

Effect of Ionic Liquid on Prototropic and Solvatochromic Behavior of Fluorescein

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The effect of a water-miscible room-temperature ionic liquid (IL), 1-butyl-3-methylimidazolium tetrafluoroborate ($[bmim][BF_4]$), on the behavior of a common and popular probe fluorescein is assessed. Depending on solubilizing milieu and conditions, fluorescein may exist in one or more of its prototropic forms (cationic, neutral (zwitterionic, quinoid, and lactone), monoanionic (phenolate and carboxylate), and dianionic) in the solution. Information regarding the various prototropic forms of fluorescein in buffer-rich aqueous IL mixtures ($[bmim][BF_4] \leq 50$ wt %, mole fraction <0.1) at different pH is obtained via UV-vis absorbance and fluorescence. The addition of $[bmim][BF_4]$ is found to cause lactonization of fluorescein in the solution. Hydrolytic instability of IL $[bmim][BF_4]$ is observed to be much lower than that of $NaBF_4$. Absorbance and fluorescence from fluorescein are observed to be insignificant when dissolved in neat $[bmim][BF_4]$, suggesting the presence of predominantly the lactone form. The IL $[bmim][BF_4]$ behaves similar to apolar media in this context. It is demonstrated that various prototropic forms of fluorescein may be generated within $[bmim][BF_4]$ by the addition of buffer of appropriate pH. Significant bathochromic shift in absorbance and fluorescence band maxima of dianionic fluorescein as concentration of $[bmim][BF_4]$ is increased correlates well with the decrease in the hydrogen-bond-donating acidity of the medium. The lack of good linear correlation between the Stokes shift and the orientational polarizability suggests the possible presence of specific Coulombic interaction between the IL cation and the fluorescein dianion within the mixture. It is established that IL $[bmim][BF_4]$ has an interesting and unique effect on the prototropic behavior of fluorescein.

Introduction

Throughout the recent decades, room-temperature ionic liquids (ILs) as novel media have garnered widespread attention and curiosity from the academic and industrial research communities because of their unusual and favorable properties.¹ Almost every named reaction in chemistry and many additional organic/inorganic/organometallic reactions have been reported to take place in thermodynamically or kinetically favorable fashion in ILs.² Novel analytical applications of ILs are emerging everyday; effective and, in some cases, truly unique deployment of ILs has been demonstrated in a variety of analysis modes encompassing electroanalysis, separation, extraction, mass spectrometry, and sensing, to name a few.³ Combined with the fact that ILs are composed entirely of cations and anions but still exist in the liquid state under ambient conditions, plethora of recent investigations concerning ILs are also due in part to their potential as environmentally benign substances. Most ILs have negligible vapor pressure in a practical sense, and can be recycled easily. As a consequence, it is logical to propose ILs as alternatives to the volatile organic compounds (VOCs).²

Recently, several research groups including our own have demonstrated that the behavior of structurally diverse probes within IL-based media sometimes exhibited unusual or unpredicted responses possibly due to specific solute–solvent interactions or to the presence of interesting solvent nanostructuring.^{4a–d} For example, the aggregation of surfactants,^{4e–h} the interaction and dimerization of common probes,^{4i–p} the behavior and mobility of dyes,^{4i–p} and biomolecular behavior^{4q–t} are frequently observed to be drastically different within ILs and (IL

+ water) mixtures. In this context, ILs may significantly alter the prototropic behavior and subsequent properties of common molecules. Toward this, we have carried out a detailed investigation of the prototropic and solvatochromic response of fluorescein, a biologically important probe, in IL-based solutions.

Fluorescein is one of the most common fluorescence probes having high molar absorptivity and fluorescence quantum yield.⁵ These desirable properties have rendered fluorescein one of the most useful and sensitive fluorescent labels.⁵ Fluorescein shows rich, diverse, and interesting protropism.⁶ As a result, the application of fluorescein requires a thorough understanding of its prototropic behavior. Depending on the conditions, one or more forms of fluorescein among the cationic, neutral (zwitterionic, quinoid, and lactone), monoanionic (phenolate and carboxylate) and dianionic may be present in the solution.⁶ The apparent protolytic constants relating the chemical activities of the cation, neutral, anion, and dianion forms are $pK_{a1} \approx 2.1$, $pK_{a2} \approx 4.3$, and $pK_{a3} \approx 6.4$ in aqueous buffer solutions.⁶ The efficiencies of the deprotonation of the various forms of fluorescein are reported to usually decrease as concentration of organic solvents, such as, methanol, ethanol, *n*-butanol, DMF, DMSO, acetonitrile, dioxane, is increased.^{7a–g} However, Ghasemi et al.^{7b} and Mchedlov-Petrossyna et al.,^{7c} respectively, have reported increased efficiency of deprotonation in the presence of ethanol and DMSO. In aqueous cationic micellar solutions of *N*-cetylpyridinium chloride and cetyltrimethylammonium chloride, the pK_{a1} decrease suggests facilitation of the deprotonation of the cationic form to the neutral form.^{7e} The reverse effect was observed within anionic micellar solutions of sodium dodecylsulfate; nonionic triton X-100 micellar media was found to have no effect on fluorescein pK_a .^{7b}

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In this work, we report the effect of a “hydrophilic” IL 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF₄]) on the prototropic equilibria of fluorescein and also on the solvatochromism of the dianionic fluorescein. In the first part of the studies, the IL is systematically added to different pH buffer solutions of fluorescein, and information regarding the various prototropic forms of fluorescein in water-rich solutions ([bmim][BF₄] mole fraction <0.1) is obtained via UV-vis absorbance and fluorescence. Fluorescein protropism in media rich in IL is investigated next, where aqueous buffer of different pH are added to [bmim][BF₄] solution of fluorescein. Solvatochromic behavior of dianionic fluorescein within aqueous [bmim][BF₄] mixture is investigated in the third part of the studies. It is clear from our studies that IL [bmim][BF₄] has an interesting and unique effect on the prototropic behavior of fluorescein.

Experimental Section

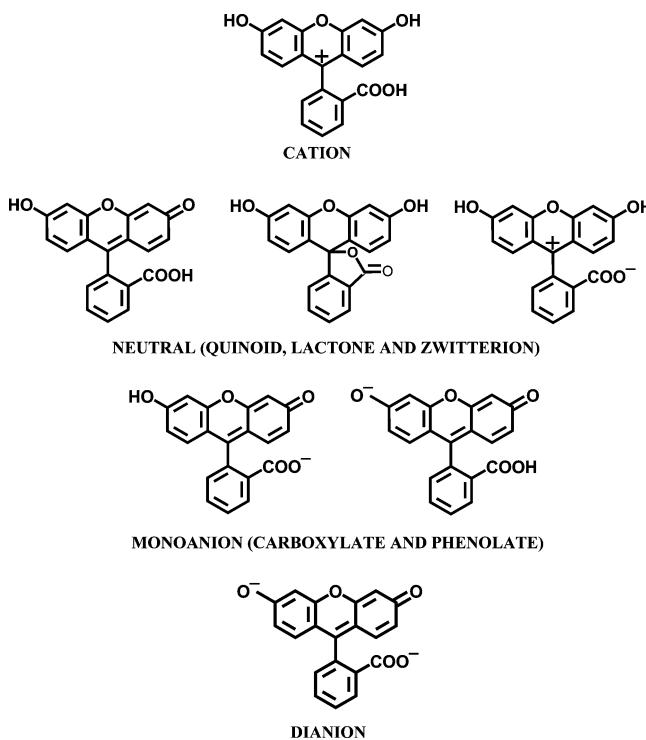
Materials. Sodium salt of fluorescein (high purity) was obtained from Acros Organics. IL [bmim][BF₄] (Merck, ultra-pure, halide content <10 ppm, water content <10 ppm) was stored under dry conditions. Doubly distilled deionized water was obtained from a Millipore, Milli-Q academic water purification system having $\geq 18 \text{ M}\Omega\cdot\text{cm}$ resistivity. Ethanol (99.9%) was obtained from SD Fine-Chem. Sodium phosphate, sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, phosphoric acid, hydrochloric acid, and sodium hydroxide were purchased from Qualigens in highest purity possible. Sodium tetrafluoroborate (NaBF₄) was purchased from Spectrochem Pvt.

Methods. Stock solutions of fluorescein were prepared in ethanol and stored under refrigeration at $4 \pm 1^\circ\text{C}$ in precleaned amber glass vials. The required amount of appropriate stock solution was taken in sample tube and dried in a gentle stream of ultrapure N₂ gas. IL [bmim][BF₄] was added to the sample tube under dry conditions to attain the desired final concentration. The sample tube was kept under dark and dry conditions for a few hours for complete solubilization of fluorescein within [bmim][BF₄]-based solutions. A precalculated amount of buffer was directly added to [bmim][BF₄] solution containing fluorescein. Different pH buffer solutions (10 mM) were prepared by proper combinations of phosphoric acid, sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, and sodium phosphate. pH adjustment was done with the help of dilute aqueous solutions of hydrochloric acid, sodium hydroxide, or both. Required amounts of materials were weighed using a Mettler-Toledo AB104-S balance with a precision of ± 0.1 mg. A Cary Varian Bio100 and Systronics 2201 double-beam spectrophotometer with variable bandwidth were used for the acquisition of UV-vis molecular absorbance data. Fluorescence spectra were acquired on model FL 3-11 Fluorolog-3 modular spectrofluorimeter with single Czerny-Turner grating excitation and emission monochromators having a 450 W Xe arc lamp as the excitation source and a PMT as the detector purchased from Horiba-Jobin Yvon. All data were acquired using 0.5 and 1 cm path length quartz cuvettes. Spectral response from appropriate blanks was subtracted before data analysis. All data were collected at least in triplicate starting from the sample preparation. All data analysis was performed using SigmaPlot v10.0 and TableCurve 2D v5.0 softwares.

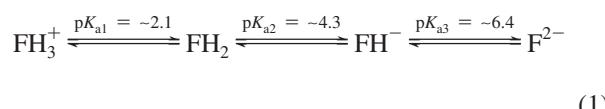
Results and Discussion

IL [bmim][BF₄] Addition to Aqueous Fluorescein Solution. **UV-vis Absorbance Behavior.** Prototropic forms of fluorescein (FH_3^+), neutral (FH_2), monoanion (FH^-), and dianion

SCHEME 1: Different Prototropic Forms of Fluorescein



(F^{2-}) present in aqueous solution depend on the pH of the solution (Scheme 1).⁶ The peak in the absorbance spectrum for dianion appears at 490 nm with a shoulder in the vicinity of 475 nm. Relatively weaker absorbance of monoanion is characterized by two bands at 472 and 453 nm of approximately the same molar absorptivity.⁶ The neutral and cationic species show absorbance maxima in the visible region at ca. 434 nm (with a side maxima at ~ 475 nm) and ca. 437 nm, respectively; the molar absorptivity of the transition for cationic species is significantly higher than that for the neutral species.⁶ The prototropic equilibria among four different species in buffer are as follows



The addition of up to 50 wt % (2.4 M, 0.07 mol fraction) [bmim][BF₄] to buffer solutions of fluorescein of pH 2.2 and 2.9, respectively, where mainly the cationic and the neutral species predominate, causes a rapid decrease in the molar absorptivity (Figures 1 and 2 and Figure S1 of the Supporting Information). In 50 wt % [bmim][BF₄]-added solution, the absorbance features of fluorescein nearly disappear. This behavior is attributed to the lactonization of the neutral quinoid or the zwitterionic forms with the addition of [bmim][BF₄] because it is already established that the lactone form of fluorescein does not show any absorbance due to the presence of sp^3 hybridization, which ruptures the conjugation of π -bonds in xanthene moiety of the fluorescein.^{6a} A decrease in the solvent hydrogen-bond-donating (HBD) ability (i.e., increased aprotic nature) is known to favor the lactonization.⁸ As reported in Table 1, the Kamlet-Taft parameter α , which represents HBD acidity of the solvent milieu, decreases as IL [bmim][BF₄] is added to the buffer solutions, thus increasing the aprotic nature of the solvent. This, in turn, facilitates lactonization within the solution.

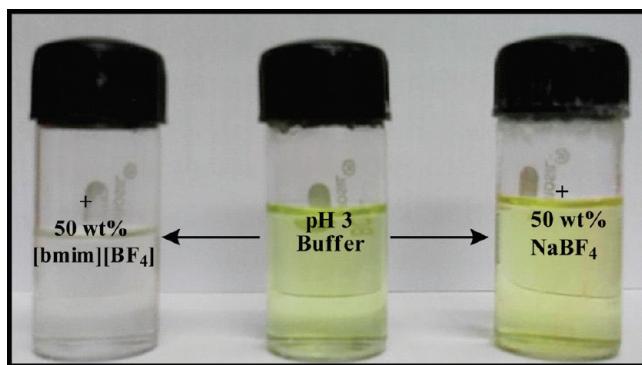


Figure 1. Fluorescein ($15 \mu\text{M}$) in pH 3 buffer (middle), NaBF_4 -added pH 3 buffer (right), and $[\text{bmim}][\text{BF}_4]$ -added pH 3 buffer (left) solutions under ambient conditions.

It is interesting to note that similar addition of salt NaBF_4 to the buffer solutions of fluorescein of pH 2.2 and 2.9, respectively, shows significantly less decrease in molar absorptivity (Figure 1 and Figure S2 of the Supporting Information). The addition of $[\text{bmim}][\text{BF}_4]$ up to 50 wt % to pH 4.0 buffer solution of fluorescein also shows a decrease in the molar absorptivity (Figure 2 and Figure S1 of the Supporting Information); however, the decrease is not as significant. It is easy to comprehend because a substantial amount of anionic FH^- is present that does not undergo any lactonization in the solution at this pH. A very different behavior is observed when NaBF_4 is added to pH 4.0 buffer solution of fluorescein; the spectra in the presence of NaBF_4 resemble more of the neutral species (Figure S2 of the Supporting Information). This is attributed to

the drop in the solution pH from 4.0 to 3.0 upon the addition of the first aliquot resulting in $[\text{NaBF}_4] = 0.22 \text{ M}$ in the solution (Table S1 of the Supporting Information). The pH change for similar additions of IL $[\text{bmim}][\text{BF}_4]$ is not that significant. At higher pH of 7.6, where dianion F^{2-} is the major species in the solution, a slight increase in the molar absorptivity is accompanied by a bathochromic shift as IL $[\text{bmim}][\text{BF}_4]$ is added to the buffer solution of fluorescein (Figure 2 and Figure S1 of the Supporting Information). The bathochromic shift, as reported in the literature, is a manifestation of the changes in the hydrogen bonding (HB) power of the cybotactic milieus (vide infra).⁹ A remarkable change from features pertaining to fluorescein dianion to that pertaining to monoanionic species is clearly seen when NaBF_4 instead of $[\text{bmim}][\text{BF}_4]$ is added (Figure S2 of the Supporting Information). This is easily explained because the pH of the buffer solution having initial pH of 7.6 decreases dramatically upon NaBF_4 addition, whereas not much change in pH of this solution is observed when IL $[\text{bmim}][\text{BF}_4]$ is added (Table S1 of the Supporting Information). Hydrolytic instability of IL $[\text{bmim}][\text{BF}_4]$ is found to be considerably less than that of NaBF_4 .¹⁰ This observation would help increase potential applications of aqueous IL solutions. Figure S1 of the Supporting Information compares the absorbance features of fluorescein in buffer solutions (panel A) with that in 50 wt % $[\text{bmim}][\text{BF}_4]$ added buffer solutions (panel B) of different pH. It is clear that absorbance features of the cationic and the neutral forms are difficult to observe, whereas those of the monoanionic and the dianionic forms dominate in the presence of 50 wt % IL $[\text{bmim}][\text{BF}_4]$.

Fluorescence Behavior. Fluorescence spectra of fluorescein in $[\text{bmim}][\text{BF}_4]$ -added different pH buffer solutions were

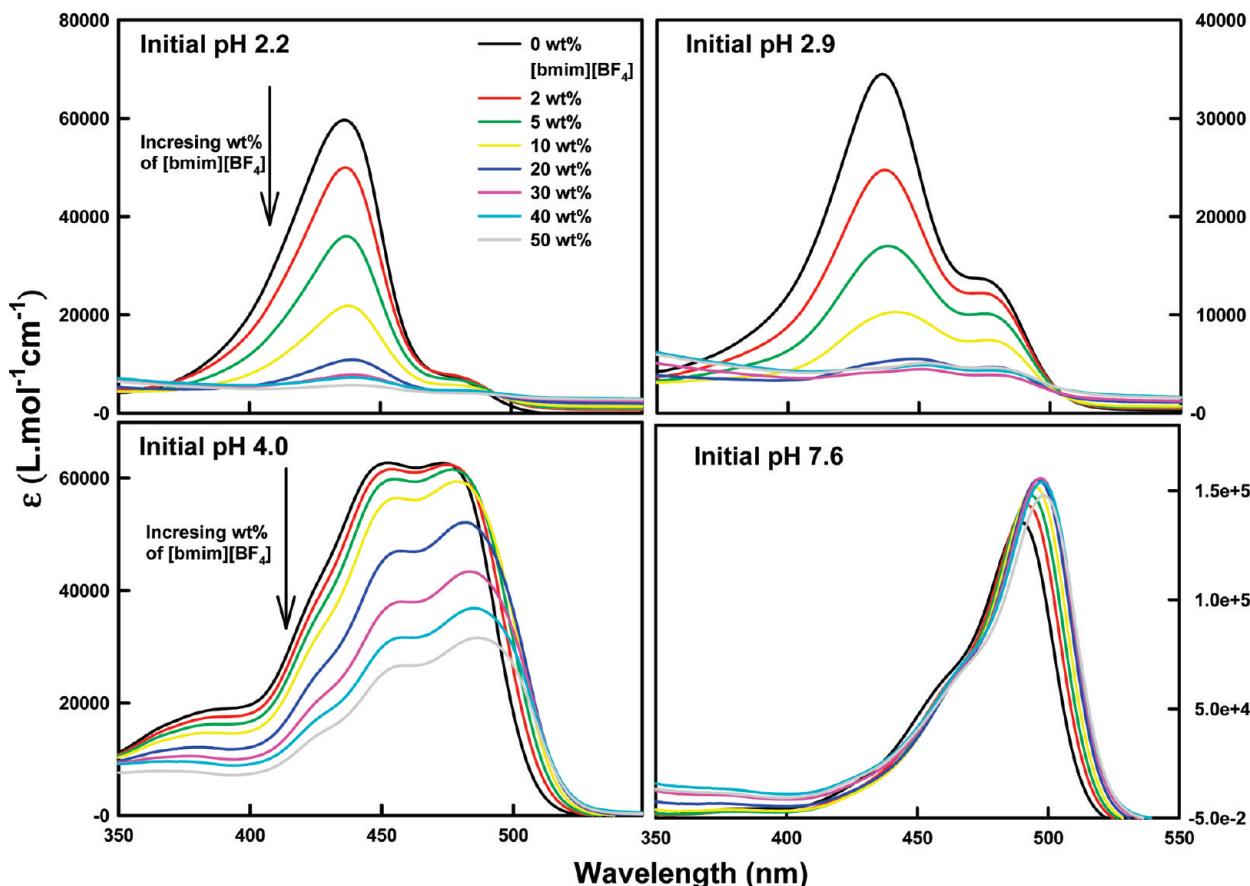
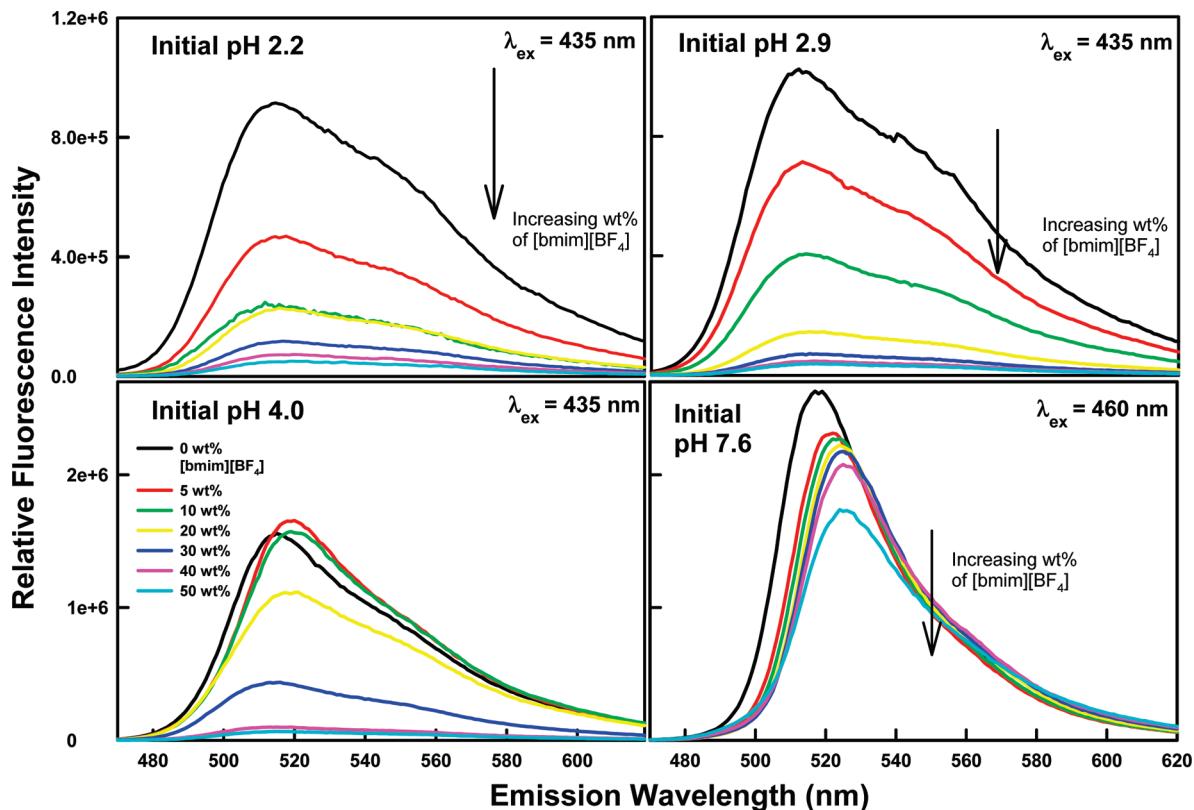


Figure 2. Absorbance spectra of fluorescein ($14 \mu\text{M}$) in $[\text{bmim}][\text{BF}_4]$ -added different pH buffer solutions under ambient conditions.

TABLE 1: Absorbance ($\lambda_{\text{max}}^{\text{abs}}$) and Fluorescence ($\lambda_{\text{max}}^{\text{em}}$) Band Maxima and Stokes Shift ($\Delta\bar{v}$) of the Fluorescein Dianion (Initial Solution pH 13.0) in the Presence of Different wt % [bmim][BF₄]^d

wt % [bmim][BF ₄] (M)	$\lambda_{\text{max}}^{\text{abs}}$ (nm)	$\lambda_{\text{max}}^{\text{em}}$ (nm)	$\Delta\bar{v}$ (cm ⁻¹)	α^a	E_T^N	π^{*a}	β^a	ϵ^b	n^c	Δf
0 (0)	490	517	1066	0.900	0.949	1.364	0.348	78.3	1.3349	0.320
10 (0.45)	496	522	1004	0.898	0.921	1.367	0.347	73.1	1.3436	0.315
20 (0.91)	498	523	960	0.897	0.891	1.370	0.345	65.8	1.3549	0.309
30 (1.34)	498	523	960	0.895	0.859	1.372	0.344	58.6	1.3665	0.304
40 (1.88)	498	524	996	0.892	0.825	1.372	0.344	51.4	1.3786	0.298
50 (2.40)	500	525	952	0.887	0.791	1.367	0.346	44.2	1.3910	0.291
60 (2.91)	500	525	952	0.880	0.762	1.355	0.350	37.0	1.4037	0.284
70 (3.45)	502	527	945	0.869	0.749	1.328	0.359	29.8	1.4169	0.274
80 (4.01)	502	528	981	0.849	0.768	1.276	0.369	22.6	1.4304	0.262
90 (4.57)	506	530	895	0.806	0.807	1.185	0.365	15.4	1.4443	0.243
95 (4.87)	508	530	817	0.764	0.798	1.107	0.371	11.8	1.4514	0.227

^a From ref 4a. ^b From ref 14. ^c From ref 15. ^d Parameters α , E_T^N , π^* , β , ϵ , n , and Δf are explained in the text.

**Figure 3.** Fluorescence emission spectra of fluorescein (7 μM) in [bmim][BF₄]-added different pH buffer solutions under ambient conditions.

acquired next (Figure 3). At pH 2.2 and 2.9, respectively, the fluorescence emission corresponds to that from the monoanion or the neutral quinoid form of fluorescein;^{6a} the fluorescence intensity decreases upon the addition of IL [bmim][BF₄]. It is well-established that in the excited state there is a rapid proton transfer from the cation to the neutral quinoid or the monoanion of fluorescein via surrounding water molecules resulting in fluorescence from the quinoid, the monoanion, or both; fluorescence emission from the cationic form is observed only at extreme high acidities (e.g., $\geq 5 \text{ M} [\text{H}^+]$).^{6b,d,f} The decrease in the fluorescence intensity in the presence of IL [bmim][BF₄] could be conveniently attributed to the decrease in molar absorptivity that results in decreased absorbance at the excitation wavelength of 435 nm. As previously proposed, this is because of the formation of the nonfluorescent lactone form. It is important to mention that care must be exercised in inferring any reduction in the emission intensity in the presence of IL [bmim][BF₄] to reduction in the fluorescence quantum yield

because this may not be the case because fluorescence quantum yield (Φ_F) is calculated taking into the consideration the absorbance at the excitation wavelength according to the expression

$$\Phi_F = \Phi_R \frac{I_F A_{R@ex\lambda} n_F^2}{I_R A_{F@ex\lambda} n_R^2} \quad (2)$$

where I_i , $A_{i@ex\lambda}$, and n_i denote integrated fluorescence intensity, absorbance at the excitation wavelength, and refractive index, respectively, and subscripts F and R represent fluorescein and a reference fluorophore whose fluorescence quantum yield is known. Fluorescence quantum yield Φ_F may not change appreciably if both I_F in the numerator and $A_{F@ex\lambda}$ in the denominator decrease to similar extents.

The fluorescence emission features of [bmim][BF₄]-added pH 4.0 buffer solution of fluorescein pertain predominantly to the

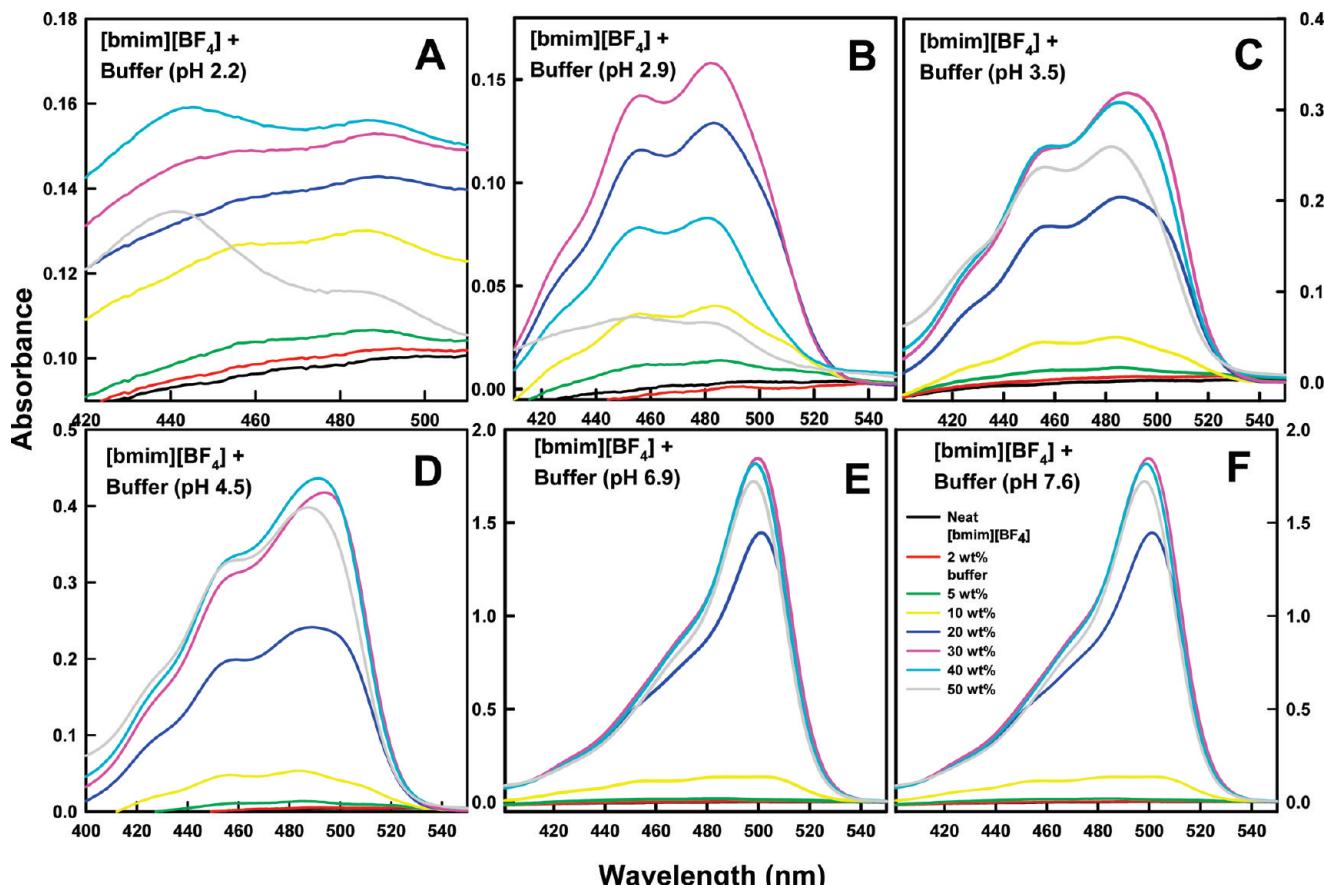


Figure 4. Absorbance spectra of fluorescein ($14 \mu\text{M}$) in different pH buffer-added $[\text{bmim}][\text{BF}_4]$ solutions under ambient conditions.

monoanionic species. The emission band becomes narrower as we increase the initial solution pH from 2.1 to 2.9 to 4.0 because of the increased [monoanionic] and decreased [quinoid]; fluorescence band maxima for the two species appear at approximately the same wavelength, but the quinoid form shows a broader fluorescence band (Figure 3).^{6a} The decrease in fluorescence intensity is not as dramatic with initial additions of $[\text{bmim}][\text{BF}_4]$ due perhaps to the smaller decrease in the optical densities at the excitation wavelength (i.e., less amount converting to lactone form because the dominant species is the monoanion in this pH range). As expected, the decrease in the fluorescence intensity upon $[\text{bmim}][\text{BF}_4]$ addition is the least when the starting pH of the buffer solution of fluorescein is 7.6 (Figure 3). The fluorescence emission features are what one would expect from the dianionic fluorescein. Because the absorbance increases upon the addition of IL, this decrease may be ascribed to the decrease in fluorescence quantum yield of the dianionic fluorescein in the presence of $[\text{bmim}][\text{BF}_4]$. Interestingly, similar to what was observed in the UV-vis absorbance spectra, a clear bathochromic shift in the emission band maxima again results as IL $[\text{bmim}][\text{BF}_4]$ is added to the solution of dianionic fluorescein.

We have compared the effect of the addition of IL $[\text{bmim}][\text{BF}_4]$ on fluorescence emission of fluorescein with that of NaBF_4 , a solid salt that is not an IL by definition. Our results clearly demonstrate the important role of $[\text{bmim}^+]$ in altering the photophysical behavior of fluorescein. The addition of NaBF_4 of similar concentrations to the buffer solutions of pH 2.2 and 3.5, respectively, results in very little change in the fluorescence intensity of fluorescein (Figure S3 of the Supporting Information). Significant changes (reduction as well as change in spectral features) in the fluorescence emission spectra upon

initial addition of 0.22 M NaBF_4 to fluorescein solutions of pH 4.5 and 7.6, respectively, are easily attributed to the reduced pH of the solution (Table S1 of the Supporting Information). Unlike IL $[\text{bmim}][\text{BF}_4]$ -added solution at higher pH, the hydrolytic instability of NaBF_4 does not allow the observation of fluorescence emission from dianionic form of fluorescein. Furthermore, it is confirmed that lactonization of fluorescein that is observed within $[\text{bmim}][\text{BF}_4]$ -added low pH solutions is not observed when NaBF_4 is added to the solutions of similar pH.

Fluorescein in Neat and Buffer-Added $[\text{bmim}][\text{BF}_4]$. Fluorescein shows almost insignificant absorbance as well as fluorescence when dissolved in neat IL $[\text{bmim}][\text{BF}_4]$ (Figures 4 and 5). Previous reports have suggested that whereas fluorescein shows interesting photophysical properties in polar protic and polar aprotic media, it is known to exist in its neutral lactonic form in the apolar media, thus showing very little absorbance or fluorescence.¹¹ It may be inferred from our investigation that fluorescein exists in the lactone form within neat IL $[\text{bmim}][\text{BF}_4]$. IL could be considered to be an apolar media in this context. Considerably lower static dielectric constants reported for ILs further support this observation (static dielectric constant of IL $[\text{bmim}][\text{BF}_4]$ is only ~ 11).¹² It could be suggested that the zwitterionic and the quinoid forms of neutral fluorescein being relatively more dipolar in nature as compared with the lactone form (Scheme 1) are not favored to exist within neat ILs.

The addition of water to fluorescein solution of IL $[\text{bmim}][\text{BF}_4]$ results in increased absorbance and fluorescence. Figure 4 presents absorbance spectra of different pH buffer-added fluorescein solutions in $[\text{bmim}][\text{BF}_4]$. It is interesting to note that as the aqueous buffer is added the absorbance spectra

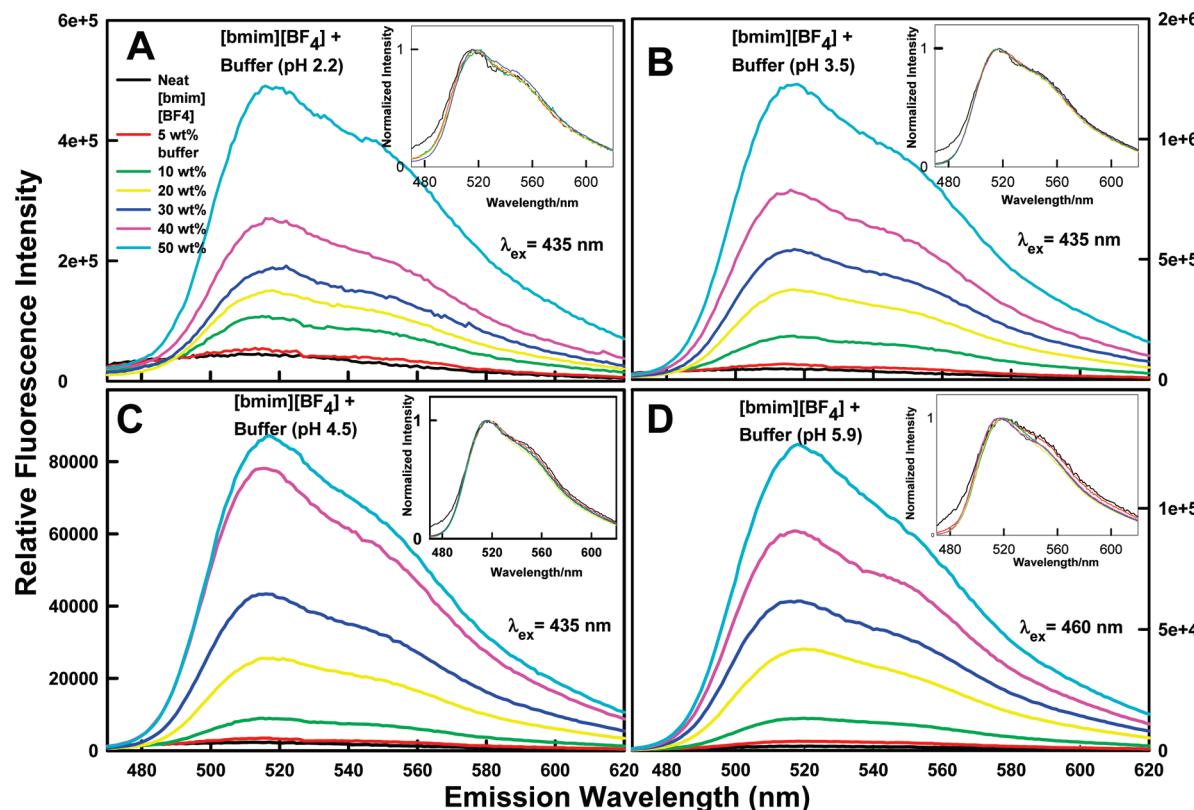


Figure 5. Fluorescence emission spectra of fluorescein ($7 \mu\text{M}$) in different pH buffer-added $[\text{bmim}][\text{BF}_4]$ solutions under ambient conditions. Insets show respective normalized fluorescence emission spectra.

become more structured; the absorbance increases to its maximum value in the presence of 30–40 wt % aqueous buffer and decreases upon further addition of buffer up to 50 wt % (equivalent to 50 wt % $[\text{bmim}][\text{BF}_4]$, as used in the experiments discussed in the previous section). As pH 2.2 buffer is added to $[\text{bmim}][\text{BF}_4]$ solution of fluorescein, it appears that the lactone form starts to convert to quinoid or zwitterionic form along with more and more cationic form manifesting itself via the growth of the absorbance band around 440 nm (Figure 4A). Interestingly, both neutral and monoanionic fluorescein seem to exist as buffer of pH 2.9 and 3.5, respectively, is added (Figures 4B,C). Whereas the presence of neutral, anion, and dianion is suggested as pH 4.5 buffer-added fluorescein solution in $[\text{bmim}][\text{BF}_4]$ (Figure 4D), the addition of pH 6.9 and 7.6 buffers, respectively, show evidence of growing absorbance corresponding to the dianionic species (Figures 4E,F). Various prototropic forms of fluorescein can be generated and observed via UV-vis absorbance from its solution in IL $[\text{bmim}][\text{BF}_4]$ by the addition of aqueous buffer of appropriate pH.

Fluorescence emission from aqueous buffer-added fluorescein solutions in $[\text{bmim}][\text{BF}_4]$ further confirms the above observation. As buffers of pH 2.2, 3.4, 4.5, and 5.9, respectively, are added, the emission features correspond to that of neutral or monoanionic fluorescein (Figure 5). This is because of the excited-state rapid proton transfer to neutral or monoanionic form (vide supra).⁶ It is surprising to learn that addition of even pH 5.9 buffer results in emission features corresponding to neutral or monoanionic species (Figure 5D); the broadened band suggests the lack of significant presence of dianionic species. The dissociation of anion to dianion appeared to be hindered in the presence of IL $[\text{bmim}][\text{BF}_4]$. A decrease in the solution pH to ca. 4.9 from initial buffer pH of 5.9 (Table S1 of the Supporting Information) is perhaps the major contributor to the absence of

emission from dianionic fluorescein. The dianionic species grows from the monoanionic as pH 7.6 buffer is added to $[\text{bmim}][\text{BF}_4]$ solution of fluorescein (Figure 6). Similar to what was observed in absorbance behavior, the neutral/monoanionic/dianionic forms of fluorescein could be generated within the IL solution of fluorescein as judiciously selected pH buffer is added.

Solvatochromism of Dianionic Fluorescein in the Presence of $[\text{bmim}][\text{BF}_4]$. The dianionic form of fluorescein shows solvatochromic behavior depending on the hydrogen-bonding capabilities of the solubilizing milieu.⁹ It was demonstrated that hydrogen bonding between fluorescein and the solvent led to a hypsochromic shift of the absorbance as well as fluorescence emission band maxima accompanied by an increase in the fluorescence quantum yield.^{9a}

The addition of IL $[\text{bmim}][\text{BF}_4]$ to fluorescein solution of pH 13 buffer results in several interesting outcomes. As the amount of IL $[\text{bmim}][\text{BF}_4]$ is increased in the solution, a bathochromic shift in both absorbance and fluorescence emission spectra is usually accompanied by a decrease in molar absorptivity and relative fluorescence intensity (Figure 7). The decrease in molar absorptivity appears to be a medium effect where the aqueous IL solution is changing from buffer-rich to $[\text{bmim}][\text{BF}_4]$ -rich composition. This decrease in molar absorptivity may result in decreased fluorescence intensity; contribution from a reduction in fluorescence quantum yield in the presence of IL $[\text{bmim}][\text{BF}_4]$ may not be ruled out either. The bathochromic shift in the absorbance as well as fluorescence emission band is fairly significant: as 95 wt % $[\text{bmim}][\text{BF}_4]$ is added to pH 13 buffer solution of fluorescein, shifts of 18 and 13 nm are

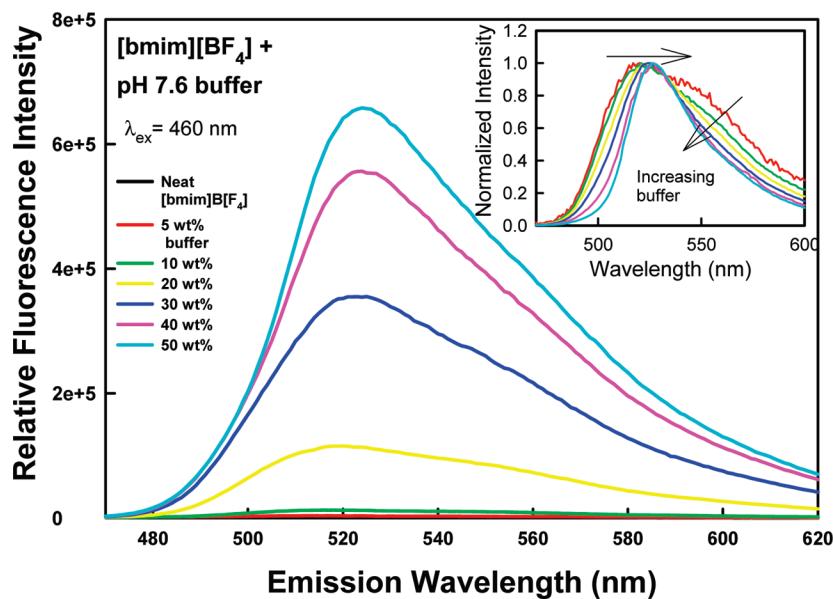


Figure 6. Fluorescence emission spectra of fluorescein ($7 \mu\text{M}$) in pH 7.6 buffer-added [bmim][BF₄] solutions under ambient conditions. Inset shows the normalized fluorescence emission spectra of the same.

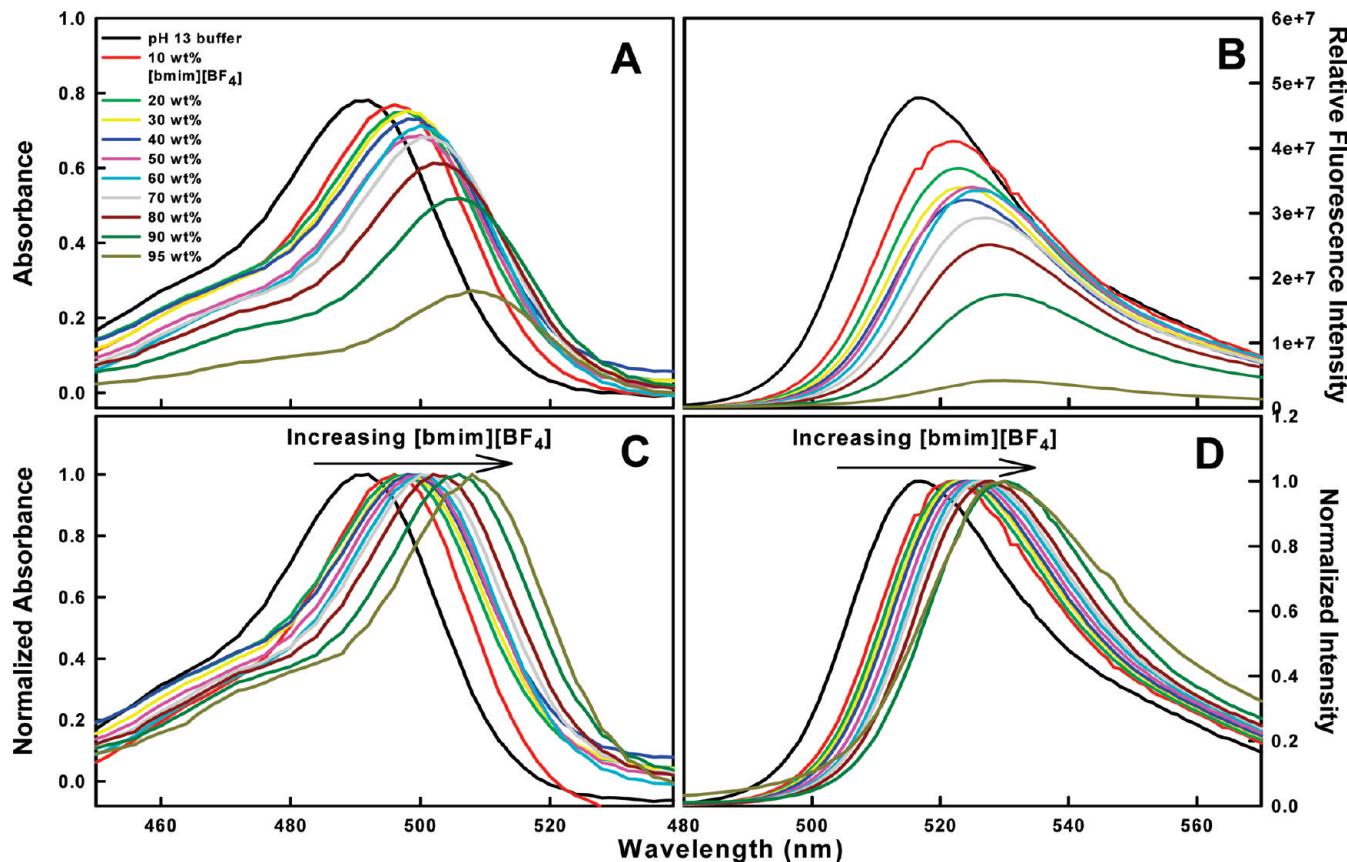


Figure 7. (A) Absorbance and (B) fluorescence emission spectra of fluorescein ($10 \mu\text{M}$) in [bmim][BF₄]-added pH 13 buffer solutions under ambient conditions. Normalized absorbance and fluorescence emission spectra are shown in panels C and D, respectively.

observed in the absorbance and fluorescence emission band maxima, respectively (Table 1).

For a positive solvatochromic probe ($\mu_{\text{ex}} > \mu_g$, where μ_{ex} and μ_g are the dipole moments of excited and the ground states, respectively), bathochromic shifts in absorbance and fluorescence bands are usually observed as the polarity of the cybotactic region is increased. We have estimated Kamlet-Taft

parameters^{13a-d} π^* (dipolarity/polarizability), α (HBD, acidity), and β (H-bond accepting (HBA) basicity), Reichardt polarity parameter^{13e} E_T^N (a combination of dipolarity/polarizability and HBD acidity), and Lippert parameter^{13f-h} Δf (orientational polarizability) of aqueous mixtures of IL [bmim][BF₄] from the values provided in the literature (Table 1).^{4a,14,15} A careful examination of the entries in Table 1 indicate that whereas π^*

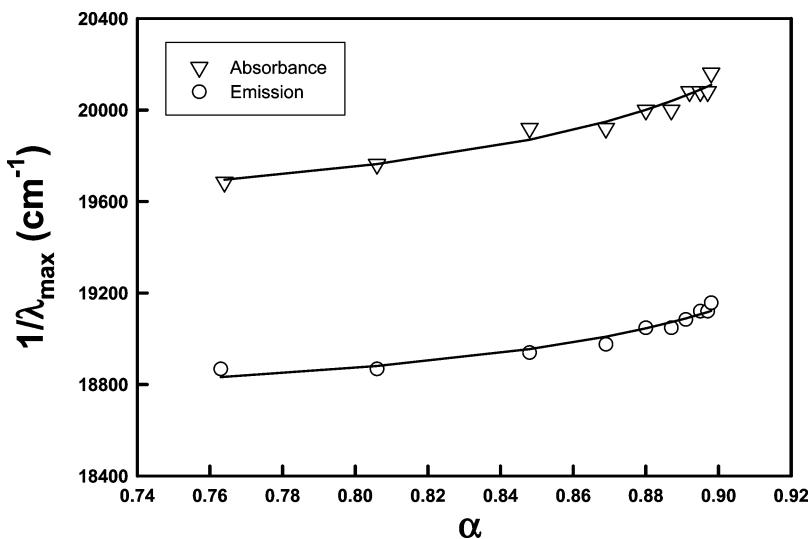


Figure 8. Absorbance and fluorescence emission band maxima (in inverse centimeters) of fluorescein dianion as a function of HBD acidity (α) of ([bmim][BF₄] + water) mixtures under ambient conditions. Solid lines show the fitting of the data according to the expression: $(1/\lambda_{\text{max}}) = a + (b/\ln \alpha)$. See the text for details.

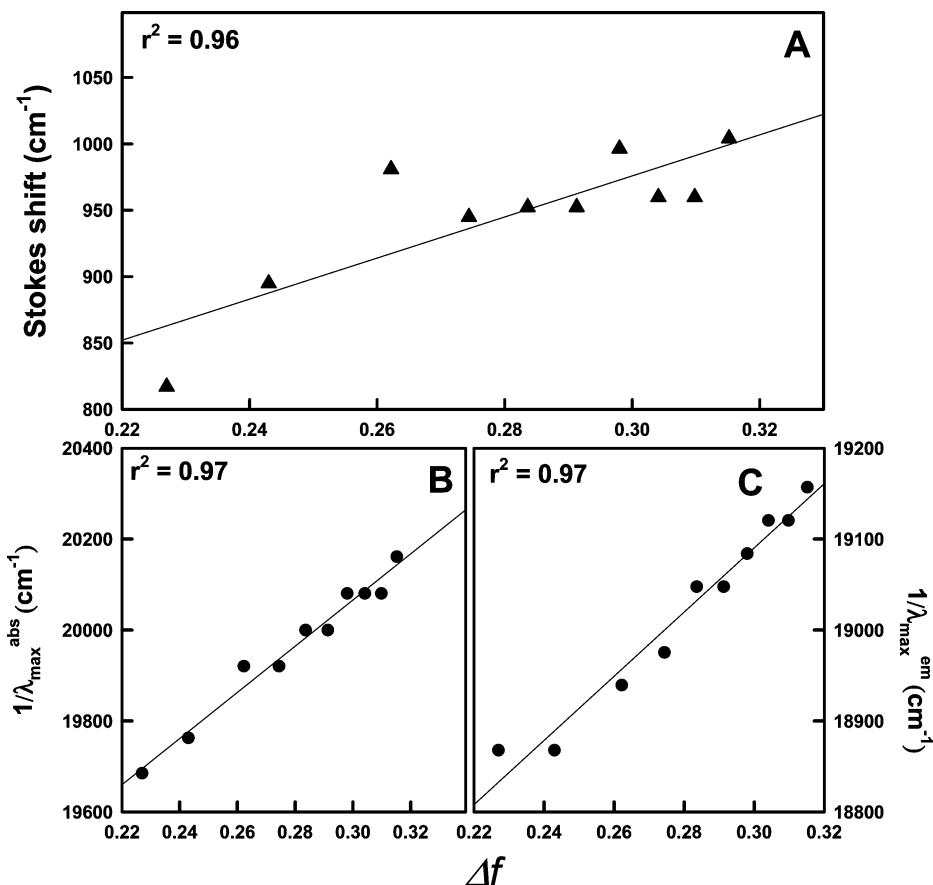


Figure 9. Stokes shift of fluorescein dianion as a function of orientational polarizability (Δf) of ([bmim][BF₄] + water) mixture (panel A) under ambient conditions. Absorbance and fluorescence emission band maxima (in inverse centimeters) as a function of Δf are shown in panels B and C, respectively.

increases slightly before decreasing significantly, suggesting decreased dipolarity/polarizability of the solution in general as IL [bmim][BF₄] is added, both absorbance and fluorescence band maxima, $\lambda_{\text{max}}^{\text{abs}}$ and $\lambda_{\text{max}}^{\text{em}}$, counterintuitively increase. Similarly, the correlations of β and E_T^N , respectively, with either $\lambda_{\text{max}}^{\text{abs}}$ or $\lambda_{\text{max}}^{\text{em}}$ are not satisfactory as well (Figure S4 of the Supporting Information).

The change in the HBD acidity (α) of the milieu, however, correlates well with the change in the $\lambda_{\text{max}}^{\text{abs}}$ and $\lambda_{\text{max}}^{\text{em}}$, respectively (Figure 8). We have already demonstrated that significant changes in the physicochemical properties of water can be achieved by the addition of very small amounts of IL.^{4d} Subsequently, we have ignored the probe responses in buffer in the absence of IL [bmim][BF₄] in our analysis. A fair-to-

good correlation was observed between $(\lambda_{\max}^{\text{abs}})^{-1}$ as well as $(\lambda_{\max}^{\text{em}})^{-1}$ and the HBD acidity (α) of the milieu requiring only two parameters (Figure 8)

$$\frac{1}{\lambda_{\max}^{\text{abs}}} = a + \frac{b}{\ln \alpha} \quad \text{with } a = 19419(\pm 41) \text{ cm}^{-1} \quad \text{and} \\ b = -74(\pm 5) \text{ cm}^{-1} \quad \text{with } r^2 = 0.96 \quad (3)$$

and

$$\frac{1}{\lambda_{\max}^{\text{ems}}} = a + \frac{b}{\ln \alpha} \quad \text{with } a = 18641(\pm 32) \text{ cm}^{-1} \quad \text{and} \\ b = -52(\pm 4) \text{ cm}^{-1} \quad \text{with } r^2 = 0.95 \quad (4)$$

As reported in the literature, the H-bonding between the fluorescein dianion and the solvent leads to hypsochromic shift of the absorbance and fluorescence spectra.⁹ Whereas Sawyer and coworkers^{9b} stated that the magnitude of the spectral shift correlated with both α and β , we have observed the correlation to be acceptable with α only as emphasized by the original work of Martin.^{9a} This is easy to comprehend because the fluorescein dianion has several HBA sites ($-\text{O}^-$, $-\text{C}-\text{O}^-$, $-\text{O}-$, and $=\text{O}$) that may involve in H-bonding with the milieu (Scheme 1). The extent of H-bonding, consequently, will depend on the HBD acidity (α) of the solvent. The lack of any real HBD site on fluorescein dianion renders the HBA basicity (β) of the solvent milieu irrelevant as observed by us.

To gain further insight into the bathochromic shifts of the absorbance and the fluorescence emission band of fluorescein dianion with increasing [bmim][BF₄] in the milieu, we estimated the Stokes shift $[\Delta\bar{\nu}(\text{cm}^{-1}) = ((1/\lambda_{\max}^{\text{abs}}) - (1/\lambda_{\max}^{\text{em}}))]$ at each addition of [bmim][BF₄] and explored its correlation with orientational polarizability (Δf) according to the Lippert–Mataga relation^{13g,h}

$$\Delta\bar{\nu} = \frac{2}{hc} \cdot \Delta f \cdot \frac{(\mu_{\text{ex}} - \mu_g)^2}{a_0^3} + \text{constant}$$

where

$$\Delta f = \left[\left(\frac{\varepsilon - 1}{2\varepsilon + 1} \right) - \left(\frac{n^2 - 1}{2n^2 + 1} \right) \right] \quad (5)$$

The ε (static dielectric constant) and n (refractive index) of aqueous [bmim][BF₄] were estimated from refs 14 and 15, respectively, and are reported in Table 1. a_0 is the radius of the cavity in which the fluorophore resides. A fair-to-good linear correlation ($r^2 = 0.96$), as suggested by the Lippert–Mataga equation above, is observed between the Stokes shifts and the orientational polarizability (Δf) (Figure 9A). It is important to mention that the Lippert–Mataga equation is only an approximation with many assumptions inherent in its derivation.^{13g,h} The fluorophore is considered to be spherical in nature and, more importantly, specific interactions with the solvent milieu are not amply considered. Deviation from the linearity is usually an indication of the presence of additional interactions that may be prevalent within aqueous IL mixtures. The presence of specific Coulombic attractive interaction between the dianionic fluorescein and the bmim⁺ within the cybotactic region may

contribute toward the observed deviation from linearity. It is perhaps worth mentioning, however, that the linear correlation improves slightly if either $(1/\lambda_{\max}^{\text{abs}})$ or $(1/\lambda_{\max}^{\text{em}})$ is used instead of Stokes shift ($\Delta\bar{\nu}$) (Figure 9B,C).

Conclusions

IL [bmim][BF₄] appears to have an interesting and unprecedented effect on the prototropic behavior of fluorescein. It causes efficient lactonization when added to buffer solutions of fluorescein. The apolar nature of this IL as a solvent is demonstrated as predominantly the lactone form of fluorescein, which is devoid of any significant optical density as well as fluorescence emission present in neat [bmim][BF₄]. Other prototropic forms having appreciable optical spectroscopic signals can be produced by the addition of buffers of appropriate pH to fluorescein solution in [bmim][BF₄]. Increasing the amount of [bmim][BF₄] in aqueous IL mixture shifts the absorbance and fluorescence emission band maxima bathochromically because of the decrease in the HBD acidity of the medium. Specific interaction, namely, of Coulombic attractive nature, between bmim⁺ and fluorescein dianion results in deviation from linearity between the Stokes shift and the orientational polarizability. IL [bmim][BF₄] is amply demonstrated to have modulated the protropism and the solvatochromism of fluorescein.

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Supporting Information Available: pH of buffer solutions after addition of different concentration of [bmim][BF₄] and NaBF₄, absorbance and emission spectra of fluorescein, and wavenumbers of absorbance and emission maxima of fluorescein dianion. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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