Basic Red 5

The ground-state pK_a value of NR in water is 6.8 [29]. Fig. 1 displayed the absorption spectra of NR in the absence and presence of SCX4 at pH 9.0. NR shows a peak absorption maximum of 446 nm, attributing to the formation of its neutral form [27]. When the concentration of SCX4 increased, the absorption band at 446 nm gradually decreased resulting in the emergence of a new absorption band at 535 nm. The appearance of this absorption band indicated the conversion of the neutral form of NR to the acidic form. Titration of NR with SCX4 resulted in a single isosbestic point at 474 nm, inferring the presence of two absorbing species in equilibrium. The isosbestic point also provided the evidence of the complexation between the acidic form of NR and SCX4. NR prefers to exist as acidic form in the presence of SCX4 through the electrostatic attraction of its positive charges with the negatively charged SCX4 as illustrated in Scheme 1.

However, the interaction of C.I. Basic Red 5 with CM-β-CD was different. Fig. 2 depicted the absorption spectra of NR with various concentrations of CM-β-CD at pH 5.5. An absorption band at 526 nm was observed in the absence of CM-β-CD, indicating that NR exhibits mainly as its acidic form at pH 5.5. This band gradually diminished, accompanying with the appearance of an absorption band at 448 nm in the addition of CM-β-CD. By contrast, this phenomenon was different from the reaction of SCX4 and NR. CM-β-CD induces the conversion of the acidic form of NR to its neutral form. An isosbestic point at 478 nm was found, indicating the presence of two types of NR in the solution. The neutral form of NR consists of the hydrophobic moiety which could penetrate into the cavity of CM-β-CD. As such, the neutral form of NR prefers to form an inclusion complex with CM-β-CD as shown in Scheme 1.

3.2. Fluorescence behavior of the inclusion complexation of C.I. Basic Red 5 with SCX4 or CM-β-CD

As shown in Fig. 3A, the fluorescence intensity of the dye markedly decreased upon the addition of SCX4 at pH 7.0. The maximum emission wavelength gradually red shifted from 611 to 624 nm. The decreased fluorescence intensity depended on the fluorescence sensitivity factor and the fluorescence quantum yield (seen in Table 1). For the same guest compound in different host

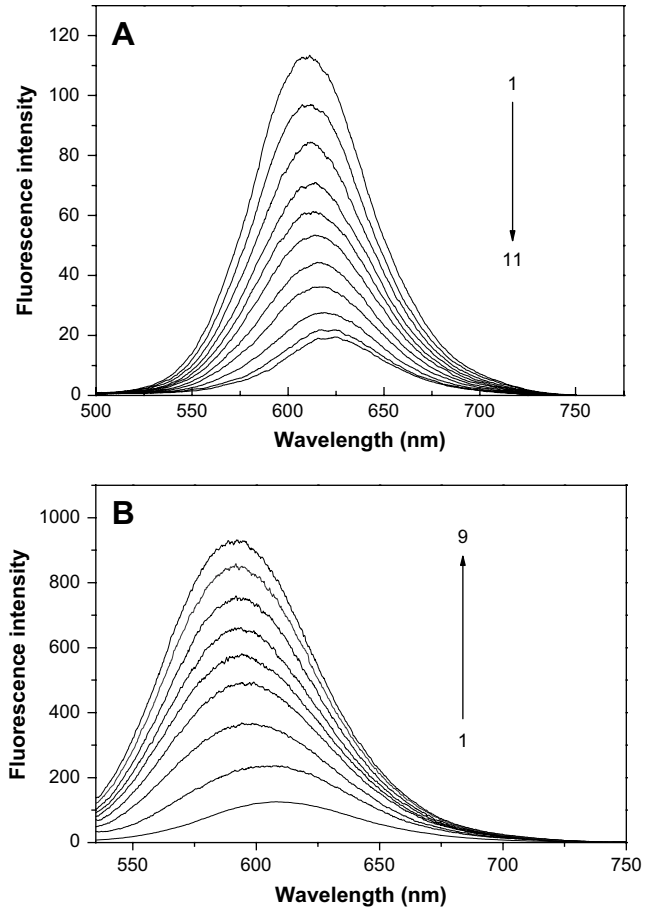


Fig. 3. A: Fluorescence spectra of 4.0×10^{-5} mol L^{-1} NR in the presence of various concentrations of SCX4 at pH 7.0. (1) 0.0, (2) 1.4×10^{-5}, (3) 4.3×10^{-5}, (4) 8.5×10^{-5}, (5) 1.4×10^{-4}, (6) 2.3×10^{-4}, (7) 3.7×10^{-4}, (8) 5.7×10^{-4}, (9) 8.5×10^{-4}, (10) 1.3×10^{-3}, and (11) 2.0×10^{-3} mol L^{-1}. B: Fluorescence spectra of 4.0×10^{-5} mol L^{-1} NR in the presence of various concentrations of CM-β-CD at pH 7.0. (1) 0.0, (2) 6.0×10^{-4}, (3) 8.0×10^{-4}, (4) 1.2×10^{-3}, (5) 2.0×10^{-3}, (6) 3.0×10^{3}, (7) 4.0×10^{-3}, and (8) 5.0×10^{-3} mol L^{-1}, (9) 6.0×10^{-3} mol L^{-1}.

Table 1
The fluorescence sensitive factor (f) and the fluorescence quantum yield (Q) at pH 7.0.

Complex	f[a]	Q[b]	Formation constants
NR		0.0042	
SCX4–NR	0.845	0.0031	10428 ± 127
CM-β-CD–NR	15.56	0.058	826 ± 34

[a] $f = (F - F_0)/F_0$, where F and F_0 represent the fluorescence intensity of NR in the presence and absence of SCX4 or CM-β-CD, respectively.
[b] The excitation wavelength is 410 nm. The reference is quinine sulfate dehydrate.

molecules, the higher the fluorescence quantum yield and fluorescence sensitivity, the stranger the fluorescence intensity. These changes were possible due to the interaction between SCX4 and NR, again implying the formation of a SCX4–NR complex. The possible mechanism was that conformational changes in the SCX4 or guest molecules. SCX4 tended to adopt different conformations upon complexation with structure-related guest and the symmetry of the guest as well as the induced-fit relationship between host and guest, which may be the main factors controlling these conformational adjustments.

Fig. 3B shows the fluorescence spectra of C.I. Basic Red 5 at pH 7.0 in the absence and presence of CM-β-CD. Complexation of CM-β-CD with the dye was, in contrast to SCX4, accompanying by a dramatic increase of the fluorescence intensity and by a large blue shift of emission peak from 611 to 575 nm. Apparently, hydrophobic part of NR could insert to the hydrophobic cavity of CM-β-CD. The experimental results show that the cavity of the CM-β-CD provided a better protective hydrophobic microenvironment from the quenching that occurred in the bulk aqueous solution. The hydrophobic interaction in the CM-β-CD cavity, restricted the internal rotation, which should lead to fluorescence enhancement and hypsochromic shift. The different behavior of NR complexation to the two hosts was attributed to the specific effects of the dominant binding modes.

3.3. Formation constant of complexes

The inclusion formation constant (K) is a measure for the complexing capacity of a host compound (H) with a guest molecule (G). The inclusion formation constants of NR with SCX4 and CM-β-CD were evaluated at different pH values, respectively. Assuming a 1:1 (H:G) inclusion model, the inclusion equilibrium is

$$H + G \overset{K_S}{\leftrightharpoons} HG \tag{1}$$

$$K_S = \frac{[HG]}{[H][G]} \tag{2}$$

where [H], [G] and [HG] represent the equilibrium concentration of the host, guest and host–guest complex, respectively. The formation constant can be obtained from the fluorescence data using a non-linear curve-fitting approach [41] as shown below:

$$\Delta F = \frac{1}{2}\left\{\alpha\left([H]_0+[G]_0+\frac{1}{K}\right) - \sqrt{\alpha^2\left([H]_0+[G]_0+\frac{1}{K}\right)^2 - 4[H]_0[G]_0\alpha^2}\right\} \tag{3}$$

where ΔF represents the change of the fluorescence intensity of NR with the addition of host compound. $[H]_0$ and $[G]_0$ denote the initial concentrations of host and guest, respectively. α is a sensitive factor of the structure change of complexation composed of host and guest

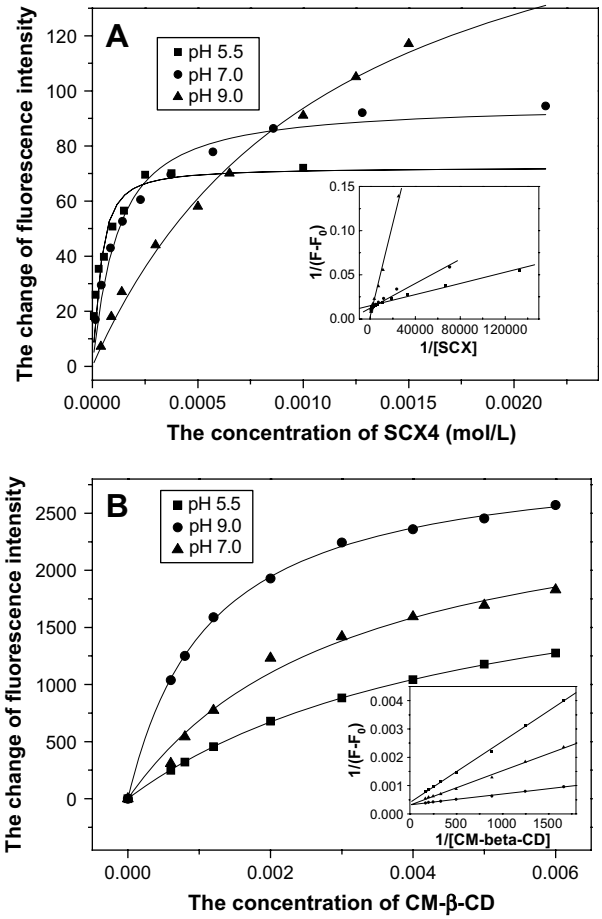


Fig. 4. A: Non-linear fitting data for NR as a function of the concentration of SCX4 at different pHs. Inset: H–B plots for NR complexed to SCX4 at different pHs. B: Non-linear fitting data for NR as a function of the concentration of CM-β-CD at different pHs. Inset: H–B plots for NR complexed to CM-β-CD at different pHs.

at the interactive course. Fig. 4A and B displayed the non-linear fitting for the reactions of SCX4 and CM-β-CD with NR at different pHs using Eq. (3), respectively. The correlation coefficients of the curves were larger than 0.99, indicating that the 1:1 complex stoichiometry for SCX4–NR and CM-β-CD systems. The Hildebrand–Benesi plots [38] were also confirmed the results (Insets A and B). The formation constants of SCX4 and CM-β-CD with NR at pH 7.0 were 10428 ± 127 and 826 ± 34 L mol^{-1}, respectively (seen in Table 1).

3.4. Influence of pH

The associations of C.I. Basic Red 5 with SCX4 or CM-β-CD were also carried out in acidic, neutral and basic media. At different pHs, the excitation and emission wavelengths of SCX–NR red shifted while CM-β-CD–NR blue shifted in contrast to the NR itself, which implying that the formation of host–guest complexation. In addition, the formation constant values were very sensitive to change of pH values (seen in Table 2). The inclusion complexation interaction

Table 2
Formation constants K (L mol^{-1}) of NR with SCX4 and CM-β-CD at different pHs.

Host	Formation constants K (L mol^{-1})		
	pH 5.5	pH 7.0	pH 9.0
SCX4	63501 ± 216	10428 ± 127	954 ± 46
CM-β-CD	205 ± 18	826 ± 34	905 ± 58

Table 3
The thermodynamic parameter of the inclusion complexes SCX4–NR and CM-β-CD–NR.

Host	T (K)	Log K	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	$T\Delta S$ (298 K) (kJ mol^{-1})
SCX4	294	4.257	−81.8	−140.8	−59.0
	303	3.701		−141.8	
	313	3.316		−143.8	
	323	2.934		−145.7	
CM-β-CD	294	2.954	−6.88	−16.6	+9.81
	303	2.900		−16.8	
	313	2.864		−17.2	
	323	2.834		−17.5	

of SCX4 with NR is the order: $K_{pH5.5} > K_{pH7.0} > K_{pH9.0}$, while for CM-β-CD with NR is the order: $K_{pH9.0} > K_{pH7.0} > K_{pH5.5}$. The different complexing trends of the two host compounds with NR indicated that the two kinds have different binding modes.

In acidic media the acidic form with positive charge of NR is predominant, the combination of negatively charged SCX4 with positively NR is superior to that of the neutral form of NR. With increasing the value of pH, the acid form gradually decreases and the formation inclusion complexation of SCX4–NR are weaked. Small NR cation can be incorporated to the SCX4, and negatively charged SO_3^- groups serve as the anchoring points. These results document the importance of electrostatic interactions because the sulfonatogroups at the upper rim of SCX4 enhance the efficiency of binding. So, we propose that the electrostatic attraction is the dominating binding mode for complexation of SCX4 with NR. While in neutral and basic media, the neutral form of NR is predominant, which is more hydrophobic than the acidic form. With increasing the value of pH, the neutral and basic forms gradually increase and the formation inclusion complexation of CM-β-CD–NR is strengthened. So, the hydrophobic interaction is the dominating binding mode for CM-β-CD–NR supramolecular system. This is consistent with the corresponding inclusion complexation of negatively charged SBE-β-CD with NR [27].

3.5. Thermodynamic parameters of inclusion complexation

The thermodynamic parameters for the inclusion complexation of the guest with host compounds are influenced by several factors: relative size between the cavity of host molecules to the guest, spatial conformation, microenvironmental hydrophobicity, electrostatic interaction, hydrogen bonding, van der Waals, and so on. The formation constants of the inclusion complexation of NR with SCX4 or CM-β-CD were determined via spectrofluorometric titration at various temperatures ranging from 21.0 to 50.0 °C. The complexation thermodynamic parameters were obtained by the slope and ordinate-intercept of van't Hoff equation [41]:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

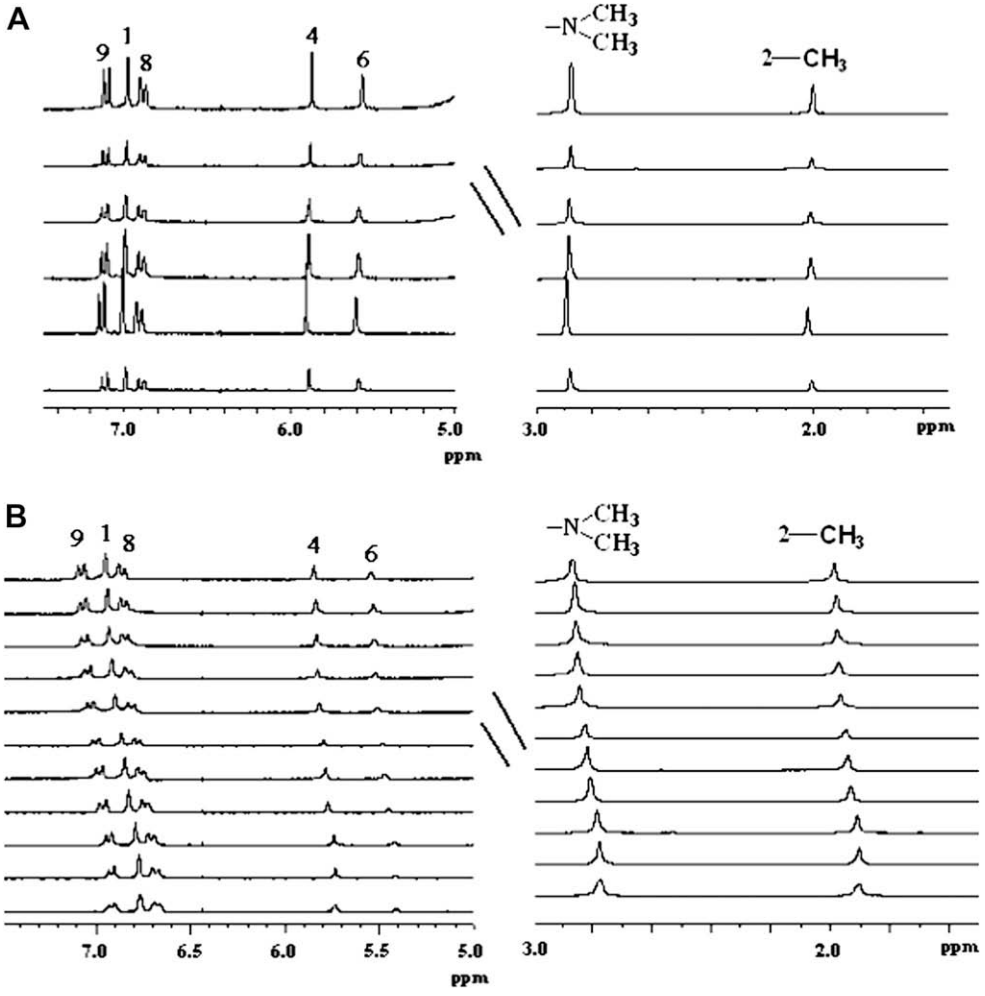


Fig. 5. A: Spectra of ^{1}H NMR titration of SCX4 with NR at 300 MHz. B: Spectra of ^{1}H NMR titration of CM-β-CD with NR at 300 MHz.

The free energy change (ΔG) was estimated from the following relationship:

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

The complexation thermodynamic parameters in Table 3 indicated that the negative sign for free energy (ΔG) means that the complexation of SCX4 with C.I. Basic Red 5 was spontaneous. The interaction process was mainly driven by the favorable enthalpic change with accompanying a small entropic loss, which is attributed to the intermolecular hosts–guest interactions, such as electrostatic interaction of NR with sulfonates and hydrogen bonding of the guest's –NH and $-NH_2$ moieties with the phenolic hydroxyl groups. And the interaction of C–H…π and N–H…π would be advantageous for the favorable enthalpy value. The entropy change originated from the arrangement of water molecules originally surrounding the host and guest molecules, and the entropic loss from the decrease in the motion freedom upon the complexation and complex-induced conformational change. However, the complexation of NR with CM-β-CD was driven by the entropic increase and a small enthalpic change. The complexation was almost driven by the entropic increase which is the typical case driven by the classical hydrophobic interaction [41]. These are consistent with the fluorescence results.

3.6. ^1H NMR studies

NMR spectroscopy is indeed the most powerful tools for the study of formation of inclusion complex between hosts and a variety of guest molecules, especially the interaction mechanism. ^1H NMR titration experiments were undertaken to confirm the binding mode between water-soluble SCX4 and CM-β-CD with NR at room temperature. The spectra of ^1H NMR titration of NR and its complex with different concentrations of SCX4 and CM-β-CD were shown in Fig. 5A and B, respectively. It is obvious that opposite ^1H NMR spectra behavior were found upon the complexation of NR with the two host molecules. The proton of NR showed little changes in the presence of SCX4 while significant changes in the presence of CM-β-CD. All the protons signals of NR moved downfield in the SCX4, which is the result of the deshielding effect by the SCX4. This phenomenon may attribute to the effect between the $-SO_3^-$ of calixarene and the $-\overset{/}{\underset{\backslash}{NH}}\oplus$ of C.I. Basic Red 5. While all the protons displayed significant upfield frequency changes to some extent in the CM-β-CD, which illustrated the shielding effect of the protons by the cavity of CM-β-CD. These different the direction and magnitude of the chemical shift changes suggested there are different binding modes: exposure the NR out the cavity of SCX4 and the inclusion of the NR inside the CM-β-CD cavity.

Graphical methods are designed to produce a linear relationship between the chemical shifts ($\Delta\delta$) and K, so that NMR data can be treated graphically. For the NMR spectra, changes in $\Delta\delta$ of the signals of NR showing the largest change as a function of added hosts were measured. Eq. (6) that describes the 1:1 binding isotherm is as following by the Benesi–Hildebrand [45]:

$$1/\Delta\delta = 1/ - \Delta\delta_{max}K[H_0] + 1/K_{max} \quad (6)$$

A plot of $1/\Delta\delta$ against $1/[H]_0$ (often referred to as a double reciprocal plot) should be linear, with a slope $1/K\Delta\delta_{max}$ and intercept $1/\Delta\delta_{max}$. The procedure is illustrated in Fig. 6, following the changes in the chemical shift of the H-9 of NR. Fig. 6 shows the linear correlation coefficients of the curves R were larger than 0.99, which indicated that the 1:1 complex stoichiometry for SCX4–NR and CM-β-CD–NR systems. Thus, the 1:1 complex stoichiometry was further confirmed by ^1H NMR.

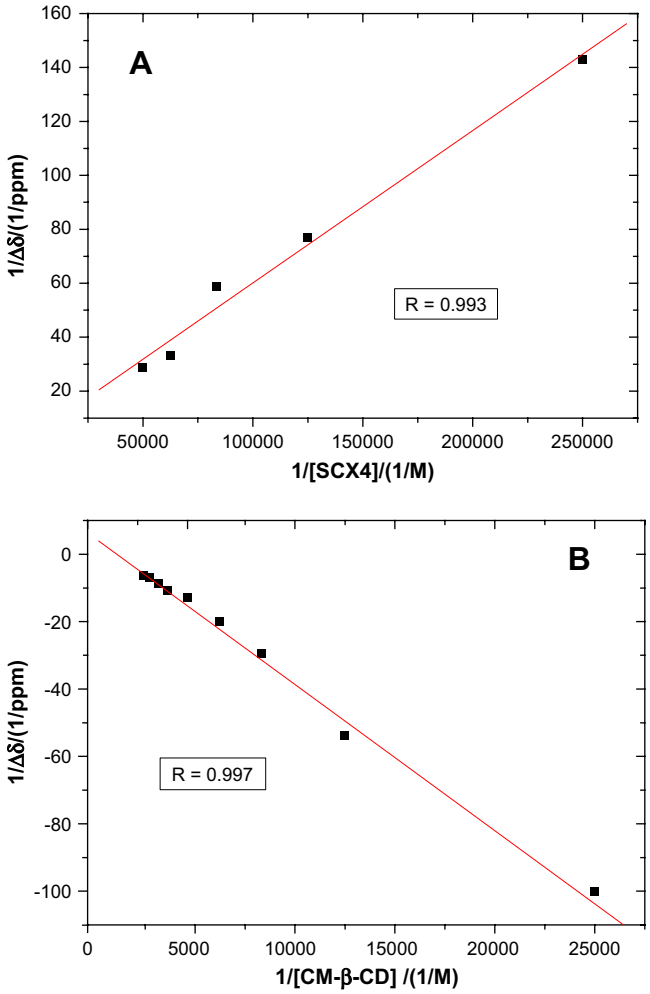


Fig. 6. A: Benesi–Hildebrand plots of ^1H NMR titration for NR complexed to SCX4. B: Benesi–Hildebrand plots of ^1H NMR titration for NR complexed to CM-β-CD.

4. Conclusion

Absorption, fluorescence and ^1H NMR investigation revealed the inclusion complexation interaction between C.I. Basic Red 5 and SCX4 or CM-β-CD. The formation constants, binding ratio, enthalpy and entropy of complexation were evaluated. The dye can form 1:1 stable inclusion complexes with two kinds of host compounds, showing distinctly opposite absorption fluorescent and ^1H NMR behavior. The absorption spectra showed that CM-β-CD prefers to combine with the neutral form while SCX interacts strongly with the acidic form of C.I. Basic Red 5. The fluorescence intensity of the dye gradually decreased upon the addition of SCX4, accompanying a bathochromic shift of the emission spectrum; in contrast, the fluorescence intensity of C.I. Basic Red 5 increased in the case of neutral complexes with CM-β-CD, accompanying a hypsochromic shift of the emission spectrum. The protons signals of NR moved downfield in the SCX4, while displayed upfield frequency changes in the CM-β-CD. This phenomenon suggested the complexation of SCX4 with NR is mainly governed by the electrostatic interaction rather than the hydrophobic interaction whereas CM-β-CD interacts with the neutral form of NR by the hydrophobic interaction. Moreover, CM-β-CD has a rigid cavity while SCX4 is conformationally very flexible. CM-β-CD and SCX4 could include NR to form host–guest complexes and alter the physical and chemical properties of C.I. Basic Red 5. Therefore, there will be potential applications in mimetic enzyme and biological probe.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 90813018) and the Youth Foundation of Shanxi Province (No. 2008021011 and No. 2007021007).

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