

Effects of Room Temperature Ionic Liquids on Fluorescence Characteristics of 17 β -estradiol and its Derivative

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Abstract Previous reports have seldom concerned about the RTILs (Room temperature ionic liquids), and their effects on derivatization reaction or derivatives. In this study, we reported that the effects of four different RTILs, i.e., [EMIM]PF₆, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆, on fluorescence spectra of 17 β -estradiol (E2), and its derivatization solvent dansyl chloride (DNSCl) and the derivative. [BMIM]PF₆ had a significant quenching effect on the fluorescence intensity of E2, suggesting the formation of [BMIM]PF₆/E2 complexes and possible buried E2 molecular in a more hydrophobic microenvironment. The estimated Stern-Volmer quenching constant ($K_{sv}=0.3519$) proved that E2 quenching caused by [BMIM]PF₆ was a dynamic quenching process. Four RTILs, with different alkyl chain-length in imidazolium cation, resulted in different quenching intensities to E2 as follows: [EMIM]PF₆ > [BMIM]PF₆ > [HMIM]PF₆ > [OMIM]PF₆. At 5 mg L⁻¹ of DNSCl, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆ increased the fluorescence intensities of E2 in water by 8.5, 7.6 and 6.1 times, respectively, and a 37-nm hypsochromic shift occurred. The fluorescence intensity for [BMIM]PF₆-extracted derivative of E2 increased more than two times compared with that for the control. In conclusion, this study demonstrated that above four hexafluorophosphate salt ionic liquids could be used in

derivatization reaction to enhance fluorescent sensitivity in E2 trace residual analysis.

Keywords Room temperature ionic liquids · Fluorescence intensity · 17 β -estradiol · Dansyl chloride · Derivative

Introduction

As a kind of new “green” solvents and soft materials, and an alternative to the conventional and environmentally detrimental volatile solvents, room temperature ionic liquids (RTILs) possess several attractive features, including low-melting temperature (below 373 K), negligible vapor pressure, excellent thermal stability, strong ability to dissolve many chemicals, high electrical conductivity, wide electrochemical window, and ability of repetitive utilization [1]. All these properties lead RTILs to a wide application in material synthesis [2], chemical reactions [3], separations [4], electrochemistry [5] and formation of ordered molecular assembly [6]. RTILs were also reconstructed for some specific applications in chemistry [7, 8]. In instrumental analytical area, RTILs were used as stationary phase in gas chromatography [9], and as mobile phase additives in capillary electrophoresis and fluorescence analysis [10, 11]. However, the mechanisms underlying the interaction between ionic liquids and fluorescent substances still remain under investigation, especially for the weak fluorescent chemicals, which may hinder the complete and most efficient utilization of these “green” solvents in fluorescent analysis of organic chemicals.

In recent years, phenolic estrogens such as estrone (E1), 17 β -Estradiol (E2), ethnylestradiol (EE2) and estradiol (E3), have been frequently studied as a group of environmental phenolic estrogens due to their structural and

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estrogenic similarities [12]. As a consequence of its large use in our daily life, E2 has made an important impact on our environment, including on animals and human health [13]. Fluorescence determination is a direct, simple and quick method for E2 residual analysis. However, due to the weak fluorescent property, E2 need to be derivatized with organic derivatization solvents prior to its fluorescent analysis. To the best of our knowledge, no data are available on interaction between ionic liquids and E2, and on application of ionic liquids in fluorescent analysis of E2. Therefore, the aim of this study is to evaluate the effect of RTILs on fluorescent behavior of E2 and its derivative, and to lay foundation for the application of RTILs in weak fluorescent chemicals.

Experimental

Materials

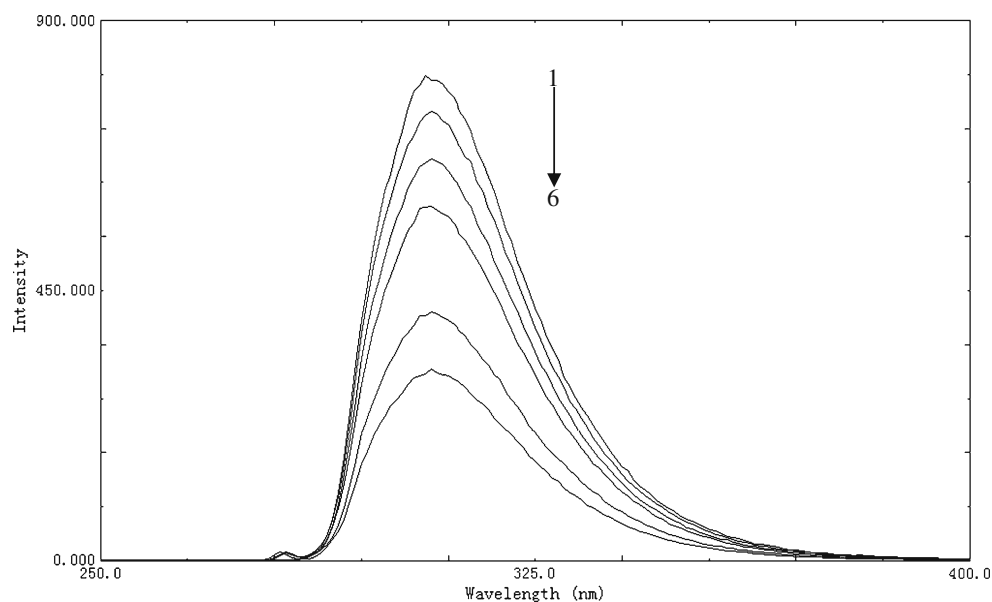
Reference compounds of 17 β -estradiol (E2), dansyl chloride (DNSCI) and RTILs: 1-ethyl-3-methylimidazolium hexafluorophosphate [EMIM]PF₆, 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM]PF₆, 1-hexyl-3-methylimidazolium hexafluorophosphate [HMIM]PF₆ and 1-octyl-3-methylimidazolium hexafluorophosphate [OMIM]PF₆ with stated purities of 99.0% were all purchased from Sigma-Aldrich (St. Louis, MO, USA). The previous standard compounds were used as received. Ultrapure water used in the study was purified with a Millipore Milli-Q plus system (Bedford, MA, USA). Methanol and acetone were LC-grade.

Methods

The stock standard solution of E2 (1,000 mg L⁻¹) was prepared in methanol and stored at -20 °C before use. The working solutions were obtained by appropriate dilution of the stock standard solution with relevant solvents or the ionic liquids. To investigate the effect of RTILs on fluorescence intensity of E2, precalculated amount of RTILs was directly added to the aqueous solutions containing E2. Samples were transferred into 1 cm² quartz cuvettes at 25 °C for fluorescence analysis. Fluorescence spectra were acquired on a model RF-5301PC spectrofluorometer (Shimadzu Corporation, Kyoto, Japan) with a 75 W Xenon arc lamp as the excitation source and single-grating monochromators as wavelength selection devices with slit width of 3 nm. All emission spectra were corrected for emission monochromator response and were background subtracted using appropriate blanks. All fluorescence data were measured in 1 cm² quartz cuvettes at 25 °C unless otherwise stated.

To study on the effects of RTILs on E2 derivative with DNSCI, the standard solution of E2 (5 mg L⁻¹) in methanol was first dried under a gentle nitrogen flow at 40 °C. The dried residue was redissolved in 200 μ L of sodium bicarbonate buffer (0.1 M, pH 10) by vortexing for 1 min. This solution was then mixed with 200 μ L of dansyl chloride in acetone (1 mg mL⁻¹) and vortexed for 1 min. The derivatization reaction was allowed to proceed for 5 min at 60 °C, and the derivative was detected immediately or extracted by 200 μ L [BMIM]PF₆. The fluorescence data for two treatments, non-extracted and extracted by [BMIM]PF₆, were compared to analyze the effects of RTILs on the derivative of E2.

Fig. 1 The fluorescence spectra of E2 in different concentrations of [BMIM]PF₆



*1 (0 mM), 2 (5 mM), 3 (10 mM), 4 (25 mM), 5 (50 mM), 6 (100 mM)

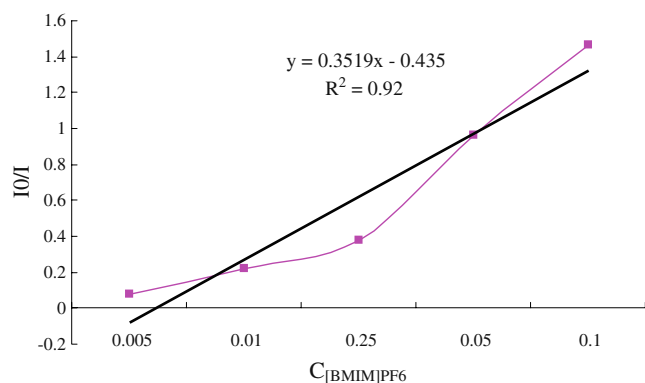


Fig. 2 Stern-Volmer plot for the binding of [BMIM]PF₆ to estradiol

Results and Discussion

Effects of [BMIM]PF₆ Different Concentrations on Fluorescence Spectra of E2

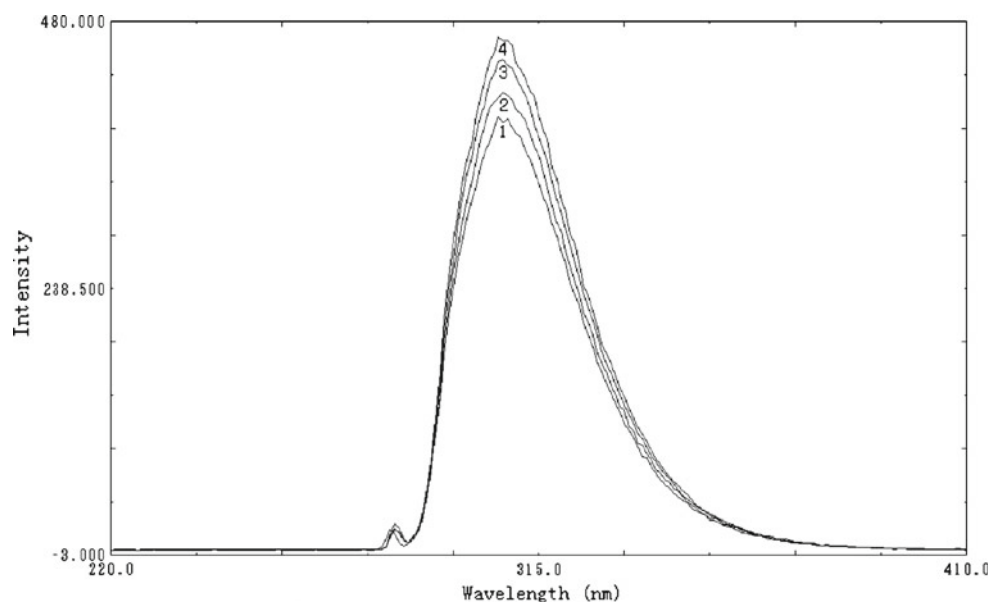
It has been reported that different concentrations of RTILs in solvents result in complex interplay due to the physicochemical properties of the solvents (e.g. viscosity, static dielectric constant, refractive index, density, polarizability, etc.), which may further affect steady-state emission behavior of the analytes [14]. At ambient conditions, [BMIM]PF₆ is much more viscous than the organic solvents such as methanol and acetonitrile. The viscosity of acetonitrile is 0.37 mN s m⁻² at 20 °C, while the viscosity of [BMIM]PF₆ is reported to be 330 mN s m⁻² at the same temperature. Considering the vast difference between the viscosity values of [BMIM]PF₆ and of the common organic solvents, we diluted [BMIM]PF₆ to a

series of concentrations (1, 5, 10, 20, 50 and 100 mM) in methanol, and then prepared 50 mg L⁻¹ of E2 with the above mixed solvents. As shown in Fig. 1, the fluorescence intensity of E2 decreased gradually with the increase of [BMIM]PF₆ concentrations from 0 to 100 mM. The fluorescence intensity was about 815.7 in absence of [BMIM]PF₆, while it decreased to 330.9 in the presence of [BMIM]PF₆ at 100 mM. Therefore, [BMIM]PF₆ had a significant quenching effect on the fluorescence of E2, which indicates the formation of [BMIM]PF₆/E2 complexes. Also it suggests that E2 molecular is likely buried in a more hydrophobic microenvironment. However, a significant hypsochromic or bathochromic shift of the emission maxima was not observed in the quenching processes. Indeed, our results were in agreement with Fletcher's observation which demonstrated the steady-state emission behavior of six polycyclic aromatic hydrocarbons along with the fluorescence quenching within [BMIM]PF₆ [15]. However, the effect of ionic liquid on fluorescent behavior of organic chemical is a debating problem. In some cases, ionic liquids may significantly improve the fluorescence intensity of organic matters. Deng et al. reported that, [BMIM]PF₆ had a prominent synergistic action to its fluorescence when the concentration of Pyronine B ranged from 1 × 10⁻⁷ to 1 × 10⁻⁴ mol L⁻¹. The similar action also occurred in the case of phenanthroline and rhodamine [16].

Fluorescence Quenching Mechanism of [BMIM]PF₆

Fluorescence quenching has been widely used to reveal the combined position of quenchers to the fluorophore. There exist two fluorescence quenchings, i.e., static and dynamic

Fig. 3 The fluorescence spectra of E2 in different kinds of RTILs



* 1 [EMIM]PF₆, 2 [BMIM]PF₆, 3 [HMIM]PF₆, 4 [OMIM]PF₆

Table 1 The excitation wavelength (EX/nm) and emission wavelength (EM/nm) of different concentrations of E2 in different ionic liquids

Ionic liquid concentrations C(mM)	[EMIM]PF ₆		[BMIM]PF ₆		[HMIM]PF ₆		[OMIM]PF ₆	
	EX	EM	EX	EM	EX	EM	EX	EM
0	282	307	282	307	282	307	282	307
5	281	306	282	307	282	306	282	307
10	281	307	282	307	282	308	282	306
25	281	307	281	307	281	307	282	307
50	281	307	281	308	282	308	281	307
100	281	306	283	308	282	307	282	308

“EX/EM” denotes the maximum excitation or emission wavelength, respectively

quenching. Static quenching refers to the formation of a ground state fluorophore–quencher complex which does not emit a photon. Dynamic quenching refers to the formation of an excited state fluorophore–quencher complex [17]. Dynamic quenching processes are described by the Stern–Volmer equation:

$$\frac{I_0}{I} = 1 + K_{sv}[Q] = 1 + K_q\Gamma_0[Q] \quad (1)$$

where I_0 and I are fluorescence intensities with or without the presence of a quencher, and K_{sv} and K_q are the Stern–Volmer quenching constant and the bimolecular quenching constant, respectively; Γ_0 is the lifetime of fluorophore in the absence of a quencher. The results of I_0/I against concentrations of [BMIM]PF₆ were shown in Fig. 2. A fair-to-good linear correlation coefficient ($r^2=0.9200$) was observed, suggesting the compliance of the Stern–Volmer relation. As reported by Geng et al. [17], the Stern–Volmer quenching constant for dynamic quenching was less than 1.0×10^3 L/mol. Therefore, the 17 β -estradiol quenching ($K_{sv}=0.3519$) caused by [BMIM]PF₆ in this study was dynamic quenching.

Effect of Ionic Liquids (ILs)

Ionic liquids affect the fluorescence spectra by transforming the features and varying fluorescence intensities. The changes of spectra are associated with the unique features

of ionic liquids and interactions between ionic liquids and fluorophores. The fluorescence enhancement or quenching effects depends on the type and concentration of fluorescent substances and features of ionic liquids [16]. To demonstrate the impact of ILs on fluorescence intensity of E2, four types of ILs with different alkyl chain-length in imidazolium cations were selected: [EMIM]PF₆, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆. Similar to [BMIM]PF₆, [EMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆ all showed quenching effects on fluorescence intensities of E2. On the other hand, the length of alkyl chain had an impact on the quenching ability of ILs, which were as in the following order: [EMIM]PF₆ > [BMIM]PF₆ > [HMIM]PF₆ > [OMIM]PF₆ (Fig. 3). The shorter alkyl chain in imidazolium cations displayed the higher quenching intensities for E2, which possibly resulted from the different microenvironment formed by varying length of alkyl chain. As summarized in Table 1, the maximum excitation wavelength of these four ILs ranged from 281–282 nm, and the maximum emission wavelength varied between 306 and 308 nm, suggesting that no hypsochromic or bathochromic shift with different ILs.

Fluorescence Intensities of DNSCl in Water and ILs

As reported by Deng et al., the interaction of strong or weak fluorescent substances with ionic liquids had significant difference. Therefore, fluorescent properties of organic

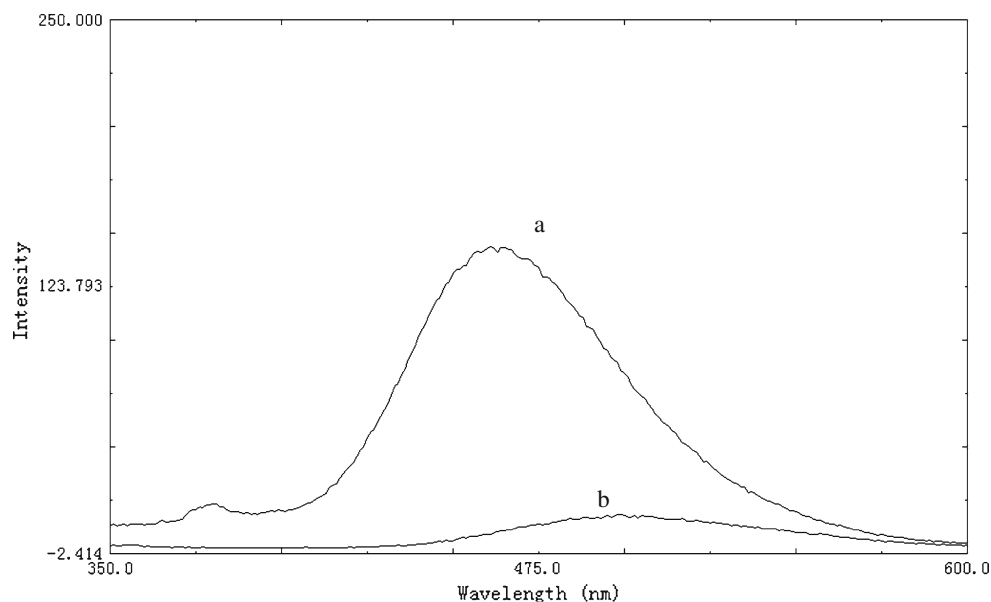
Table 2 The comparison of fluorescence behavior of dansyl chloride (DNSCl) in water solution and ionic liquids

DNSCl concentrations		5 mg L ⁻¹	10 mg L ⁻¹	25 mg L ⁻¹	50 mg L ⁻¹	100 mg L ⁻¹
DNSCl - Water	EX/EM	318/498	320/497	320/497	320/494	321/496
	FI	16.585	35.098	77.257	111.009	125.115
DNSCl - [BMIM]PF ₆	EX/EM	332/461	331/463	330/465	334/463	ND
	FI	141.750 (8.5)	262.391 (7.5)	565.593 (7.3)	873.386 (7.9)	
DNSCl - [HMIM]PF ₆	EX/EM	331/460	332/459	332/460	335/462	336/465
	FI	125.396 (7.6)	248.878 (7.1)	533.759 (6.9)	844.366 (7.6)	917.220 (7.3)
DNSCl - [OMIM]PF ₆	EX/EM	332/458	331/456	334/458	334/462	341/460
	FI	101.342 (6.1)	219.213 (6.2)	435.903 (5.6)	778.874 (7.0)	808.405 (6.5)

“EX/EM” denotes the maximum excitation or emission wavelength, respectively

“FI” indicates the fluorescence intensity

Fig. 4 The fluorescence spectrum of dansyl chloride in [BMIM]PF₆ (a) and water solution (b)



chemicals may lead to varying effects within ionic liquids. As a weak fluorescent substance, E2 is often derivatized with derivatization solvents prior to fluorescent analysis. Dansyl chloride, a type of strong fluorescent substance, is often used to derivatize E2 to enhance fluorescence response [18–20]. Therefore, DNSCI was selected as a representative of strong fluorescent substances to study the effects of ionic liquids. As shown in Table 2, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆ in 5 mg L⁻¹ of DNSCI increased the fluorescence intensity of E2 by 8.5, 7.6 and 6.1 times, respectively, compared with those in water. Similarly, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆ in 50 mg L⁻¹ of DNSCI increased the intensity by 7.9, 7.6 and 7.0 times, respectively. These results demonstrated that DNSCI had an enhancement effect on the fluorescence intensity of E2. However, the increase of DNSCI concentration from 5 to 100 mg L⁻¹ did not lead to significant change of RTILs effects on E2 intensity, which ranged from 6.1 to 8.5. The maximum emission peak for DNSCI was at 498 nm in water samples, while it was at 461 nm in [BMIM]PF₆,

indicating the occurrence of 37-nm hypsochromic shift (Fig. 4). In conclusion, [BMIM]PF₆ had enhancement effects for strong fluorescence chemicals and quenching effects for weak fluorescence chemicals. In line with our findings, Fletcher et al. also observed that the emission from alternant PAHs was quenched while that from nonalternant PAHs was not [15].

Effect of [BMIM]PF₆ on the Derivative of E2

As shown in Fig. 5, E2-Dansyl was the reaction product combined from E2 and DNSCI by oxygen atom. Consequently, the derivatization product possessed higher fluorescence intensity than its parent compound E2. After derivatization, the fluorescence intensities of the derivative extracted with [BMIM]PF₆ was 256.06, while that of the non-extracted derivative, was 806.59. The fluorescence intensity for [BMIM]PF₆-extracted derivative increased more than two times compared with that for nonionic liquid-extracted derivative. The emission maxima was

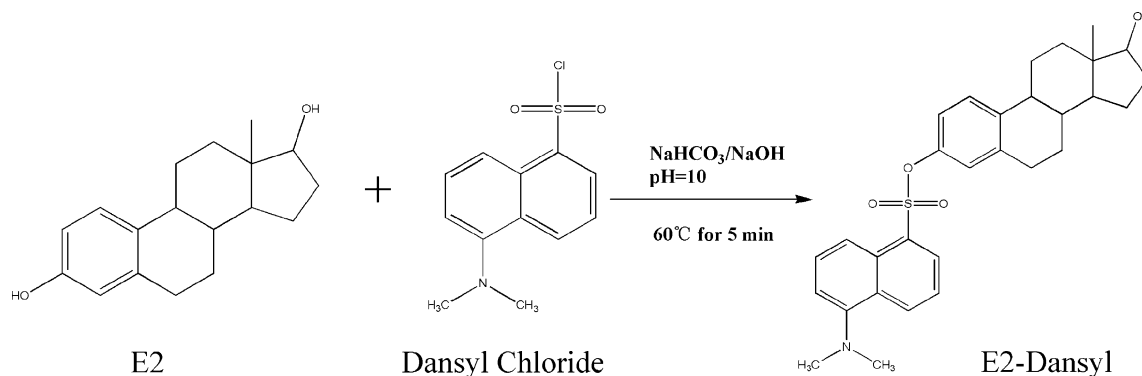


Fig. 5 The derivatization reaction of E2 with dansyl chloride (DNSCI)

480 nm in nonionic liquid-extracted derivative, while it was 465 nm in [BMIM]PF₆-extracted derivative, revealing an occurrence of 15-nm hypsochromic shift. This result further proved that [BMIM]PF₆ had an enhancement effect on strong fluorescent derivatives, but quenching effect on weak ones. To the best of our knowledge, this is the first report on the effects of ionic liquids on fluorescence intensities of derivatives of phenolic estrogens. We are currently synthesizing the purity standards of derivatization product of phenolic estrogens to further study the effects of ionic liquids and to apply ionic liquids with enhancement fluorescence effects in fluorescent or chromatographic analysis of phenolic estrogens at trace levels.

Conclusions

In the absence of RTILs, the fluorescence intensity of E2, at fortified level of 50 mgL⁻¹, was quenched by 40.5% in the presence of [BMIM]PF₆ (100 mM) than in the absence. [BMIM]PF₆ had significant quenching effect on the fluorescence of E2, demonstrating the formation of [BMIM]PF₆/E2 complexes and possible submerge of E2 molecular in a more hydrophobic microenvironment. The Stern-Volmer quenching constant (*K*_{sv}) was 0.3519, suggesting that E2 quenching process by [BMIM]PF₆ was dynamic quenching. With regard to hexafluorophosphate salt ionic liquids [C_nMIM]PF₆, the different alkyl chain-length in imidazolium cation led to the different quenching effects on E2, which possibly resulted from the varying hydrophobic microenvironment. Among [EMIM]PF₆, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆, [EMIM]PF₆ possessed the highest quenching efficiencies, while [OMIM]PF₆ had the lowest. As for the strong fluorescent derivatization solvent (DNSCI) of E2, [BMIM]PF₆, [HMIM]PF₆ and [OMIM]PF₆ increased the fluorescence intensities of E2 in DNSCI by approximately 8.5, 7.6 and 6.1 times, respectively, than those in water, and an occurrence of 37-nm hypsochromic shift was observed. After E2 was derivatized by DNSCI, the fluorescence intensity for [BMIM]PF₆-extracted derivatization product of E2 increased more than two times compared with that for the control. The current study lays foundation for the application of [C_nMIM]PF₆ in derivatization reaction to enhance fluorescent analytical sensitivity of weak fluorescent chemicals such as E2.

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