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Fluorescence Enhancement of Imidazolium Ionic Liquid by Its Confinement on PVC for In Situ Selective Quantification of Hemoglobin

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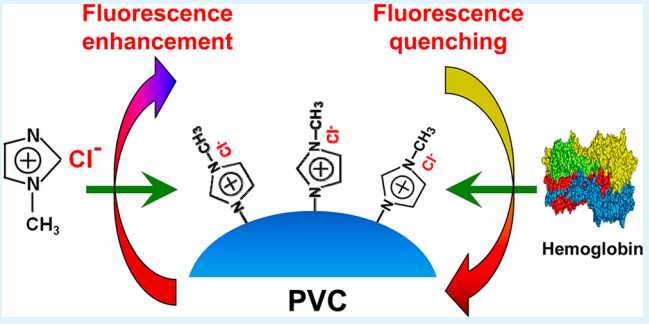
ABSTRACT: A hydrophilic ionic liquid (methylimidazolium chloride, NmimCl)-polyvinyl chloride ionomer (NmimCl-PVC) was prepared by immobilizing and confining N-methylimidazole onto PVC chains. The NmimCl-PVC ionomer exhibits a 4-fold enhancement on the fluorescence intensity with respect to that of NmimCl, attributing to the confinement of ionic liquid by the PVC chain. The fluorescence is excitation-dependent with a maximum at λ_{em} 430 nm when excited at 325 nm. In addition, the fluorescence intensity of NmimCl-PVC ionomer increases remarkably with the loading ratio of N-methylimidazole in the range of 4.3−15.1%. The fluorescence quantum yield and lifetime were derived to be 0.112/7.1 ns for the NmimCl-PVC ionomer and 0.063/8.8 ns for NmimCl. Furthermore, hemoglobin is selectively adsorbed by NmimCl-PVC and causes significant fluorescence quenching of the ionomer via dynamic quenching and energy transfer between NmimCl-PVC and hemoglobin. A solid surface fluorimetric procedure was developed for surface adsorption and preconcentration of hemoglobin followed by in situ detection. A linear dynamic range of 0.3−26.2 μg mg^{-1} is achieved with a detection limit of 0.1 μg mg^{-1}. Regarding hemoglobin in aqueous solution, the linear range 5−300 μg mL^{-1} is achieved along with a detection limit of 2 μg mL^{-1}.



KEYWORDS: *ionic liquid, immobilization/confinement, fluorescence enhancement, hemoglobin*

1. INTRODUCTION

Imidazolium ionic liquids are found to be fluorescent induced by the imidazolium moiety, and their fluorescence efficiencies are generally very low; that is, the fluorescence quantum yields of the bare imidazolium ionic liquids, including 1-butyl-3-methylimidazolium hexafluorophosphate ($BmimPF_6$), 1-butyl-3-methylimidazolium tetrafluoroborate ($BmimBF_4$) and 1-ethyl-3-methylimidazolium tetrafluoroborate ($EmimBF_4$), are between 0.005 and 0.02 when excited at 360 nm.[1−3] The fluorescent efficiency could be raised by improving the structure of ionic liquid or grafting functional group. A symmetrical hydrophilic ionic liquid 1,3-butylimidazolium chloride (BbimCl) was found to be highly fluorescent with a quantum yield of 0.523 in aqueous medium when excited at 315 nm, attributing to its symmetrical plane conjugating structure.[4] A quinolizinium ionic liquid with an unbranched cation core has been reported to exhibit very strong fluorescence at 334 nm with a quantum yield of >99%. The small bicyclic quinolizinium plays important role in the quantum yields.[5]

The physical, chemical as well as optical characteristics of the ionic liquids vary greatly with the local microenvironment around their functional moiety, and these features could be regulated by the immobilization of ionic liquid moiety onto appropriate inorganic or organic solid supports.[6−12] It has been reported that the fluorescence intensity of an imidazolium dicyanamide ionic liquid confined into mesoporous silica gel was significantly increased in comparison with that of the pure ionic liquid.[13] The stronger $\pi-\pi$ conjugated interaction among the dicyanamide anions and π conjugated association formed between dicyanamide anion and imidazolium cation may contribute to the enhancement of the fluorescence emission. In addition, the cationic imidazolium moiety of the ionic liquid can be employed as the fluorophore by immobilizing it in conjugated polymers. It is known that conjugated polymers have light-harvesting properties and are able to coordinate the action of a large number of absorbing units with efficient intrachain and interchain energy transfer mechanisms to amplify the fluorescence sensing signals.[14] A series of conjugated polymers containing ionic liquid were used to design fluorescent assay protocols for sensing DNA-related events within a wide range.[15−17] In summary, the design and synthesis of high fluorescent efficiency ionic liquids or their derivatives are greatly desired for highly sensitive detection of biomacromolecules in the field of biological studies.

In the present work, we have tried to regulate the fluorescence behavior of a hydrophilic ionic liquid (i.e., methylimidazolium

Received: September 19, 2013
Accepted: October 28, 2013
Published: October 28, 2013

chloride) by immobilizing N-methylimidazole onto the surface and into the bulk of the particulate PVC. The fluorescent properties of the obtained ionomer of NmimCl-PVC were carefully investigated. The fluorescence intensity of the NmimCl-PVC ionomer was dramatically increased compared with the pure ionic liquid NmimCl. This indicated that the immobilization or confinement of ionic liquid moiety onto an appropriate solid support is an effective approach to manipulate the fluorescent performance of the ionic liquids. It further demonstrated that the obtained NmimCl-PVC ionomer exhibits favorable selectivity for the adsorption of hemoglobin, which causes significant quenching of the fluorescence.[18] Therefore, a solid surface fluorimetry for the direct determination of hemoglobin was developed based on this observation.

2. EXPERIMENTAL SECTION

Chemicals. The proteins used in the present study include lysozyme from chicken egg white (Lys, L2879, isoelectric point pI 11), bovine hemoglobin (Hb, H2500, pI 6.9), immunoglobulin G from human serum (IgG, I4506, pI 5.8), transferrin (Trf, T3309, pI 5.9), and bovine albumin serum (BSA, A3311, pI 4.7). These proteins were purchased from Sigma (St. Louis, MO) without further purification. Commercial PVC particles with an average molecular weight of 58000 (nominal particle size of ca. 150 μm) were obtained from Shenyang Chemical Co. (Shenyang, China), and N-methylimidazole was the product of Kaile Chemicals (Linhai, China). N-methylimidazolium chloride (NmimCl) and 1-vinyl-3-ethylimidazolium bromide (VeimBr) were purchased from Shanghai Cheng Jie Chemicals (Shanghai, China) and used as received. 2,2′-Azobis-isobutyronitrile (AIBN, 99%) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Other chemicals employed were at least of analytical reagent grade unless otherwise specified. Deionized water of 18 MΩ cm^{-1} was used throughout.

Preparation of Immobilized Ionic Liquids/Ionomers. The NmimCl-PVC ionomer was prepared by following a previously reported procedure.[18] Shortly, 5 g of PVC particles and various amounts of N-methylimidazole (4.5, 9, 18, 36, 72 mL, corresponding to N-mim/PVC molar ratios of 1:2, 1:1, 2:1, 4:1, 8:1) were dissolved in 50 mL of toluene. Each portion of the reaction mixture was stirred at 70 °C for 48 h. The separation of the resulted NmimCl-PVC was facilitated by adding an excessive amount of methanol/water (50%, v/v, 100 mL) under sonication. The NmimCl-PVC ionomer was thoroughly washed with water and ethanol alternatively to eliminate any residues of N-methylimidazole and toluene. The product was finally filtered and dried at room temperature for overnight under vacuum.

For the preparation of $NmimPF_6$–PVC ionomer, the counteranion of the NmimCl-PVC ionomer was readily exchanged via a general anion-exchange procedure to achieve the $NmimPF_6$–PVC ionomer. Short description of the procedure: 10 mL of HPF_6 was added dropwise into 5 g NmimCl-PVC, and the mixture was stirred for 1 h at room temperature. The resulting solid was filtered and washed with pure water until a neutral washing solution was obtained. The $NmimPF_6$–PVC ionomer was then dried under vacuum.

Polymerized ionic liquid poly(VeimBr): 4.9 g of VeimBr was dissolved in 30 mL of chloroform. After the addition of 2% AIBN, the mixture was stirred at 60 °C for 6 h. The precipitates were filtered and washed with chloroform. The obtained poly(VeimBr) was finally dried under vacuum.

Protein Adsorption by NmimCl-PVC Ionomer. 100 mg NmimCl-PVC ionomer was used to extract proteins in 6 mL of aqueous solution (pH 7.0, regulated by Tris-HCl). The mixture was shaken vigorously for 10 min to facilitate protein adsorption on the ionomer, followed by phase separation with centrifugation for 5 min at 3000 rpm. The concentrations of proteins left in the aqueous phase before and after adsorption were obtained by measuring the absorbance at the characteristic absorption wavelengths of the proteins, that is, 408 nm for Hb, 280 nm for Lys, BSA, Trf, and Ig G, by using a U-3900 spectrophotometer (Hitachi High Technologies, Japan).

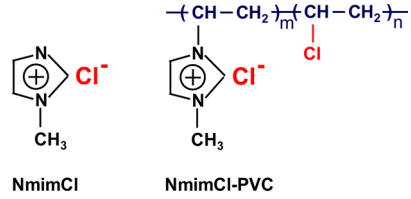
Before performing in situ surface fluorimetric detection, the NmimCl-PVC ionomer after hemoglobin adsorption was washed thoroughly by using Na_2CO_3–$NaHCO_3$ buffer (pH 10.5, 0.2 mol L^{-1}), Na_2HPO_4–NaH_2PO_4 buffer (pH 11.5, 0.2 mol L^{-1}), and water to eliminate possible residues of coexisting protein species.

Spectral Measurements. A F-7000 fluorospectrophotometer (Hitachi High Technologies, Japan) is used for fluorescence measurement. The particulate ionomer was collected in a cell and detected with a slit width of 5 nm and a scan speed of 1200 nm min^{-1}. The scanning voltage of lamp is set to 500 V. The fluorescence quantum yield was obtained with a FLS920 spectrometer (Edinburgh, U.K.) with barium sulfate as control. The ionomer and pure ionic liquid were excited at 325 nm and 365 nm, respectively. The fluorescence decay profiles were measured by a FluoroMax4 fluorospectrophotometer (HORIBA Jobin Yvon Inc., France) with time-correlated single-photon counting (TCSPC) technique. The FluoroMax4 fluorospectrophotometer was also used to confirm the fluorescence quantum yield of the bare ionic liquid. A 325 nm NanoLED was used as the excitation source with performing deoxygenation of the sample.

3. RESULTS AND DISCUSSION

Fluorescence Behaviors of the NmimCl-PVC Ionomer. *3D Fluorescence Spectra.* The molecular structure of native ionic liquid NmimCl and the produced NmimCl-PVC ionomer are illustrated in Scheme 1.

Scheme 1. Molecular Structures of Ionic Liquid NmimCl and NmimCl-PVC Ionomer



For the purpose of elucidating the variations of fluorescence before and after immobilization of the ionic liquid moiety, the fluorescence behaviors of NmimCl and NmimCl-PVC ionomer are investigated. Figure 1 showed the three-dimensional (3D) fluorescence spectra of native ionic liquid NmimCl and NmimCl-PVC ionomer with an N-methylimidazole immobilization ratio of 15.1%. It is obvious that a fluorescence intensity of ca. 2200 was observed for NmimCl at λ_{em} = 410 nm with excitation at λ_{ex} = 365 nm (Figure 1A). However, a much higher fluorescence intensity of ca. 8750 was recorded for the NmimCl-PVC ionomer, corresponding to a 4-fold improvement with respect to that of the bare NmimCl. It is noticeable that the maximum wavelength for the emission of NmimCl-PVC ionomer was red-shifted to λ_{em} = 440 nm with excitation at λ_{ex} = 325 nm (Figure 1B). Theoretical elucidations provide suitable interpretation for this observation. It has been previously reported that there exist various structures in pure ionic liquids with short- and long-range spatial correlations of cation–anion and cation–cation pairs, that is, the associated species. The confinement of associated species tends to cause realignment of cations and anions of the ionic liquid.[13] In addition, there exists a lot of π stacking associations through surface overlapping of imidazolium ring in a limited space.[19] In the NmimCl-PVC ionomer, grafting of N-methylimidazole leads to the confinement of ionic liquid moiety by PVC chain, which contributes to the increment of π stacking associations. As a result, remarkable enhancement on the fluorescence emission of the NmimCl-PVC ionomer was observed.

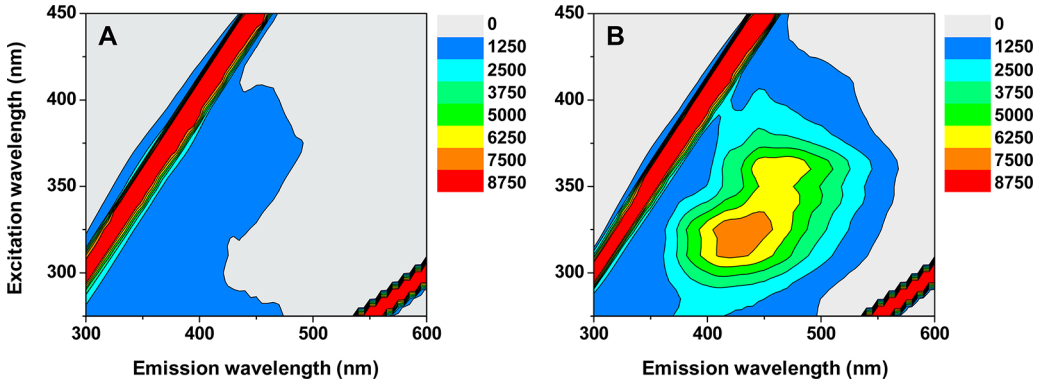


Figure 1. 3D fluorescence spectra of the native NmimCl (A) and NmimCl-PVC ionomer (B). The slit width for the excitation and emission is 5 nm, and the voltage of lamp is 500 V.

Excitation Wavelength-Dependent Emission Behavior for NmimCl-PVC Ionomer. As is known that the emission of most neat imidazolium ionic liquids exhibit excitation wavelength dependence at longer wavelength region. This was attributed to the existence of energetically different associated species.[20] By means of changing the excitation wavelength, a slightly different associated species is excited and an emission characteristic of this particular species is observed. It is illustrated in Figure 2

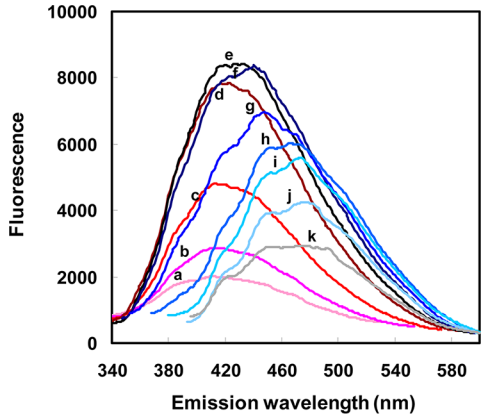


Figure 2. Excitation wavelength-dependent emission behavior of the NmimCl-PVC ionomer. Excitation wavelength λ_{ex} at (a) 285 nm, (b) 295 nm, (c) 305 nm, (d) 315 nm, (e) 325 nm, (f) 335 nm, (g) 345 nm, (h) 355 nm, (i) 365 nm, (j) 375 nm, (k) 385 nm. The slit width for the excitation and emission is 5 nm, and the voltage of lamp is 500 V.

that the fluorescence behavior of the NmimCl-PVC ionomer is similar to that of the neat imidazolium ionic liquid, where the fluorescence behavior is strongly dependent on the excitation wavelength. When excited at λ_{ex} = 285−315 nm, NmimCl-PVC exhibits an emission band centered at ca. 420 nm. However, as the excitation wavelength shifted to longer wavelengths (λ_{ex} > 325 nm), the fluorescence maximum starts to shift toward longer wavelength with progressive decrease of the overall fluorescence intensity. This clearly indicated that there exists energetically different associated species in the NmimCl-PVC ionomer.

In order to further investigate the impact of N-methylimidazole immobilization on the fluorescence spectra, a comparison of the emission behavior of the 1-vinyl-3-ethylimidazolium bromide monomer (VeimBr) and its polymerized derivative (poly(VeimBr)) is performed. As illustrated in Figure 3, the maximum fluorescence emission shifted from 445 to 415 nm after the polymerization process. This indicated an increase of the higher energetic associated species with the content of π stacking associations in the poly(VeimBr) and thus results in blue shift of the fluorescence emission wavelength.

The Dependence of Fluorescence Intensity on Grafting Ratio. In the preparation of the NmimCl-PVC ionomer, various molar ratio of N-methylimidazole/PVC (i.e., 1:2, 1:1, 2:1, 4:1 and 8:1) were investigated.[18] Consequently, N-methylimidazole immobilization/grafting ratios of 4.3%, 7.4%, 10.8%, 15.1%, and 13.7% were achieved respectively. A maximum grafting ratio of 15.1% was obtained by using a molar ratio of N-methylimidazole/PVC = 4:1, and no further improvements on the immobilization ratio were observed at an even higher N-methylimidazole/PVC molar ratio.[18] Figure 4 showed the fluorescence spectra of the NmimCl-PVC ionomer with various immobilization ratios N-methylimidazole by exciting at λ_{ex} 325 nm. A significant enhancement on the fluorescence intensity was observed with the increase of the immobilization ratio from 4.3% to 15.1%. In this respect, a previous study has physically confined 1-ethyl-3-methylimidazolium dicyanamide ($EmimN(CN)_2$) into mesoporous silica gel, which resulted in a great enhancement of the fluorescent emission of dicyanamide based ionic liquids within 5−15% (m/m) ionic liquid loading. When further increasing the ionic liquid loading to >15%, the loaded ionic liquid moiety tends to aggregate on the external surface of the silica gel, and a decrease of the fluorescent emission was encountered.[13] In our present case, the intensity of fluorescence emission of the NmimCl-PVC ionomer was largely dependent on the loading of ionic liquid on PVC. This observation is in agreement with that reported in the literature within N-methylimidazole grafting ratio of 4.3−15.1%.

Figure 3 illustrates an extreme example for the fluorescent behavior of a polymerized ionic liquid with a 100% grafting ratio of the ionic liquid moiety. Where a relatively small increase of ca. 39% of the maximum fluorescence intensity for poly(VeimBr) was obtained with respect to that of the monomer VeimBr, that is, a fluorescence intensity of 3550 for the polymer versus 2550 for the monomer. This further indicated that on one hand the π stacking associations contribute to the increase of the fluorescence intensity, on the other hand, the self-absorption of the bound ionic liquid and the energy transfer between the fluorophores through collisions result in the reduction of emission intensity. As a whole, the bound ionic liquid with too high an immobilization/

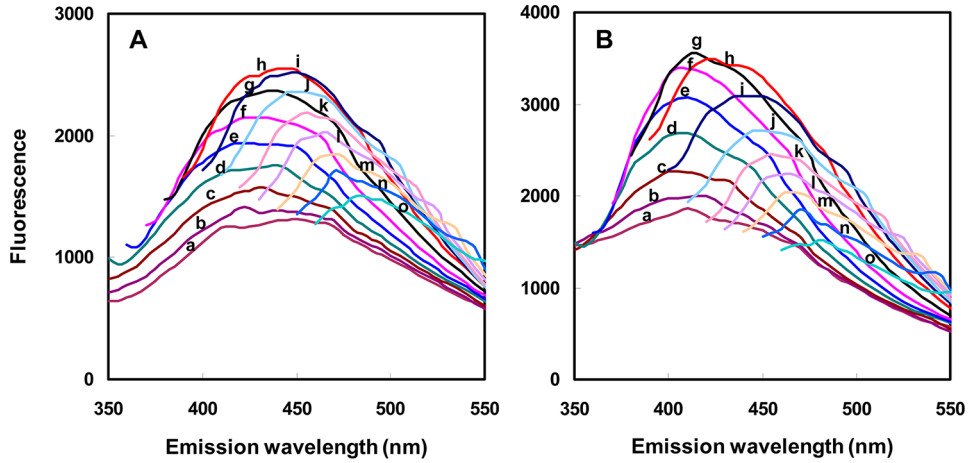


Figure 3. Excitation wavelength-dependent emission of VeimBr (A) and poly(VeimBr) (B). Excitation wavelength at λ_{ex} (a) 285 nm, (b) 295 nm, (c) 305 nm, (d) 315 nm, (e) 325 nm, (f) 335 nm, (g) 345 nm, (h) 355 nm, (i) 365 nm, (j) 375, (k) 385 nm, (l) 395 nm, (m) 405 nm, (n) 415 nm, (o) 425 nm. The slit width for excitation and emission is 5 nm; the lamp voltage is 500 V.

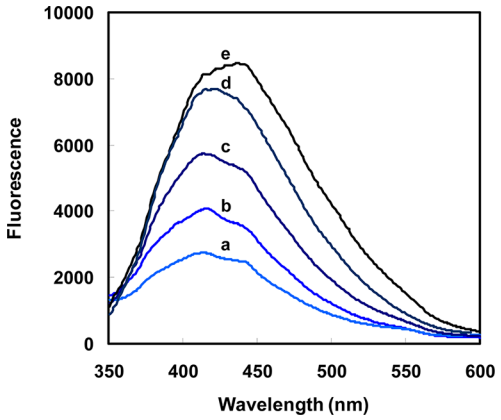


Figure 4. Fluorescent spectra of the NmimCl-PVC ionomer with various immobilization ratios: (a) 4.3%, (b) 7.4%, (c) 10.8%, (d) 13.7%, (e) 15.1%. λ_{ex} = 325 nm; the slit width for the excitation and emission is 5 nm; the voltage of lamp is 500 V.

grafting ratio tends to deteriorate the fluorescence intensity to different extent depends on the self-absorption as well as the intraionomer energy transfer. For the ensuing experiments, the NminCl-PVC ionomer with a grafting ratio of 15.1% was used.

The fluorescence emission quantum yield and fluorescence lifetime are typical and important spectroscopic parameters of the singlet state, depends strongly on the interaction of a molecule with its microenvironment.[21] The fluorescence quantum yields and fluorescence lifetimes of the native NmimCl and NmimCl-PVC ionomer were derived to be 0.063/8.8 ns and 0.112/7.1 ns, respectively, which clearly demonstrated that the confinement of ionic liquid moiety on a suitable solid support results in the enhancement of both fluorescence intensity and the quantum yield itself. This provides further support for the aforementioning explanations.

Influence of Anionic Moiety on the Fluorescence Behavior. The chemical properties of inorganic salts generally depend much more closely on the anionic moiety rather than the cationic counterpart.[22] For the NmimCl-PVC ionomer, although the cationic moiety (i.e., methylimidazolium) related to the strong fluorescence emission of PVC confined imidazolium ionic liquid, variation of the anionic counterpart contributes even more to its fluorescence. After replacing the anionic chloride with a weak coordinating anion hexafluorophosphate (PF_6^-), a significant increase of the fluorescence intensity for the NmimPF$_6$-PVC ionomer was achieved with respect to that of

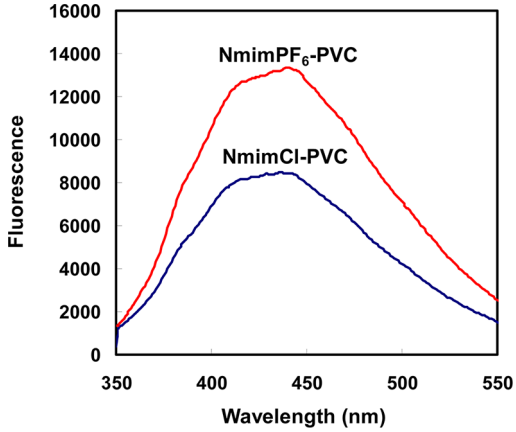


Figure 5. Fluorescent spectra of the NmimCl-PVC and NmimPF$_6$-PVC ionomers. λ_{ex} = 325 nm; the slit width for the excitation and emission is 5 nm; the voltage of lamp is 500 V.

the NmimCl-PVC ionomer as illustrated in Figure 5, where ca. 1.6-fold increment of the fluorescence intensity was demonstrated. This observation is probably related to the lower nucleophilicity of PF_6^- with respect to Cl^-, which resulted in the decrement of the electron density of imidazolium ring. This well facilitates the fluorescence emission of the PVC confined NmimPF$_6$-PVC ionomer.[23]

Quantification of the Adsorbed Hemoglobin by Fluorimetry. *Fluorescence Quenching of NmimCl-PVC Ionomer by Hemoglobin.* Our further experiments have shown that the NmimCl-PVC ionomer is able to adsorb some protein species in aqueous solution. During the adsorption process, the interaction between the protein species and the fluorophores of the PVC confined ionic liquid have the tendency to affect the fluorescent behavior of the NmimCl-PVC ionomer. In general, this interaction can result in fluorescence quenching, corresponding to the decrease of the fluorescence quantum yield of the fluorophore.[24,25] In the present case, 100 mg of the NmimCl-PVC ionomer was used to extract proteins of 100 μg mL^{-1} in 6 mL of aqueous solution at

pH 7. The interaction of hemoglobin with the ionomer might involve coordination between the iron atom in heme group and the cationic ionic liquid moiety as demonstrated previously.[18] In the present case, the ionomer may behave as an anionic exchange resin. The NmimCl-PVC with retained protein species was isolated by centrifugation and applied for in situ solid surface fluorescence detection. The fluorescence quenching effect parameter (E) of the NmimCl-PVC ionomer by certain amount of a specific protein was expressed according to the following equation:

$$E = 1 - F/F_0$$

F_0 is the fluorescence intensity of the NmimCl-PVC ionomer in the absence of protein, while F is the fluorescence intensity recorded for the NmimCl-PVC/protein complex after protein adsorption. As illustrated in Table 1, the adsorption efficiencies of Hb, Lys, IgG, Trf, and BSA on NmimCl-PVC were 93%, 97%, 5%, 3%, and 3%. While the fluorescence quenching effect parameters for these proteins were derived to be 98%, 5%, 0%, 2%, and 2%. These results well indicated that the presence of Hb causes significant quenching on the fluorescence of the NmimCl-PVC ionomer. However, Lys, IgG, Trf, and BSA cause virtually no quenching at the same conditions. This observation provides a selective approach for the quantification of hemoglobin in human body fluids and other biological samples.

Table 1. Adsorption Efficiencies and Fluorescence Quenching Effect of Proteins on NmimCl-PVC

protein	Hb	Lys	BSA	Trf	IgG
adsorption efficiency (%)	93	97	5	3	3
fluorescence quenching (%)	98	5	0	2	2

Mechanisms of Fluorescence Quenching. Fluorescence quenching might be dynamic, resulting from collisions between the fluorophore and the quencher, or be static, arising from the formation of a ground-state complex between the fluorophore and the quencher. The detection of fluorescence lifetime is the most accurate method to judge the type of quenching.[26] The fluorescence decay curves of NmimCl-PVC ionomer and the NmimCl-PVC/Hb complex were illustrated in Figure 6.

The fluorescence decay profile was fitted by a sum of exponentials as in the following:

$$R(\tau) = \sum B_i \, e^{-\tau/\tau_i}$$

$R(\tau)$ denotes the multiexponential decay function, B_i is the pre-exponential factor, and τ_i is the decay time. The fluorescence parameters of the NmimCl-PVC ionomer and the NmimCl-PVC/Hb complex are listed in Table 2. The average lifetime was calculated according to the following equation, where $B_i\tau_i$ reflects the contribution of the ith component to the steady-state fluorescence intensity.

$$\tau = \sum B_i\tau_i / \sum B_i$$

The fluorescence lifetimes of the NmimCl-PVC ionomer and the NmimCl-PVC/Hb complex were derived to be 7.1 and 5.3 ns, respectively. The decrease of lifetime after the adsorption of hemoglobin might demonstrate an overall dynamic quenching of the fluorescence for the NmimCl-PVC ionomer.

Figure 7 showed the emission spectrum of NmimCl-PVC ionomer in combination with the absorption spectrum of hemoglobin. The heavy overlap of the emission and absorption spectra indicated that excitation energy transfer might take place between the NmimCl-PVC ionomer and Hb molecule. Fluorescence resonance energy transfer (FRET) is an electrodynamic phenomenon that occurs between the primarily excited molecule and its neighbors. By Förster theory of dipole−dipole energy transfer, the efficiency of energy transfer is related to the distance between the donor and the acceptor. The distance r between Hb and NmimCl-PVC ionomer in the present case was calculated by the following equation:[27,28]

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6}$$

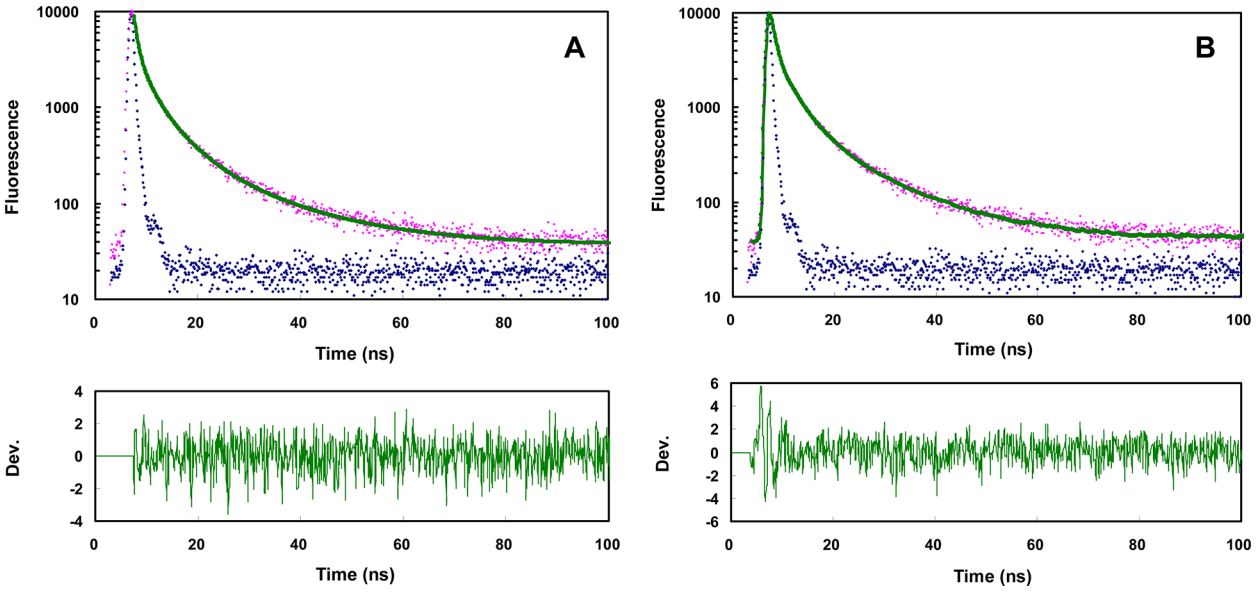


Figure 6. Fluorescence decay curves of (A) NmimCl-PVC and (B) NmimCl-PVC/Hb. λ_{ex} = 325 nm, λ_{em} = 430 nm; the slit width for the excitation and emission is 5 nm; the voltage of lamp is 500 V. The blue dots demonstrate the instrumental response. The pink dots are the real measured fluorescence decay. The green curve is the fitting results. The bottom is the profile for the residuals.

Table 2. Fluorescence Parameters of the NmimCl-PVC Ionomer and the NmimCl-PVC/Hb Complex

	B_1	τ_1 (ns)	B_2	τ_2 (ns)	B_3	τ_3 (ns)	B_4	τ_4 (ns)
NmimCl-PVC	2468.976	22.1965	1254.235	54.16771	324.9122	156.4833	4890.924	6.258341
NmimCl-PVC/Hb	0.03673	33.40802	0.175864	6.637447	0.007224	121.4574	0	0

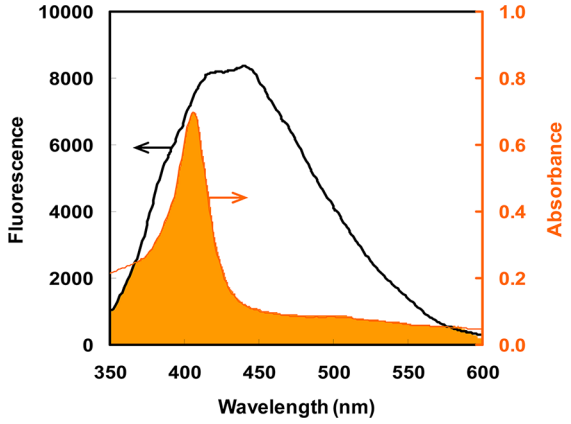


Figure 7. Overlap of NmimCl-PVC ionomer fluorescence spectrum and Hb absorption spectrum. λ_{ex} = 325 nm; a slit width for the excitation and emission is 5 nm; the voltage of lamp is 500 V; Hb aqueous solution: 100 μg mL^{-1}.

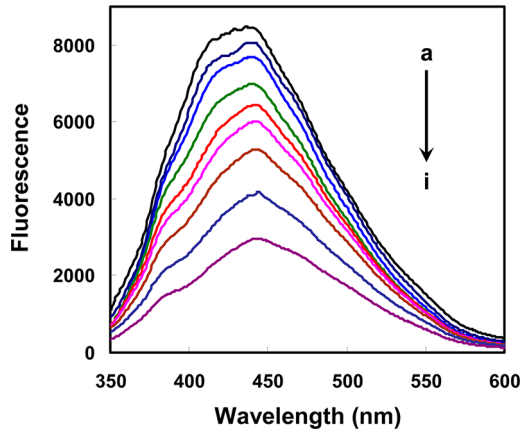


Figure 8. Dependence of fluorescence quenching of NmimCl-PVC ionomer on the concentration of hemoglobin. Hb concentration (μg mL^{-1}) from a to i: a, 0; b, 30; c, 60; d, 120; e, 180; f, 240; g, 300; h, 400; i, 600. Hb in 6 mL Tris-HCl buffer at pH 7 was adsorbed by 100 mg of NmimCl-PVC ionomer with a 15.1% grafting ratios. λ_{ex} = 325 nm; the slit width of excitation and emission is 5 nm; the voltage of lamp is 500 V.

E, F, and F_0 are the same as described previously; r is the average distance between NmimCl-PVC and Hb. R_0 is the critical distance at a transfer efficiency of 50%, as deduced by

$$R_0^6 = 8.79 \times 10^{-25} K^2 n^{-4} \varphi j$$

K^2 is the orientation factor related to the geometry of NmimCl-PVC ionomer and Hb of dipoles (2/3 for random orientation in fluid solution), n is the average refracted index of the medium (1.336 for protein aqueous solution), φ is the fluorescence quantum yield of the NmimCl-PVC ionomer, J is the effect of spectral overlap between the emission spectrum of the NmimCl-PVC and the absorption spectrum of Hb, as calculated by the following equation:

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 \mathrm{d}\lambda}{\int_0^\infty F(\lambda)\mathrm{d}\lambda}$$

$F(\lambda)$ is the corrected fluorescence intensity of the NmimCl-PVC ionomer in the wavelength of λ, $\varepsilon(\lambda)$ is the extinction coefficient of Hb at λ.

In the present case, φ = 0.050 was found for the NmimCl-PVC ionomer, while J, R_0, and E were derived to be 4.46 × 10^{-13} cm^3 L mol^{-1}, 3.98 nm, and 0.145. Thus, r = 5.36 nm was deduced based on the above equations. As the donor-to-acceptor distance was located within the 2−9 nm region, the energy transfer from NmimCl-PVC ionomer to hemoglobin was assumed to occur with high probability.[25]

Quantification of Hb Adsorbed on the Ionomer and in Aqueous Solution. Figure 8 illustrated the significant fluorescence quenching effect caused by the adsorption of hemoglobin onto the NmimCl-PVC ionomer within the range 5−600 μg mL^{-1}. In addition, the shape of the fluorescence peak and the maximum emission wavelength keep constant with excitation at λ_{ex} 325 nm. This observation provides a promising potential by using the NmimCl-PVC ionomer as a fluorescence probe for sensing the adsorbed Hb.

The quantity of adsorbed hemoglobin (Q, μg mg^{-1}) was calculated in the following:

$$Q = \frac{(C_0 - C_1)V_{aq}}{M_{IL}}$$

C_0 and C_1 represent the concentration of Hb in aqueous solution before and after adsorption by NmimCl-PVC, V_{aq} and M_{IL} stand for the volume of the aqueous phase and the amount of NmimCl-PVC used for protein adsorption.

The experiments have demonstrated that Q and E showed excellent linear relationship. A linear regression equation of F/F_0 = −0.0213C + 0.989 was achieved within 0.3−26.2 μg mg^{-1}, and a detection limit of 0.1 μg mg^{-1} was derived along with a RSD of 2.6% at 7.2 μg mg^{-1}.

NmimCl-PVC is a suitable adsorbent for the selective extraction and preconcentration of hemoglobin from other protein species in biological sample matrices.[18] 100% of Hb was adsorbed on the NmimCl-PVC ionomer within a concentration range of 5−300 μg mL^{-1}; that is, the quantity of Hb in the sample solution is equal to the amount absorbed on the NmimCl-PVC in this range. Regarding hemoglobin in aqueous solution, the linear range of the solid surface fluorimetric procedure is 5−300 μg mL^{-1}, with a detection limit of 2 μg mL^{-1}. It was previously demonstrated that 1,3-butylimidazolium chloride (BBimCl) exhibited high fluorescence, and it was applied for the sensing of hemoglobin as a fluorescence probe,[4] which offered a detection limit of 7.3 × 10^{-8} mol L^{-1} (5 μg mL^{-1}). The symmetrical plane conjugating structure of the ionic liquid contributed to the sensitive detection. As for the case of NmimCl-PVC ionomer, the detection limit is similar with respect to that of BBimCl due to the confinement of ionic liquid on the polymer support.

In practice, 10 μL of human whole blood was directly diluted with 10 mL of deionized water without any further pretreatment to ensure the concentration of Hb fall into the range

5−300 μg mL^{-1}. 6.0 mL of the diluted sample was used to mix with 100 mg of NmimCl-PVC for facilitating the extraction by shaking vigorously, and thereafter, the two phases were separated by centrifugation. Afterward, the NmimCl-PVC was washed with Na_2CO_3−$NaHCO_3$ buffer (pH 10.5, 0.2 mol L^{-1}), Na_2HPO_4−NaH_2PO_4 buffer (pH 11.5, 0.2 mol L^{-1}), and water to eliminate any loosely retained protein species on the ionomer. Then, the fluorescence intensity of NmimCl-PVC/Hb was detected and the quantity of Hb adsorbed on the NmimCl-PVC as well as the residual amount of Hb in the aqueous solution was calculated. Spiking recoveries of 95−102% for Hb in the human whole blood samples were obtained as listed in Table 3.

Table 3. Results for Hemoglobin Determination in Human Whole Blood Samples

sample	found (μg mL^{-1})	spiked (μg mL^{-1})	recovery (%)
human blood 1	124 ± 5	100	102
human blood 2	144 ± 9	100	95

4. CONCLUSIONS

The confinement of hydropholic ionic liquid methylimidazolium chloride (NmimCl) by polyvinyl chloride (PVC) produces an ionomer (NmimCl-PVC) with greatly enhanced fluorescence intensity with respect to the native ionic liquid. The fluorescence enhancement is attributed to the rearrangement of the anions/cations as well as the increase of π stacking. This observation not only provided useful information on the elucidation of the relationship between the structure of the ionic liquid and its fluorescence nature but also offered an approach for improving the fluorescence intensity by fluorophore confinement. In addition, the ionomer can selectively adsorb hemoglobin and result in significant dynamic quenching on its fluorescence. This provides a potential for in situ detection of hemoglobin by developing a solid surface fluorimetric procedure integrating hemoglobin preconcentration on the ionomer.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors appreciate financial support from the Natural Science Foundation of China (No. 21105008, 21275027, and 21235001), the Program of New Century Excellent Talents in University (NCET-11-0071), and the Fundamental Research Funds for the Central Universities (N110305005, N110705002, and N110805001).

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