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The joint effects of room temperature ionic liquids and ordered media on fluorescence characteristics of estrogens in water and methanol

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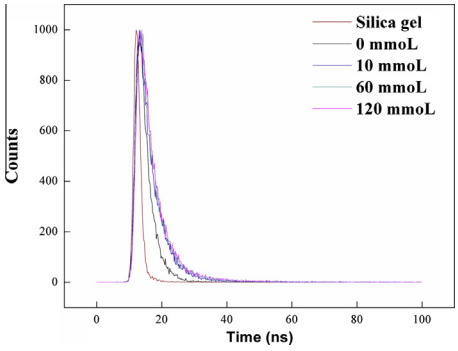
HIGHLIGHTS

- Both β-CD and CTAB could sensitize the fluorescence of EE2 and E2.
- Inclusion ratio of the complex was 1:1.
- Typical RTILs including $[C_nMIM]BF_4$ and $[C_nMIM]PF_6$ (n = 4, 6).
- The fluorescence lifetimes were 2.50–2.53 and 4.03–4.13 ns for EE2 and E2.
- The quenching process was demonstrated to be a dynamic quenching mechanism.

GRAPHICAL ABSTRACT

The fluorescence lifetimes of EE2 were 2.50 and 4.13 ns, respectively, in water and methanol, and they increased gradually with increasing of β-CD concentrations.



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ABSTRACT

This study investigated the steady-state and time-resolved fluorescence properties of 17α-ethinylestradiol (EE2) and 17β-estradiol (E2) in the presence of ordered media (β-cyclodextrins (β-CD) and cetyltrimethylammonium bromide (CTAB)). In addition, we analyzed the effects of four room temperature ionic liquids (RTILs) on the fluorescence intensities (FIs) of EE2/β-CD and E2/β-CD inclusion complexes in methanol. Both β-CD and CTAB enhanced the fluorescence of EE2 and E2. The FIs of EE2 and E2 with β-CD or CTAB in methanol were greater than those in water, possibly resulting from decreased oxygen-quenching in H_2O molecules. β-CD and CTAB may form inclusion complexes with estrogen in both water and methanol. The inclusion ratio of the complex was 1:1 and the inclusion constant (K) values in water were greater than those in methanol. The fluorescence lifetimes were 2.50 and 4.13 ns for EE2 and 2.58 and 4.03 ns for E2 in aqueous solution and methanol, respectively. The changing trend of fluorescence lifetimes for EE2 and E2 in β-CD or CTAB was similar to the steady-state FIs. The four RTILs had a significant quenching effect on the FIs of EE2/β-CD and E2/β-CD, and the quenching process for EE2/β-CD and E2/β-CD by RTILs was demonstrated to be a dynamic quenching mechanism. Fluorescent data obtained from these complex systems provide a theoretical foundation for understanding the interaction mechanisms between ordered media and RTILs in the analysis of estrogens.

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Introduction

Cyclodextrins (CDs) are a family of compounds made up of sugar molecules bound together in different ring structural formations: α, β, and γ-types, which consist of 6, 7 and 8 glucose units, respectively [1]. They are cyclic oligosaccharides formed enzymatically from starch by *Bacillus macerans*. β-CD is the most widely used CD type, which forms a doughnut-shaped structure with a hydrophobic cavity due to the shield of C–H to two ring atoms of hydrogen (H-3 and H-5) and an oxygen atom with a circle glycosidic bond inside the cavity. In contrast, β-CD has a hydrophilic exterior because of hydroxyl gathering at the lateral border of the CD molecule [2,3]. Because of these properties, β-CD can enhance the fluorescence intensities (FIs) of many compounds with weak fluorophores [4–6]. The cavity can incorporate nonpolar molecules as guests to form inclusion complexes by van der Waals forces. β-CD is non-toxic, edible and chemically stable [7], leading to its widespread application in several fields, such as development of analytical techniques [8,9], chromatography [10,11], enzyme–subtract interactions [12], pharmacy [13] and enhanced performance of fullerenes in biomedical applications [14,15]. In this research, we chose β-CD as one of the fluorescence-sensitizing agents to study the interaction between β-CD and two typical estrogens, 17β-estradiol (E2) and 17α-ethinylestradiol (EE2).

Surfactant molecules are amphiphilic in nature having hydrophilic (head) and hydrophobic moieties. The hydrophobic component is generally a long chain hydrocarbon or aromatic ring [16]. Based on hydrophilic groups, surfactants can be classified as ionic (surface-active) or non-ionic (not surface-active). Cetyltrimethylammonium bromide (CTAB) is an important cationic surfactant that is widely used in analytical fields, industrial applications [17–19] and catalysis [20,21]. In fluorescence analysis, CTAB is often used as a fluorescence-enhancing agent. Previous studies showed that CTAB can have varying effects on chemicals with different fluorophores [22,23]. Therefore, it is necessary to study the interaction mechanism(s) between CTAB and organic chemicals to advance their application in analytical fields. Room temperature ionic liquids (RTILs) are organic salts, which are liquid at ambient temperatures and possess an appreciable liquid range. RTILs have several attractive features, including low-melting temperature (below 373 K), negligible vapor pressure, and excellent thermal stability. These properties make RTILs ideal candidates for "green chemistry" applications.

Both surfactants and β-CDs belong to ordered media having a significant effect on molecular luminescence, and thus they are widely used in luminescence analysis [4,5,22]. In recent years, many studies have examined the interaction between surfactants and β-CD or their derivatives [24,25]. However, there is a paucity of information concerning interactions between RTILs and ordered media (surfactants or β-CD). If RTILs and ordered media have co-synergistic effects, their combination may markedly improve the detection sensitivity of weakly fluorescent substances. Thus, there is great potential opportunity for enhancing fluorescence characteristics through a better understanding of the interaction between RTILs and ordered media.

Estrogens are among the most potent endocrine-disrupting compounds (EDCs) found in wastewater. The four estrogens most commonly found in wastewater include three natural steroids (17β-estradiol (E2), estrone (E1) and estriol (E3)) and one synthetic compound (17α-ethinylestradiol (EE2)) that is used in contraceptives and hormone replacement therapy [26]. Among these estrogens, E2 and EE2 have three to seven orders of magnitude greater estrogenic potencies than the other EDCs identified in wastewater [27]. The lowest observable effect concentration for E2 affecting production of vitellogenin in juvenile female rainbow trout is 14 ng/L [27]. Purdom and coworkers reported that less than 1 ng/L of EE2 can stimulate male rainbow trout to produce vitellogenin [28], while Lange and coworkers found that a concentration of 4 ng/L EE2 can cause failure in the male fathead minnow to develop normal secondary sexual characteristics [29]. Furthermore, freshwater worms can bioaccumulate EE2, making possible a transfer to benthivores and subsequent secondary poisoning of predators. Estrogen concentrations of 1–500 ng/L are commonly found in wastewater effluents. With dilution, receiving surface waters typically contain E2 and EE2 up to 20–30 ng/L [30]. Thus, these estrogen concentrations are environmentally relevant [26] and it is critically important to develop a simple and reliable method for the determination of estrogen in environmental matrices. As a quick and effective method, fluorescence techniques are often used for quantification and analyzing the properties of fluorescing compounds. The aim of this work was to evaluate the effects of β-CD and CTAB on the fluorescence intensities (FIs) and time-resolved fluorescence spectra of EE2 and E2, and further to provide a mechanistic understanding of their interactions. Due to their green chemistry properties, we also investigated the effects of RTILs on the fluorescence properties of EE2 and E2 in β-CD for

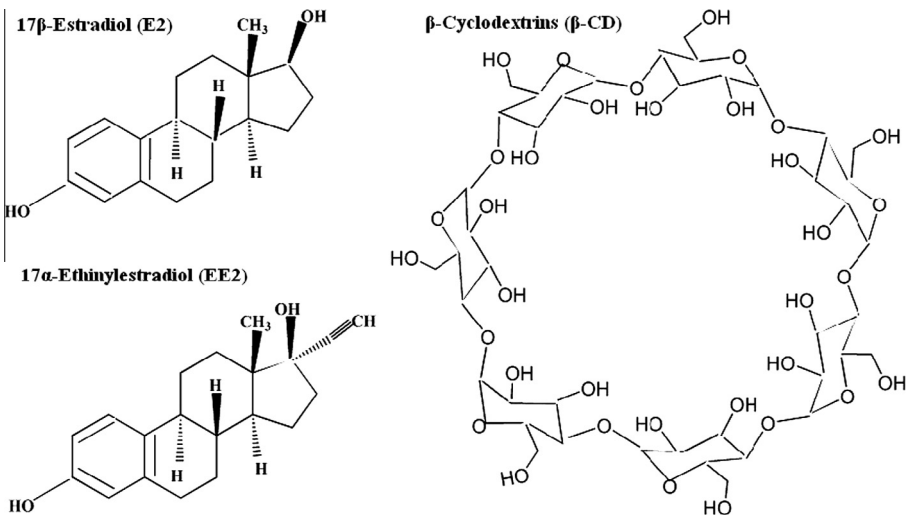

Fig. 1. The molecular structures of EE2, E2 and β-CD.

possibly application in enhancing the fluorescence properties of estrogens.

Materials and methods

Materials

Analytical grade 17α-ethinylestradiol (EE2) and 17β-estradiol (E2) were purchased from Sigma-Aldrich (St. Louis, MO, USA), β-CD from TCI (Shanghai, China), and CTAB and methanol (chromatographic grade) from Jinshan Reagent Corporation, Wenzhou, China. The chemical structures of EE2, E2 and β-CD are shown in Fig. 1. RTILs with purities of 99.0% were obtained from Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China). The four RTILs were 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM]BF_4$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($[C_6MIM]BF_4$), 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM]PF_6$) and 1-hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM]PF_6$).

Analytical instrumentation

A RF-5301PC model spectrofluorometer was used to measure all fluorescence spectra (Shimadzu Corporation, Tokyo, Japan). The spectrofluorometer has a 150 W Xenon arc lamp as the excitation source and a single-grating monochromator with a slit width of 5 nm for wavelength selection. Fluorescence lifetimes were determined at 18 ± 1 °C using a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., UK) equipped with a temperature-controlled circulator (Julabo, Germany).

Methods

A stock solution (100 mg L^{-1}) was prepared by dissolving 5 mg of EE2 or E2 in 50 mL methanol and stored at 4 °C before use. Working solutions were made by appropriate dilution of the stock solution with ultrapure water or methanol. A series of experimental concentrations for β-CD and CTAB were set at 0, 0.02, 0.06, 0.18, 0.5 mM and 0, 5, 10, 60, 120 mM, respectively. Working solutions for all RTILs were 0, 10, 30, 60 and 90 mM and the tested concentration of estrogen was 0.2 mg L^{-1}. The test solution (20 μL) was added to 1 cm^2 quartz cuvettes for fluorescence determination. The excitation and emission wavelengths for EE2 and E2 were 280 nm and 308 nm, respectively. The samples were excited at 280 nm and the emission spectra recorded from 290 to 412 nm. All fluorescence spectra were corrected for the solvent blank and the fluorescent lifetime curves were corrected using a low concentration of silica gel reference solution at the same experimental conditions.

Results and discussion

Effects of β-CD on fluorescence spectra of EE2 and E2

EE2 and E2 have very similar structures, differing only in the C17 substituent; E2 has a hydroxyl group at C17, while EE2 has an ethinyl group (Fig. 1). Both compounds have phenol rings, which are responsible for the ca 280 nm $^1\pi\pi^*$ absorption [31]. The ethinyl group of EE2 adds a very weak $^1n\pi^*$ absorption at slightly higher wavelengths, resulting in the slightly higher FIs of EE2 relative to those of E2 when they are at the same concentration

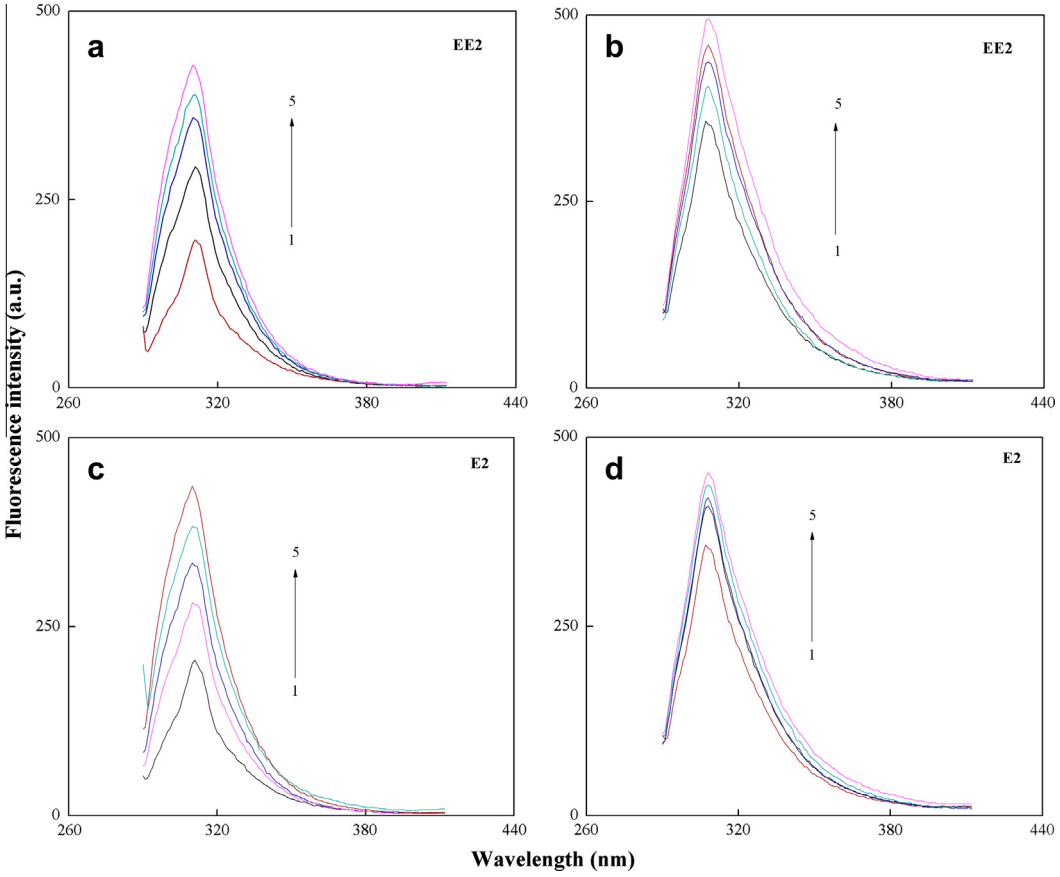

Fig. 2. The fluorescence spectra of EE2 and E2 with different concentrations of β-CD in water (a and c) and methanol (b and d). *β-CD concentrations: 1 (0 mM), 2 (0.02 mM), 3 (0.06 mM), 4 (0.18 mM) and 5 (0.5 mM).

in methanol or water (Figs. 2 and 3). Following excitation in the lowest energy absorption band of E2 and EE2, a strong emission is observed at 308 and 312 nm, respectively [32]. Fig. 2 shows the steady-state emission spectra of EE2 and E2 in the absence and presence of different concentrations of β-CD with an excitation wavelength at 280 nm in water and methanol. The FIs of EE2 and E2 (0.2 mg L^{-1}) increased significantly with increasing concentrations of β-CD (0, 0.02, 0.06, 0.18, 0.5 mM). In addition, no obvious hypsochromic or bathochromic phenomenon of the emission maxima was observed in the fluorescence-enhancing process. Previous reports showed that the fluorescence enhancement of citrinin by β-CD was due to formation of an inclusion complex [33]. Estrogen is an acidic hormone with a conjugated planar structure, which has natural fluorescence and similar fluorophores as citrinin. When EE2 or E2 enters into the β-CD cavity, the polarity of the microenvironment in the complex decreases and causes a larger $S_1 \sim S_0$ energy gap. The increased energy gap causes a significant reduction in the rate of internal conversion, which is the dominant non-radioactive decay pathway competing with fluorescence. When EE2 or E2 is incorporated into the β-CD cavity, the rotational freedom and vibrational level relaxation (VR) caused by the solvent molecules are significantly reduced [3]. Moreover, the number of deactivated EE2 and E2 molecules caused by VR is greatly decreased [3]. Consequently, the FIs of EE2 and E2 show a large increase.

β-CD can incorporate a large number of guest molecules in its interior and form non-covalent inclusion complexes. Complexes of β-CD with completely or partially incorporated guest molecules yield interesting spectroscopic effects. If the change in free energy of the complex is higher in its excited state than its ground state, it indicates that a stronger inclusion complex is formed in the excited state compared to its ground state [34]. In addition, the structure of the inclusion complex (EE2 (E2)-β-CD) is different from that of EE2 (E2) and β-CD. Further study is warranted to determine the structure of 4-t-OP-β-CD by means of Fourier Transform Infrared (FTIR), 1H Nuclear Magnetic Resonance (^{1}H NMR) and Scanning Electron Microscope (SEM) techniques in order to fully understand the mechanistic effects of β-CD on fluorescence characteristics.

Effects of CTAB on fluorescence spectra of EE2 and E2

CTAB, a cationic surfactant, is often used as a fluorescence-enhancing agent to improve the detection sensitivity of weakly fluorescent chemicals, especially when it is combined with β-CD [35]. As shown in Fig. 3, no changes of the fluorescent spectra of EE2 or E2 in aqueous solution and methanol were observed in various concentrations of CTAB solution. However, the FIs of EE2 and E2 (0.2 mg L^{-1}) increased with increasing concentrations of CTAB (0, 5, 10, 60, 120 mM). In aqueous solution, the FIs of EE2 and E2 at a CTAB fortification level of 120 mM increased more than 2-fold as compared with the control. However, the FIs increased only 30–40% in methanol with the addition of CTAB. The addition of CTAB changes the microscopic properties of the aqueous solution, which provides a protective microenvironment for the excited singlet state of EE2 and E2 molecules. EE2 and E2 are dispersed and linked into the microemulsion droplets, thereby effectively shielding their molecules. This kind of protective microenvironment reduces fluorescent quenching due to self-quenching and external quenching mechanisms [36]. Therefore, this microenvironment can lead to a large decrease in the rate constant for the excited singlet state of non-radiative deactivation, and improve the fluorescence quantum yield, thus enhancing the FIs of EE2 and E2.

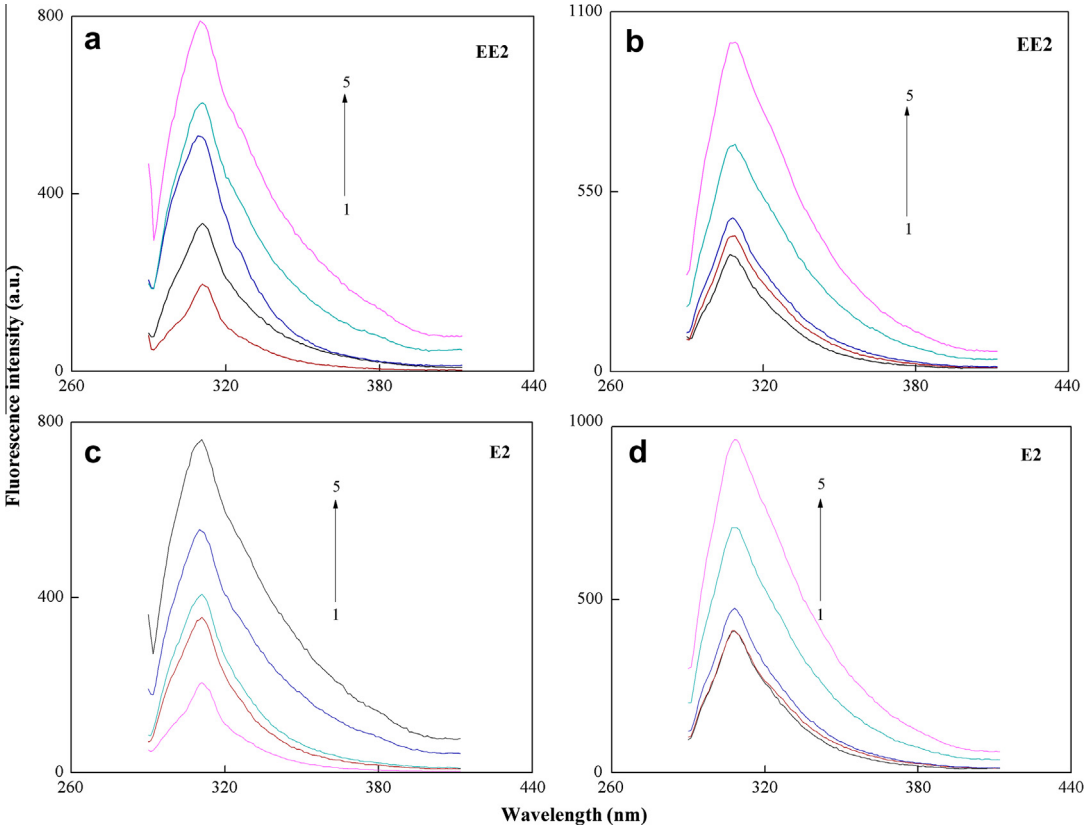

Fig. 3. The fluorescence spectra of EE2 and E2 with different concentrations of CTAB in water (a and c) and methanol (b and d). *CTAB concentrations: 1 (0 mM), 2 (5 mM), 3 (10 mM), 4 (60 mM) and 5 (120 mM).

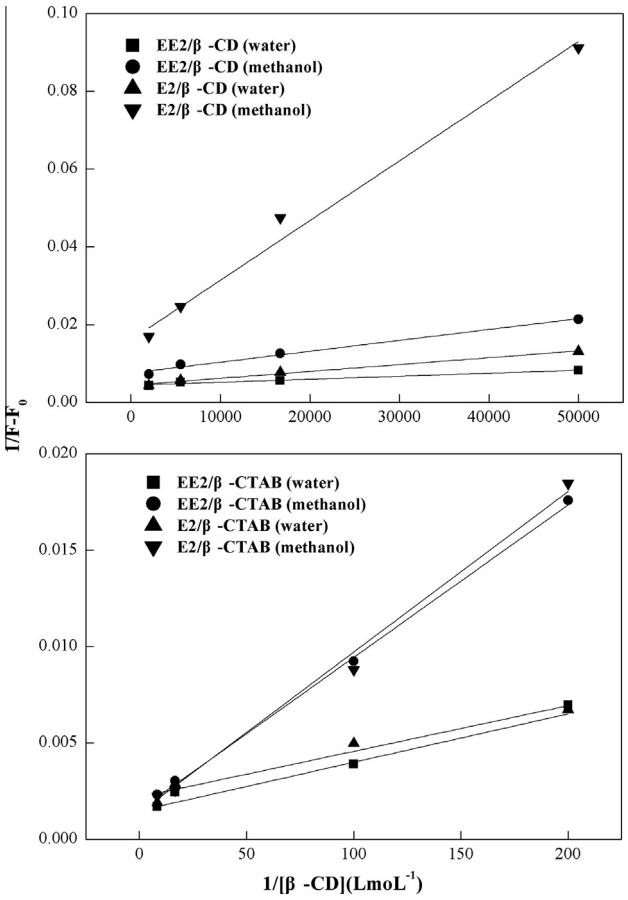

Fig. 4. Plot of $1/F - F_0$ versus $1/[G]$ for EE2 or E2/β-CD (a) and EE2 or E2/CTAB (b) inclusion complexes in methanol and water.

Solvent effects on FIs of EE2/β-CD, E2/β-CD, EE2/CTAB and E2/CTAB inclusion complexes

Water and methanol were chosen as representative solvents to determine solvent effects on FIs of inclusion complexes. Figs. 2 and 3 show the comparative results for the FIs of EE2/β-CD, E2/β-CD, EE2/CTAB and E2/CTAB in different percentages of water (a and c) and methanol (b and d). The FIs of EE2/β-CD and E2/β-CD in methanol were higher than those in water. For example, the FIs of EE2 in methanol and water were approximately 970 and 780 (a.u.), respectively, when the fortified concentration of CTAB was 120 mM. Both methanol and water have tetrahedron structure (V form) and possess angles of 109° for SP^3 bonds. However, water molecules have lone pair electrons, resulting in a smaller bond angle (104.5) and higher H-bond donating capacity [37]. It was reported that the 308 nm band could shift to higher wavelengths (lower energies) as the dielectric/H-bonding ability of the solvent increases [32]. The changing FIs and shifts of the 308 nm band were found to vary as a function of solvent [32]. In this investigation, because water has higher polarity and H-bonding capacity, higher FIs and wider blue-shifts (ca 2 nm) for EE2 and E2 were observed in water than in methanol (Figs. 2 and 3). The energy of the $^1\pi\pi^*$ state at C17 is not expected to significantly affect the absorption at 280 nm. However, the $^1n\pi^*$ state for the ethinyl group is significantly affected by the presence of hydrogen-bond donors. By adding water, the H-bond donating capacity increases which increases the energy of the $^1n\pi^*$ state, while decreasing the energy of the 308 nm emission band resulting from 280 nm absorption [32]. Therefore, the highest FIs were observed for EE2 in methanol, as compared to water (Fig. 2). In addition, these results may be due to a vibrational coupling interaction between EE2 (E2) and its surrounding water molecules, with the fluorescence energy partially released as heat to the solution [3]. When EE2 and E2 are incorporated into the cavity of β-CD, the hydrophobic microenvironment provided by β-CD and methanol molecules prevents water molecules from quenching EE2 (E2) fluorescence by hydrogen bonds resulting in a stronger fluorescence signal. The above observations have a similar mechanism to those for inclusion complexes of EE2/β-CD and E2/β-CD, i.e., decreasing the possibility of oxygen-quenching processes by H_2O molecules.

Stoichiometry and inclusion constant

The stoichiometry and association constant for the inclusion complex were studied using FI data collected in the absence and presence of β-CD or CTAB. Assuming that the composition of the inclusion complex is 1:1, the following expression can be used to determine the inclusion constant (K) [35]:

$$\frac{1}{F - F_0} = \frac{1}{F_1 - F_0} + \frac{1}{K[G](F_1 - F_0)}$$

where F_0 and F_1 denote the FIs of EE2 or E2 in the absence and presence of β-CD or CTAB, respectively; F is the FI for each host molecule (EE2 and E2) concentration tested; $[G]$ denotes the FI of β-CD or CTAB; and K is the inclusion constant of the complex. Evidence for the existence of a 1:1 complex was obtained from fitting a double-reciprocal plot of $1/F - F_0$ versus $1/[G]$ (Fig. 4). Table 1 summarizes the regression equation, inclusion constant (K) values and linear correlation coefficient. K is an important parameter for describing the inclusion behavior and can reflect the binding intensity of β-CD and CTAB with EE2 or E2. As shown in Table 1, the $K_{\beta\text{-CD}}$ values for the 1:1 complex stoichiometry of β-CD with EE2 or E2 was larger than for the 1:1 complex stoichiometry of CTAB with EE2 or E2 in different solvents. In addition, we also observed that the K values for the inclusion complex in water were greater than those in methanol. The larger K values imply a more stable complex structure as a function of the guest molecules ease of entering into the host cavity leading to decreased quenching efficiency. Moreover, formation of more than one peak for an analyte in the presence of β-CD or CTAB would indicate formation of a mixture rather than complex structure [36]. In this investigation, we only detected one fluorescent peak for the analyte, and thus conclude that a complex rather than a mixture was formed.

Table 1
The regression equation and inclusion constants of complexes in different solvents.

Solvents	Complex	Regression equation	R^2	$K/(\times 10^4\ \mathrm{L\ mol^{-1}})$
Water	EE2/β-CD	$y = 8.99 \times 10^{-8}\,x + 0.0044$	0.992	4.89
	E2/β-CD	$y = 1.75 \times 10^{-7}\,x + 0.0045$	0.992	2.57
	EE2/CTAB	$y = 2.54 \times 10^{-5}\,x + 0.0016$	0.991	6.3×10^{-3}
	E2/CTAB	$y = 2.1 \times 10^{-5}\,x + 0.0026$	0.992	1.24×10^{-2}
Methanol	EE2/β-CD	$y = 2.84 \times 10^{-7}\,x + 0.0074$	0.993	2.6
	E2/β-CD	$y = 1.56 \times 10^{-6}\,x + 0.0141$	0.994	0.9
	EE2/CTAB	$y = 8.33 \times 10^{-5}\,x + 0.0008$	0.999	9.6×10^{-4}
	E2/CTAB	$y = 8.46 \times 10^{-5}\,x + 0.0014$	0.996	1.65×10^{-3}

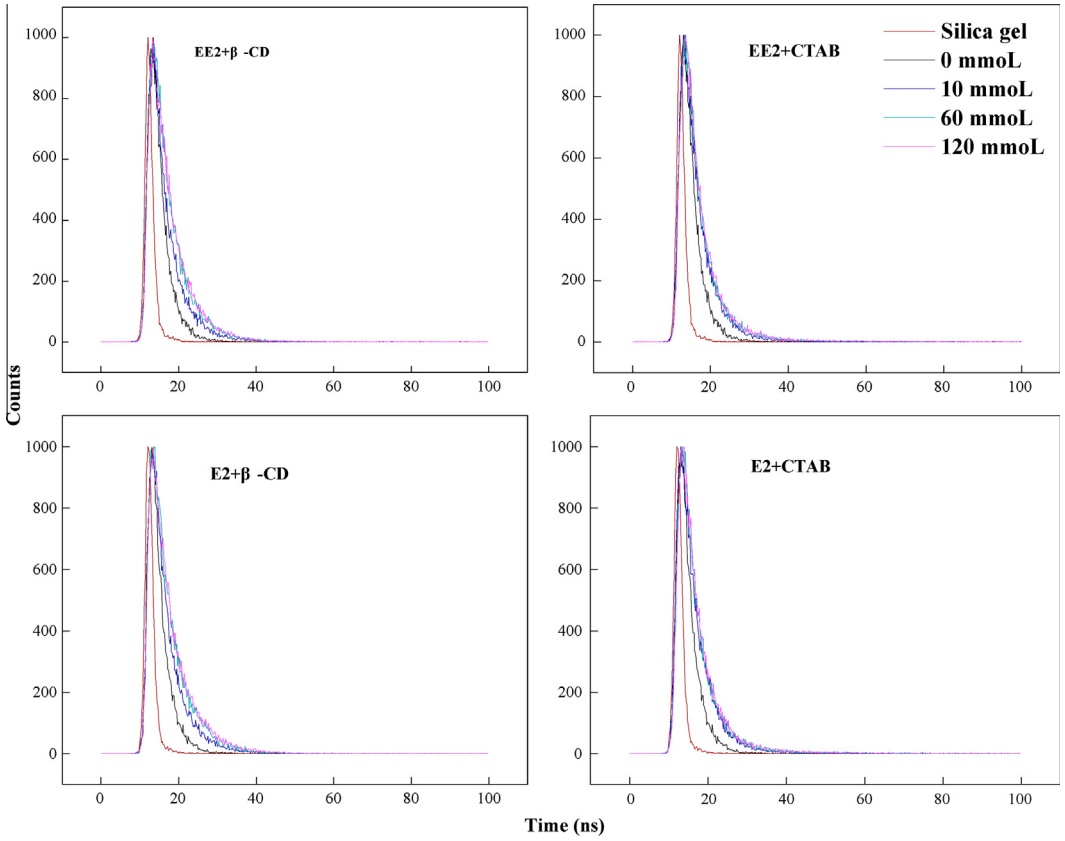

Fig. 5. The time-resolved fluorescence of EE2 and E2 with β-CD or CTAB in water ($\Delta\lambda$ = 20 nm).

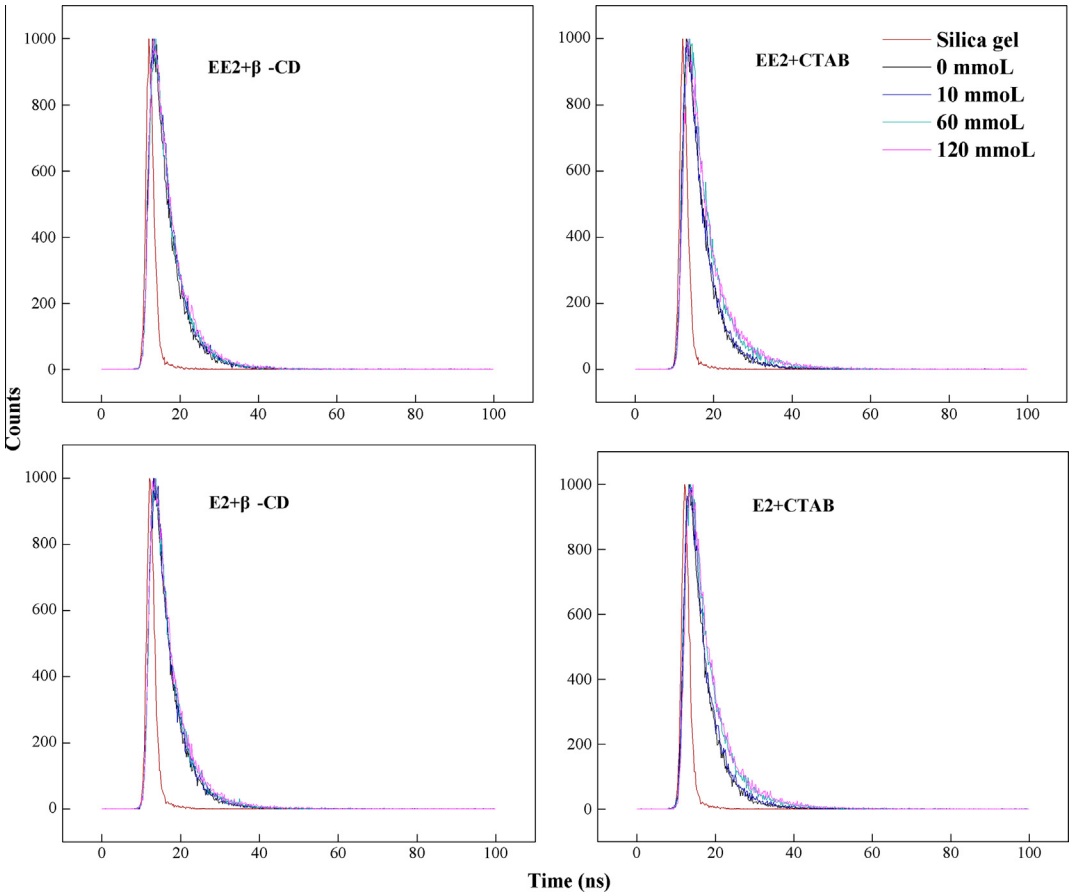

Fig. 6. The time-resolved fluorescence of EE2 and E2 with β-CD or CTAB in methanol ($\Delta\lambda$ = 20 nm).

Table 2
Time-resolved fluorescence spectra of EE2 in the absence and presence of different concentrations of β-CD or CTAB (λ_{ex} = 280 nm, λ_{em} = 308 nm; [EE2] = 0.2 mg L^{-1}).

Complexes	C (mmol/L)	Water		Methanol	
		τ (ns)	x^2	τ (ns)	x^2
EE2/β-CD	0	2.50	1.002	4.13	0.999
	0.02	4.19	1.001	4.26	0.984
	0.06	4.73	0.999	4.41	1.001
	0.18	4.94	1.002	4.67	1.003
EE2/CTAB	10	4.37	1.000	4.29	1.000
	60	5.29	1.000	5.19	1.006
	120	5.63	1.001	5.52	1.124

Table 3
Time-resolved fluorescence spectral data of E2 in the absence and presence of different concentrations of β-CD or CTAB (λ_{ex} = 280 nm, λ_{em} = 308 nm; [E2] = 0.2 mg L^{-1}).

Complexes	C (mmol/L)	Water		Methanol	
		τ (ns)	x^2	τ (ns)	x^2
E2/β-CD	0	2.58	1.005	4.08	0.971
	0.02	4.32	1.001	4.25	1.002
	0.06	4.93	1.001	4.46	1.000
	0.18	5.33	1.002	4.63	1.000
E2/CTAB	10	4.17	1.002	4.37	1.000
	60	4.35	1.110	5.29	1.000
	120	4.64	1.133	5.63	1.001

In addition to the above analysis, previous literature reported that the stoichiometry of the inclusion complex could be judged by surface tension measurements [38,39] and structural characterization by means of FTIR, ^{1}H NMR and SEM techniques [40,41]. Guest molecules with long hydrophobic chains provided convincing evidence for penetration into the CD cavity and showed a 1:2 stoichiometry [42]. Li and coworkers found that two β-CD tend to incorporate one ionic liquid C_{16}TPB molecule to form a C_{16}TPB@2β-CD complex. The aggregates of C_{16}TPB@2β-CD, as consistent building blocks, can construct bilayer membranes at the appropriate concentration [43]. However, surface tension measurements revealed that there were two kinds of inclusion formations having 1:1 and 1:2 stoichiometry for β-CD–[C_{12}MIM]PF_6 and that only the tails of [C_{12}MIM]PF_6 were incorporated into the β-CD cavity. Hydrophobicity played a crucial role in supporting the formation of β-CD–[C_{12}MIM]PF_6 inclusion complexes, and hydrogen bonding was responsible for maintaining the channel structure [39]. Because both ILs and 4-t-OP can form inclusion complexes, a three-dimensional complex among IL, EE2 (E2) and β-CD possibly formed in this experiment. Further studies are required to determine the possible formation of a three-dimensional structure of EE2 (E2)-β-CD-ILs using techniques such as FTIR, ^{1}H NMR and SEM.

Time-resolved fluorescence lifetime in ordered media

Time-resolved fluorescence profiles for EE2 and E2 in ordered media (β-CD or CTAB) are shown in Fig. 5 (aqueous solution) and Fig. 6 (methanol). The fluorescence lifetimes were 2.50 and 4.13 ns for EE2 (Table 1) and 2.58 and 4.03 ns for E2 (Table 2) in aqueous solution and methanol, respectively. Additionally, fluorescence lifetimes increased gradually with increasing ordered media (β-CD and CTAB) concentrations. In general, the changing trend for fluorescence lifetimes of EE2 and E2 were similar to those for steady-state FIs (Figs. 2 and 3) (see Table 3).

Quenching effects of RTILs on EE2/β-CD and E2/β-CD fluorescence

At ambient conditions, ionic liquids are much more viscous than organic solvents, such as methanol. For example, the viscosity of methanol is 0.58 mPa s at 20 °C while the viscosities of [C_4MIM]PF_6 and [C_4MIM]BF_4 are 430 and 154 mPa s, respectively, at the same temperature. Considering the large difference between the viscosity of RTILs and common organic solvents, a series of RTIL concentrations (10, 30, 60 and 90 mM) were prepared in methanol. As shown in Figs. 7 and 8, the FIs of EE2/β-CD and E2/β-CD gradually decreased with increasing RTILs concentrations from 0 to 90 mM. The four RTILs had a significant quenching effect on the fluorescence of EE2/β-CD and E2/β-CD. However, no obvious hypsochromic or bathochromic phenomenon for the emission maxima was induced by quenching processes.

Interactions between ionic liquids and β-CD

The spectral behavior of neat RTILs exhibits excitation wavelength-dependent, two-component emission. The 340–360 nm emission band is observed when neat RTILs are excited at short excitation wavelengths. However, as the excitation is shifted to longer wavelengths, which correspond to the tail portion of the absorption band, the fluorescence maximum starts to shift toward longer wavelengths with a progressive decrease in the overall intensity [44]. An interesting feature of the emission spectra is the shift of the emission maximum toward a longer wavelength with increasing excitation wavelength [45]. For neat [C_4MIM][PF_6], the estimated value of the fluorescence quantum yield (λ_{exc} = 360 nm) is about 5×10^{-3}, and the major component (~90%) of the decay consists of a lifetime in the range of 470–590 ps [44]. At the maximum excitation wavelength of 276 nm, no changing phenomena were observed for the emission maxima shape at ca. 430 nm for neat [C_8MIM]BF_4 and neat [C_8MIM]PF_6, and ca. 440 nm for neat [C_8MIM]Cl) in the presence of β-CD (Fig. 9A). However, different phenomena were apparent for the FIs of different ILs with addition of β-CD. The FIs of neat [C_8MIM]PF_6 and [C_8MIM]Cl did not vary prominently, while the FI for neat [C_8MIM]BF_4 was significantly increased in the presence of β-CD [44]. Fig. 9B shows the FIs of neat β-CD, neat [C_8MIM]BF_4 and the mixture of β-CD and [C_8MIM]BF_4.

The four ILs ([C_4MIM]PF_6, [C_6MIM]PF_6, [C_4MIM]BF_4 and [C_6MIM]BF_4) all exhibited quenching effects on the FIs of EE2 (E2)-β-CD complexes. This may be attributed to both RTILs and EE2 (E2) forming inclusion complexes with β-CD, and thus a competition between ILs and 4-t-OP. In most cases, ILs have a higher probability of being incorporated into the β-CD cavity [40], and

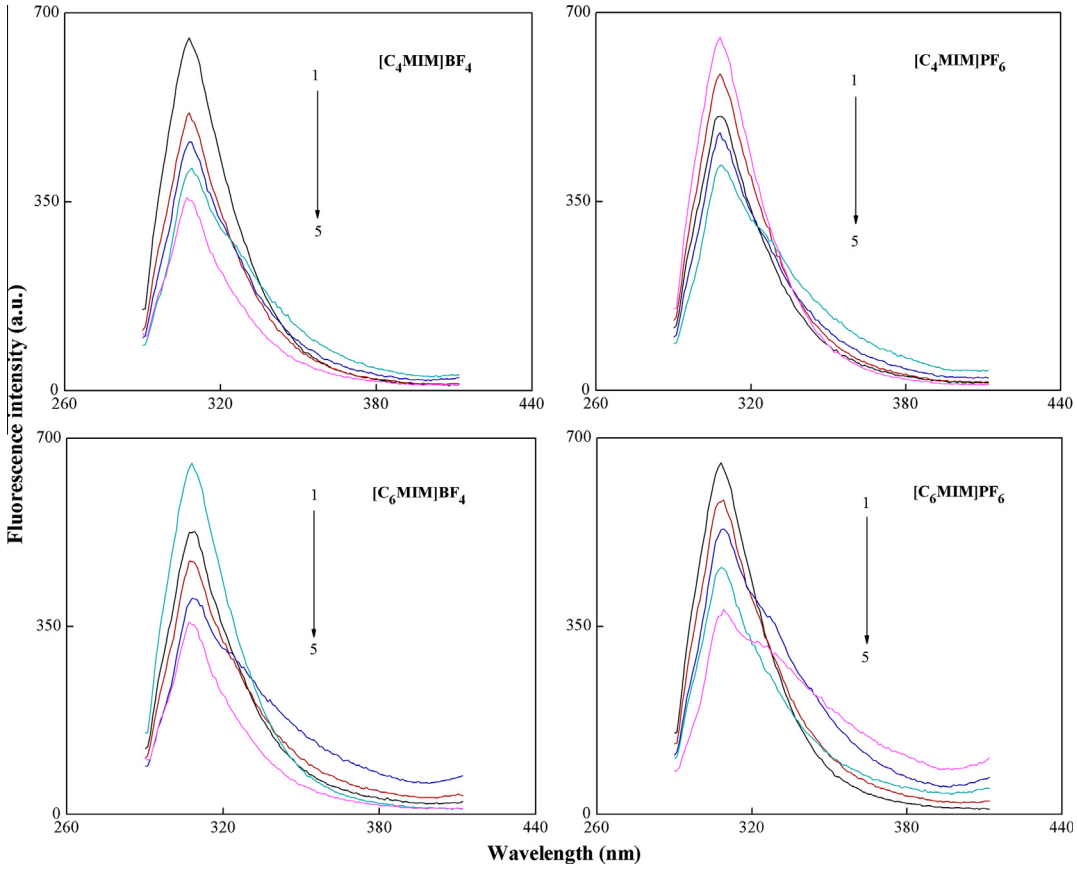

Fig. 7. The fluorescence spectra of EE2/β-CD in four RTILs with different concentration. *RTILs concentrations: 1 (0 mM), 2 (10 mM), 3 (30 mM), 4 (60 mM) and 5 (90 mM).

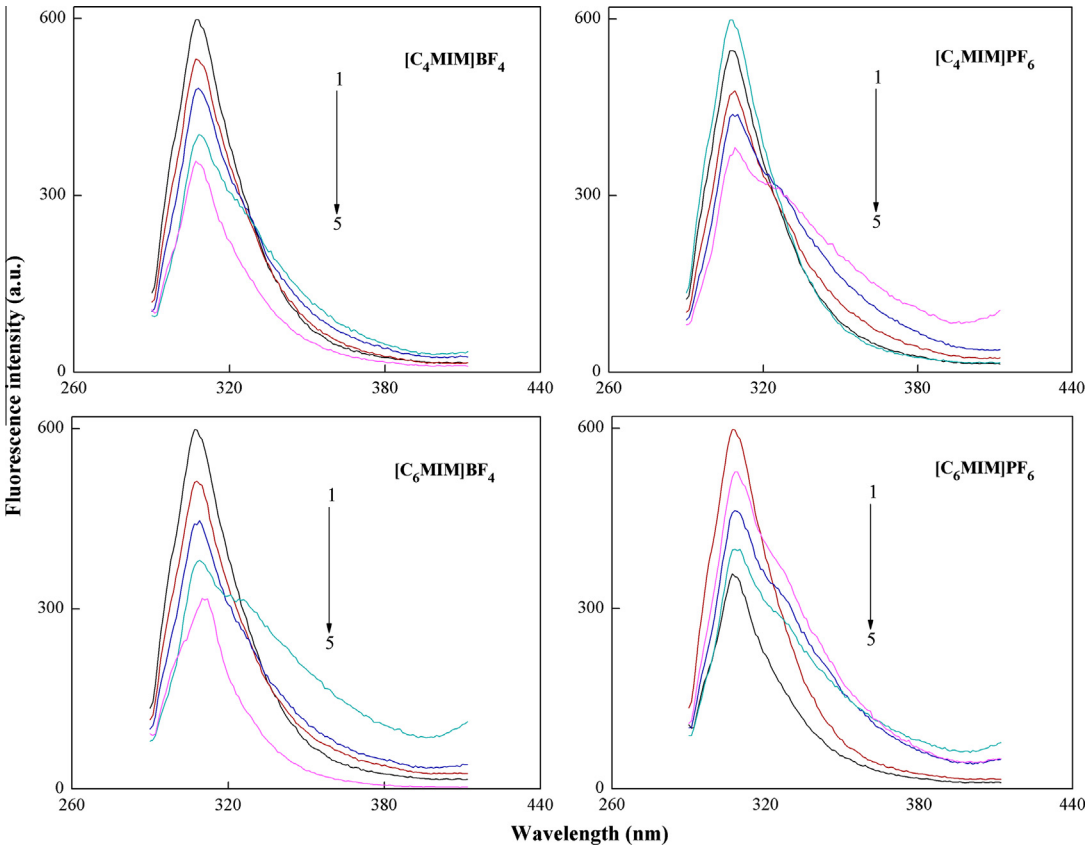

Fig. 8. The fluorescence spectra of E2/β-CD in four RTILs with different concentrations. *RTILs concentrations: 1 (0 mM), 2 (10 mM), 3 (30 mM), 4 (60 mM) and 5 (90 mM).

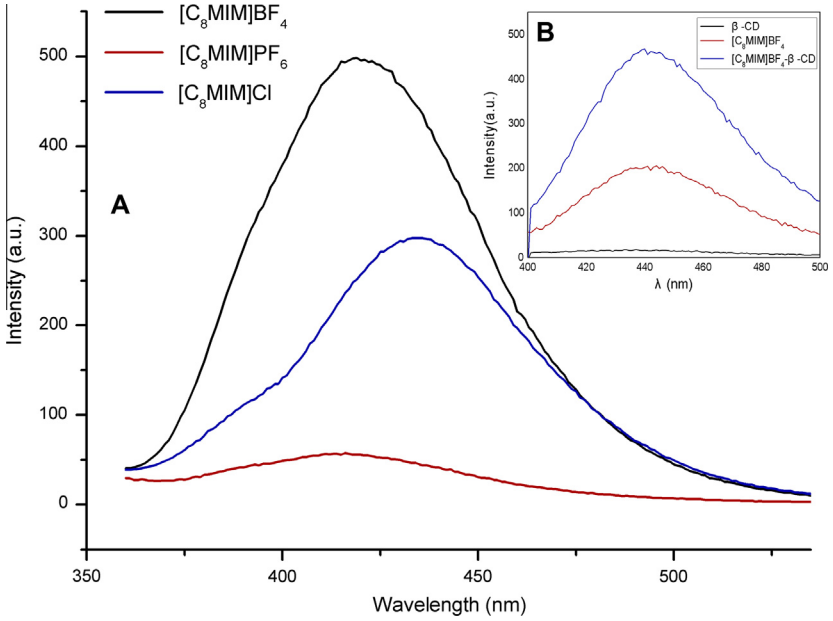

Fig. 9. Emission spectra of ILs in aqueous solution (A) and the effect of β-CD on neat $[C_8MIM]BF_4$ (B).

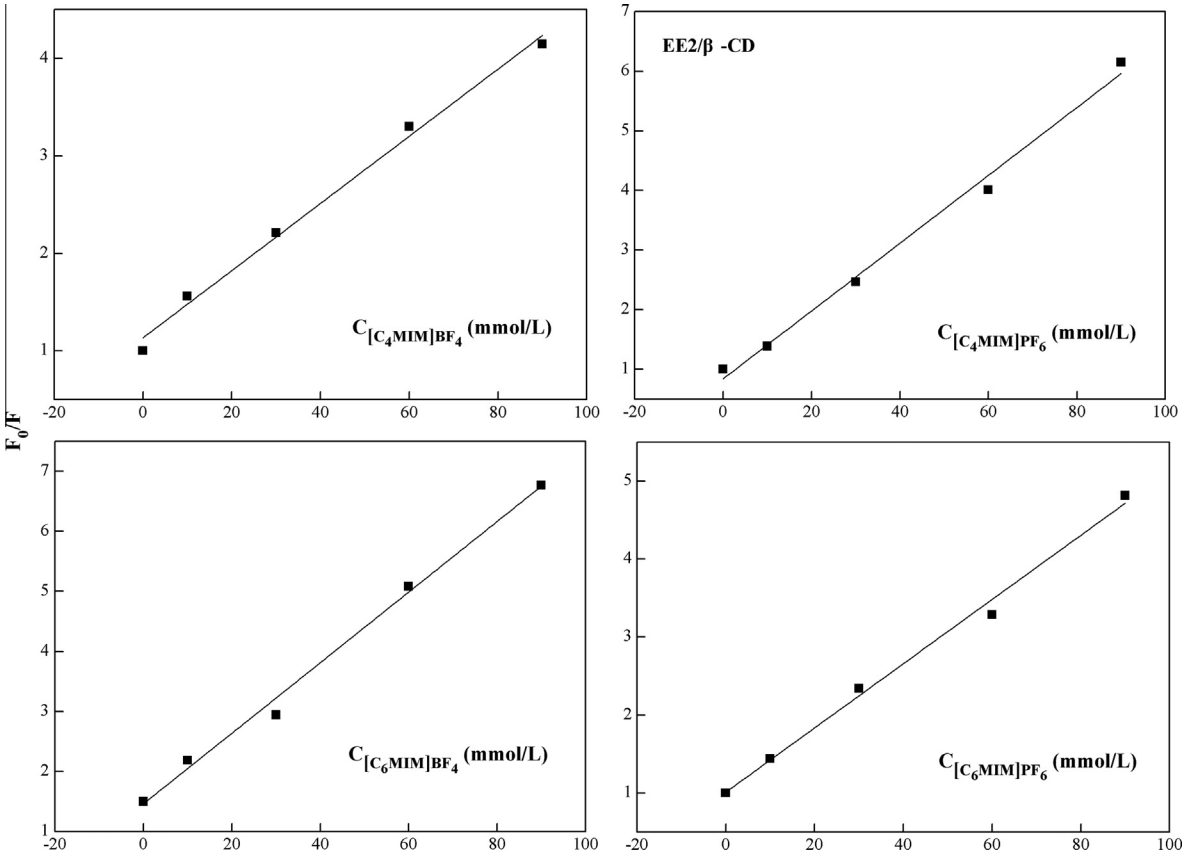

Fig. 10. The Stern–Volmer plot for the binding of RTILs to EE2/β-CD.

the similar size of the β-CD cavity and imidazolium cation play an important role in the complexation process [41]. The building block structure of the C_{16}TPB@2β-CD complex was found in aqueous solution, which can form a sheet-like hydrogel with multi-responsive properties [43]. β-CD and $[C_4MIM]PF_6$ can also enhance the solubility of each other due to formation of inclusion complexes of 1:1 or 1:2 stoichiometry and the entrance of $[C_4MIM]^+$ into the hollow β-CD cavity [46].

It has also been reported that the alkyl side chain on the imidazolium ring enters into the hollow cavity [47] while the imidazolium ring cations do not enter [48,49]. The interaction between $[C_4MIM]BF_4$ and β-CD was studied by ^{19}F NMR,

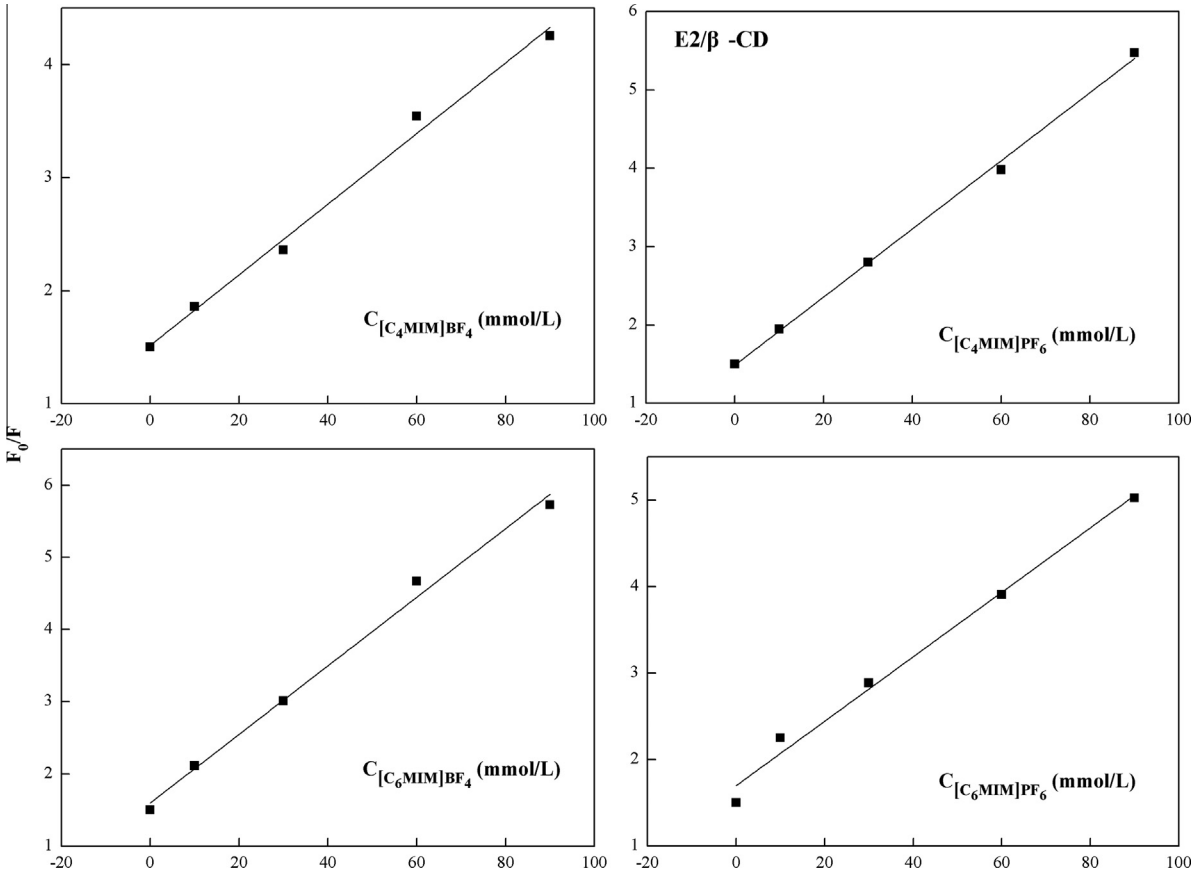

Fig. 11. The Stern–Volmer plot for the binding of RTILs to E2/β-CD.

Table 4
The Stern–Volmer equation of EE2/β-CD and E2/β-CD.

Complexes	RTILs	Stern–Volmer equation	R^2	$K_{sv}/(\times 10^4\ L\ mol^{-1})$
EE2/β-CD	$[C_4MIM]BF_4$	$y = 0.0345x + 1.1338$	0.9933	0.0345
	$[C_6MIM]BF_4$	$y = 0.0588x + 0.9636$	0.9944	0.0588
	$[C_4MIM]PF_6$	$y = 0.0569x + 0.8427$	0.9927	0.0569
	$[C_6MIM]PF_6$	$y = 0.0412x + 1.0111$	0.9939	0.0412
E2/β-CD	$[C_4MIM]BF_4$	$y = 0.0313x + 1.0161$	0.9927	0.0313
	$[C_6MIM]BF_4$	$y = 0.0475x + 1.0979$	0.9935	0.0475
	$[C_4MIM]PF_6$	$y = 0.0435x + 0.9883$	0.9981	0.0435
	$[C_6MIM]PF_6$	$y = 0.0372x + 1.1984$	0.9898	0.0372

showing that the fluorin signals of $[C_4MIM]BF_4$ were shifted upfield by addition of β-CD. The fluorin signals of BF_4^- were also shifted upfield with addition of β-CD, suggesting that the anion from the RTILs interacted with β-CD [49]. There are two pathways for complexation between RTILs and β-CD, in which either the cation or anion first interacts with β-CD. If one of them interacts with β-CD very weakly, 1:2 inclusion complexes should be minimal and 1:1 inclusion complexes are dominant. However, if both the cation and anion of an IL strongly interact with β-CD, 1:2 inclusion complexes are prominent [40,41]. According to the above analyses, RTILs have a strong possibility of being incorporated into the hollow β-CD cavity, which decreases the incorporation of EE2 or E2 into β-CD. This will further lead to a weak protection effect of β-CD from collision quenching of 4-t-OP molecules in aqueous solution, and thus the FIs of EE2 (E2)-β-CD complexes were markedly quenched with addition of RTILs.

Fluorescence quenching mechanism of RTILs

Fluorescence quenching is usually used to obtain information about the structure and dynamics of fluorescent molecules. There exist two kinds of fluorescence quenching, i.e., static and dynamic quenching. Static quenching refers to formation of a ground-state fluorophore–quencher complex which does not emit a photon. Dynamic quenching refers to formation of an excited-state fluorophore–quencher complex [50]. In this study, the FI of estrogen decreased because of molecular interaction with RTILs, which act as a fluorescence quencher. Fluorescence quenching is generally described by the Stern–Volmer equation:

$$F_{aq}/F_{pq} = 1 + K_q\tau_0[Q] = 1 + K_{sv}[Q]$$

where F_{aq} and F_{pq} are the FIs of the fluorophore in the absence and presence of quenchers, respectively; K_{sv} and K_q are the Stern–Volmer quenching constant and the bimolecular quenching constant,

respectively; τ_0 is the lifetime of the fluorophore in the absence of a quencher; and [Q] is the quencher concentration. Plots of F_{aq}/F_{pq} versus concentrations of RTILs are shown in Figs. 10 and 11. The strong linear correlation coefficients ($R^2 > 0.99$) and the quenching constants (K_{sv}) are listed in Table 4. As reported by Geng et al. [50], the Stern–Volmer quenching constant for dynamic quenching was less than 1.0×10^3 L mol^{-1}. Therefore, we infer that fluorescence quenching of EE2/β-CD and E2/β-CD by the four RTILs was a dynamic quenching mechanism.

Conclusions

This study investigated the effects of ordered media on fluorescence characteristics of EE2 and E2 in water and methanol, and the effects of four RTILs on the FIs of EE2/β-CD and E2/β-CD inclusion complexes in methanol. The main conclusions are summarized as follows: (i) the FIs of EE2 and E2 increased with increasing β-CD and CTAB concentrations due to formation of inclusion complexes; (ii) the FIs of EE1 and E2 with β-CD or CTAB in methanol were greater than those in water, resulting from the decreased possibility of oxygen-quenching processes in H_2O molecules; (iii) the inclusion ratio of β-CD or CTAB with EE2 and E2 was 1:1 in different solvents, and the K values for the inclusion complex in water were greater than those in methanol; (iv) the lifetimes of EE2 and E2 increased gradually with the addition of β-CD or CTAB, which were in good agreement with those of steady-state FIs; and (v) the four RTILs had quenching effects on the FIs of EE2/β-CD and E2/β-CD, and the quenching process of EE2/β-CD and E2/β-CD by RTILs was demonstrated to be a dynamic quenching mechanism.

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