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Ionic liquid–polyvinyl chloride ionomer for highly selective isolation of basic proteins

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ABSTRACT

Hydrophilic ionic liquid–polyvinyl chloride (PVC) hybrids were prepared by immobilizing N-methylimidazole (N-mim) to PVC chains in toluene. The NmimCl–PVC hybrids were characterized by FT-IR, ^{1}H NMR, surface charge analysis and elemental analysis. The immobilization ratio, i.e., the percentage of chloride on PVC chain reacting with N-mim to form the hybrid, varies from 4.3% to 15.1% by increasing the N-mim/PVC molar ratio. The most distinct feature of the hybrid is its excellent selectivity for adsorbing basic proteins by effective suppression of the non-specific protein adsorption by pure PVC, and a higher immobilization ratio facilitates better selectivity. In Tris–HCl buffer, 100 μg mL^{-1} of basic proteins, i.e., lysozyme (Lys), cytochrome c (cyt-c) and hemoglobin (Hb), were favorably adsorbed with efficiencies of 97%, 98% and 94% by the hybrid with an immobilization ratio of 15.1%, while the adsorption of acidic proteins, i.e., bovine albumin serum (BSA), transferring (Trf) and immunoglobulin G (IgG) were negligible. The retained Lys, cyt-c and Hb could be readily recovered by elution with phosphate buffer, carbonate buffer and SDS solution with efficiencies of 89%, 87% and 84%, respectively. Another feature of the hybrid is the significant improvement of the biocompatibility characterized by the maintenance of the activity of hemoglobin after adsorption and elution process. The practical usefulness of the hybrid was demonstrated by selective isolation of hemoglobin from human whole blood.

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1. Introduction

Ionic liquids (ILs) have attracted extensive attentions in the recent years. Their intrinsic non-molecular natures give rise to unique physico-chemical properties, i.e., nonflammability, low volatility, negligible vapor pressure, high thermal stability, a wide electrochemical window, ease of recirculation and manipulation [1–3]. Recently, ionic liquid immobilization opens novel applications in catalysis [4,5], electrochemical analysis [6,7] and separation sciences [8,9]. The immobilized ionic liquids generally exhibit preferable properties as compared to their pure counterparts, e.g., higher catalytic efficiency [4], easier to recover the catalyst, tunable hydro-philicity or -phobicity of the surface by changing the length of side-chains of the cationic moieties and improvement on the adsorption capacity of the ionic liquid.

Generally, ionic liquids can be coated onto the surface of solid support with [10] or without [11] covalent bonding, and the latter is most frequently used attributed to the favorable performance of the materials. Some of the commonly used solid supports include inorganic materials, i.e., silica [12], molecular sieves [13], carbon nanotubes [14], carbon nanofiber/sintered metal fiber [15], porous alumina [16] and organic materials, i.e., polystyrene [17,18]. A immobilization protocol generally includes the functionalization of the surface of solid support to introduce certain functional groups, followed by covalent bonding of the ionic liquid onto the solid support [19,20]. In some cases, no ionic liquid was used during this process but ionic complexes/ionic liquids were generated after immobilization or covalent bonding [5,13,21]. In practice, functional groups containing chloride ion were most frequently introduced onto the solid support prior to covalent bonding of the ionic liquid moieties.

Polyvinyl chloride (PVC) possesses abundant chlorine atoms on both the surface and the bulk. Thus, it could be a potential material for reacting with imidazole or similar substance to generate bound ionic liquids. PVC has been modified by chemical and/or physical processes, such as surface coating [22], surface crosslinking with ultraviolet radiation or plasma treatment [23], and surface grafting with hydrophilic polymers [24]. When considering protein adsorption for the purpose of biological investigations, the poor biocompatibility of the hydrophobic PVC surface not only leads to significant non-specific retention of proteins, but also denatures the protein species [25]. In this respect, it is highly demanded to modify the PVC material in order to suppress its non-specific adsorption of various proteins and thus improve its selectivity for protein isolation from complex sample matrixes. Meanwhile, the improvement on its biocompatibility is required for the elimination

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of protein denaturation during the pretreatment process [26]. Taking into account that surface immobilization can only improve the performance of the material to a limited extent, new modification approaches are required.

In the present work, we report a simple one-step modification protocol for immobilizing the N-methylimidazole moiety onto both the surface and the bulk of the PVC material to generate bound hydrophilic ionic liquid for the purpose of protein adsorption. This bulk immobilization protocol significantly improved the selectivity for the adsorption of basic proteins by effective suppression of non-specific adsorption of protein species. In addition, the hybrid provides favorable biocompatibility characterized by the avoidance of protein denaturation during the adsorption process.

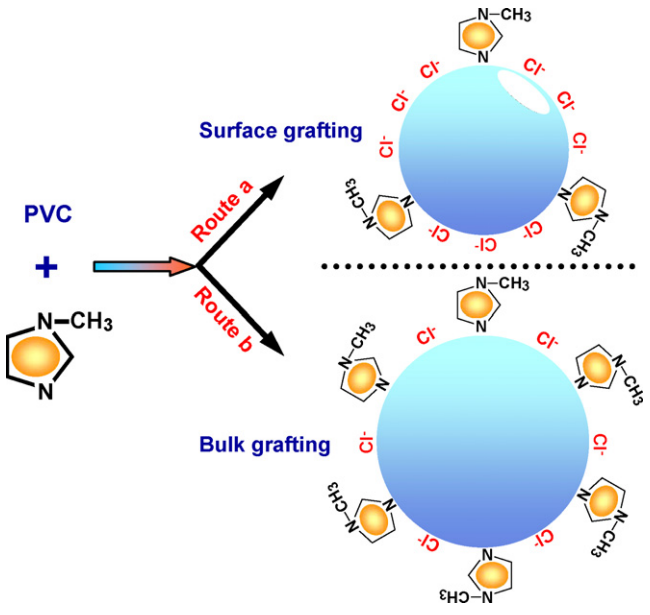
2. Experimental

2.1. Materials

Lysozyme from chicken egg white (Lys, L2879, isoelectric point pI 11), cytochrome c from horse heart (cyt-c, C7752, pI 10.3), 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) were purchased from Sigma (St. Louis, MO) and were used without further purification. Commercial PVC particles with an average molecular weight of 58,000 (nominal particle size of ca.150 μm) were purchased from Shenyang Chemical Co. (China) and N-methylimidazole was purchased from Kaile Chemicals (China). Other chemicals employed were at least of analytical reagent grade. Deionized water of 18 MΩ cm^{-1} was used throughout.

2.2. Preparation of the NmimCl ionic liquid–polyvinyl chloride hybrid

For providing comparative information, surface immobilization of N-methylimidazole onto PVC was also investigated in addition to the bulk grafted NmimCl ionic liquid–PVC hybrids. The one-step preparation of both categories of materials was illustrated in Scheme 1.



Scheme 1. The two pathways for the one-step preparation of the NmimCl–PVC hybrids.

Surface immobilization of N-methylimidazole onto PVC: 4.5 mL of N-methylimidazole and 5 g of PVC was added to a 50 mL flask. The immobilization of N-methylimidazole onto PVC surface was facilitated by stirring at 70 °C for 48 h. Afterwards, the solid (NmimCl–PVC) was separated by centrifugation at 3000 rpm for 10 min, followed by thorough washing with ethyl acetate and water alternatively. The product was finally dried *in vacuo* at room temperature for overnight. In this case, the PVC particles remain their original morphology, i.e., ca. 150 μm in diameter.

NmimCl ionic liquid–PVC hybrid (*bulk immobilization*): In order to obtain NmimCl ionic liquid–polyvinyl chloride hybrid with higher immobilization ratio, more thorough reaction between N-methylimidazole and PVC was conducted by extending the PVC in toluene. 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 to ensure sufficient reaction between N-methylimidazole and PVC. The separation of the NmimCl ionic liquid–polyvinyl chloride hybrid (NmimCl–PVC) was facilitated by adding an excessive amount (100 mL) of methanol/water (50%, v/v) under sonication. The hybrid 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. In order to facilitate the future use for protein adsorption, a thin layer of the ionomer was coated onto the surface of solid support by adding appropriate amount of silica beads (150 μm in diameter) into the reaction mixture accompanying the addition of methanol/water under sonication. In the present case, a thickness of ca. 590 nm of the thin layer is obtained.

The characterizations of the materials were conducted by FT-IR spectra recorded on a PerkinElmer FT-IR spectrometer (Wellesley, MA, USA) and ^{1}H NMR spectra recorded in $(CD_3)_2SO$ at 293 K with chemical shifts referenced to tetramethylsilane (TMS) on a Bruker Avance 500 spectrometer (Bruker, Switzerland). In addition, the surface charge property of the hybrid material was investigated by measuring Zeta potential within pH 3–11, by using a Nano Zetasizer (Malvern, England).

2.3. Protein adsorption by the ionic liquid–PVC hybrids

20 mg of each hybrid was used to extract proteins in 3 mL of aqueous solution (within a range of 15–100 μg mL^{-1}) in a 5 mL centrifuge tube. Blank runs were also carried out in the absence of the hybrid to ensure that no protein loss by their adsorption on the centrifuge tube. The mixture was shaken vigorously for 10 min to facilitate protein adsorption, followed by phase separation with centrifugation for 5 min at 3000 rpm. The concentrations of proteins in the aqueous phase before and after adsorption were obtained by measuring the absorbance at their characteristic absorption wavelengths (408 nm for Hb and cyt-c, 280 nm for Lys, BSA, Trf and Ig G) with a T_6 UV–vis spectrophotometer (Purkinje General Instruments, Beijing, China). The adsorption efficiency (E) was thus calculated based on the protein concentrations in the aqueous solution before and after adsorption, respectively.

The elution of proteins from the hybrids after adsorption was performed by using Na_2CO_3–$NaHCO_3$ buffer (pH 10.5, 0.2 M), Na_2HPO_4–NaH_2PO_4 buffer (pH 11.5, 0.2 M) as well as 0.5% (m/v) SDS aqueous solution.

2.4. The activity of hemoglobin after elution from the hybrid

Hemoglobin catalyzes the oxidation of 2-methoxyphenol by hydrogen peroxide and gives rise to a color product with a maximum absorption wavelength at 470 nm [27]. The color formation

rate is proportional to the oxidation rate of guaiacol. The operations were described as follows.

2 μmol of guaiacol was added into an aqueous solution in a small vial containing 10–100 μg hemoglobin. The mixture was stirred for 15 min before the addition of 9 μmol of H_2O_2 to initiate the reaction and the variations of absorbance with time (*A*–*t* curve) was recorded. The slope of the *A*–*t* curve provides information about the activity of hemoglobin.

3. Results and discussion

3.1. Preparation and characterization of the hybrids

As illustrated in Scheme 1 (Route a), the nitrogen atom at position 3 of the N-methylimidazole replaces a chlorine atom via nucleophilic substitution and bound to the PVC chain on the surface. As a result, the cationic $Nmim^+$ and the chloride anion form counterparts of the hydrophilic NmimCl ionic liquid.

Generally, surface immobilization gives rise to very low immobilization ratios of N-methylimidazole. Therefore, the low degree of modifications by surface immobilization provides limited potentials for the improvement on surface performance, e.g., surface selectivity and biocompatibility in the present case. The use of toluene dissolves the polymer and extends its chains and thus facilitates the reaction of the N-methylimidazole with the bulk of PVC [28]. Consequently, the bulk immobilization of N-methylimidazole onto PVC entails much higher degree of modification (Scheme 1, Route b). It is worth mentioning that the present one-step modification procedure is not only much simpler and straightforward, but the hybrid also provides excellent selectivity and biocompatibility for protein adsorption as detailed in the following sections.

3.2. Characterization of the hybrids

The FT-IR spectra of pure PVC, N-methylimidazole, surface and bulk immobilized NmimCl–PVC hybrids were investigated. The experimental results indicated that an absorption band at 1518 cm^{-1} was observed for both the surface and the bulk modified NmimCl–PVC hybrids, attributed to the vibration of C=C bond of the imidazole ring (C=C vibration from the end groups of the PVC was not observed) [29]. In addition, the out-of-plane C–H bending of imidazole ring at 740 cm^{-1}, in-plane imidazole ring bending at 828 cm^{-1} and imidazole H–C–C and H–C–N bending at 1076 cm^{-1} were also observed.

The ^{1}H NMR spectra of the above materials were illustrated in Fig. 1. The band from the N-bound methyl group at 3.62 ppm, the bands from protons 2, 4, 5 in the midazolium ring at 6.87, 7.09 and 7.56 ppm [30] as well as the characteristic bands arising from PVC at 2.28 ppm (CH_2) and 4.44 ppm (CH–Cl) [31] were clearly identified (a and b). For the surface modified NmimCl–PVC, the band of N-bound methyl group was identified with a little shift to higher field from 3.62 to 3.81 ppm attributed to the bonding of the nitrogen atom with the PVC chain, and the small intensity of the band indicates a very low immobilization ratio of N-methylimidazole (c). On the other hand, the same band for that of the bulk modified NmimCl–PVC was more obvious (d), corresponding to a much higher immobilization ratio. In addition, the bands of protons 2, 4 and 5 were almost not identifiable in the surface modified NmimCl–PVC because of the low immobilization ratio, however, those in the bulk modified NmimCl–PVC were identified. The strong bands at 2.50 and 3.31 ppm were attributed to DMSO and water, respectively.

The experiments have indicated that a low immobilization ratio on the surface offers very limited improvements on the selectivity of protein adsorption and biocompatibility and more details were given in the following sections. In this respect, it is highly

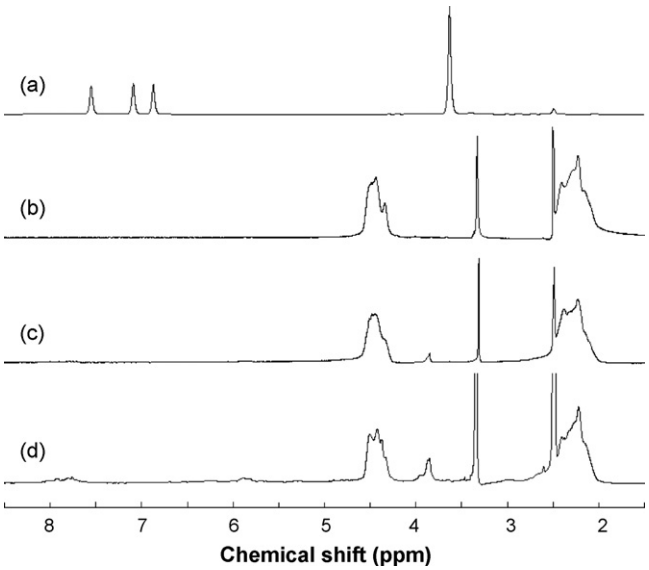


Fig. 1. The ^{1}H NMR spectra of (a) N-methylimidazolium; (b) PVC; (c) the surface modified NmimCl–PVC; (d), the bulk modified NmimCl–PVC with a N-mim/PVC molar ratio of 4:1.

desirable to increase the degree of immobilization by using bulk modification. In a highly swelling medium with a proper solvent, the polymer dissolves and facilitates the diffusion of the modifier, i.e., N-methylimidazole in this particular case, into the bulk of the polymer leading to much more extensive interactions between the polymer chain and the N-methylimidazole moiety. For the case of PVC, toluene has been proven to be a suitable solvent for this purpose.

Further experiments indicated that the bulk immobilization ratio of N-methylimidazole depends strongly on the variation of the N-mim/PVC molar ratio in toluene. In this particular case, the immobilization ratio or the degree of modification (either surface or bulk modification) could be readily derived from the ^{1}H NMR spectra based on the integrals of proton signals in CH–Cl and N–CH_3 groups [26]. It illustrated that a limited surface immobilization ratio of 2.8% was achieved using a N-mim/PVC molar ratio of 1:2, and no improvements were observed by further increasing the molar ratio up to 8:1. For bulk modification, N-mim/PVC molar ratios of 1:2, 1:1, 2:1, 4:1 and 8:1 give rise to bulk immobilization ratios of 4.3%, 7.4%, 10.8%, 15.1% and 13.7%, respectively. It can be seen that a maximum of 15.1% was observed with a molar ratio of 4:1. No further improvements on the immobilization ratio were observed at an even higher molar ratio of 8:1. These results were further demonstrated by CHN elemental analysis.

3.3. The adsorption of proteins by the NmimCl–PVC hybrids

The suitability of the NmimCl–PVC hybrids for protein isolation was investigated. 100 μg mL^{-1} Lys, cyt-c, Hb, IgG, Trf and BSA in 3 mL of aqueous solution were treated by 20 mg of the hybrids with pure PVC particles as a comparison. The adsorption efficiencies of the various protein species were summarized in Table 1.

The non-specific adsorption of proteins by PVC particles was well illustrated by the substantial adsorption of both acidic and basic proteins. On the other hand, however, selective adsorption of basic proteins was achieved after N-methylimidazole immobilization, and the increase of the immobilization ratio significantly improves the selectivity. It can be seen that a bulk immobilization ratio of 10.8% and higher entails almost complete adsorption of the basic protein species from a mixture of various proteins with

Table 1
The adsorption efficiencies of proteins by the NmimCl–PVC hybrids. 100 μg mL^{-1} proteins in 3 mL of Tris–HCl buffer (pH 7.0, 0.1 M) were treated with 20 mg of pure PVC particles and the hybrids with various N-mim grafting ratios.

Material (N-mim/PVC molar ratio)	N-mim grafting ratio	Protein adsorption efficiency (%)					
		Lys	cyt-c	Hb	IgG	Trf	BSA
Pure PVC		88	75	93	67	46	59
NmimCl–PVC (surf)	2.8%	67	52	54	15	13	31
NmimCl–PVC (bulk, 1:2)	4.3%	74	77	72	12	10	7
NmimCl–PVC (bulk, 1:1)	7.4%	86	91	87	9	7	0
NmimCl–PVC (bulk, 2:1)	10.8%	95	95	94	4	2	0
NmimCl–PVC (bulk, 4:1)	15.1%	97	98	94	5	2	0
NmimCl–PVC (bulk, 8:1)	13.7%	94	95	92	5	3	2

adsorption efficiencies of ca. 95%, while the retention of acidic protein species remains negligible.

Fig. 2 illustrates the dependence of protein adsorption efficiencies on the pH of sample solution at an ionic strength of 0.1 M by 20 mg NmimCl–PVC hybrid with a bulk immobilization ratio of 15.1% for N-methylimidazole. When pH exceeds the isoelectric point of a protein species, a significant decline of the adsorption efficiency was observed for that particular protein. Thus, the adsorption of the three acidic protein species becomes negligible at pH > 6. On the contrary, the adsorption efficiency of the basic proteins remain high, i.e., 97%, 95% and 93% for Lys, cyt-c and Hb, respectively. This could be well attributed to the electrostatic interactions between the protein species and the surface of the NmimCl–PVC hybrid. This has been further demonstrated by the variation of zeta potential on the surface of the hybrid as a function of pH. The isoelectric point (IP) of the NmimCl–PVC ionomer is 5.8, which is similar to those of the acid proteins used for the present investigations. The ionomer and the acid proteins are both positively charged in acidic medium, while they convert to be negatively charged in basic medium. That is, the surface charges of the ionomer and the acid proteins are the same within the whole pH range studied, i.e., pH 3–12. Thus, the poor adsorption of acid proteins is mainly due to the electrostatic compulsion. On the contrary, the opposite charge property of the ionomer and the basic proteins facilitates their favorable adsorption.

Hemoglobin might be an exception in this particular case. At pH 7.0, both hemoglobin and the surface of the hybrid material are neutral. The high adsorption efficiency of Hb, i.e., ca. 94%, is not due to electrostatic interactions, but could partly be attributed to hydrophobic interaction. This has been proven by the results in Fig. 3 that the increase of ionic strength in a certain range gives rise to a slight increase of the adsorption efficiency of hemoglobin. Another important driving force for the high adsorption efficiency of hemoglobin at higher pH could be the coordination interaction between the iron atom in its heme group and the cationic ionic liquid moiety as previously demonstrated by circular dichroism (CD) spectra and ^{57}Fe Mossbauer spectra [32].

Fig. 3 further illustrates that the variation of ionic strength of the sample solution leads to significant change of the adsorption efficiency of protein species. At pH 7.0, the increase of ionic strength within a small range of 0–0.3 mol L^{-1} NaCl results in an effective suppression of the adsorption of acidic proteins. The adsorption efficiencies of acidic proteins immunoglobulin G, transferring and bovine albumin serum were well suppressed to a negligible level of 6% at an ionic strength of >0.1 mol L^{-1} NaCl. Meanwhile, however, favorable adsorption efficiencies of >94% were achieved for the basic protein species, including hemoglobin.

The performance of selective isolation of basic protein species in the presence of acidic proteins by the NmimCl–PVC hybrid was further demonstrated by the separation of Hb and BSA, as illustrated in Fig. 4. Hb and total amount of proteins were quantified by measuring the absorbance at 408 and 280 nm, respectively. After calculation, the results indicated that the surface grafted NmimCl–PVC hybrid adsorbed 84% of Hb and 43% of BSA (Fig. 4b). On the other hand, when using the bulk modified NmimCl–PVC hybrid with an immobilization ratio of 15.1%, the soret absorption band of heme group in Hb at 408 nm was almost completely disap-

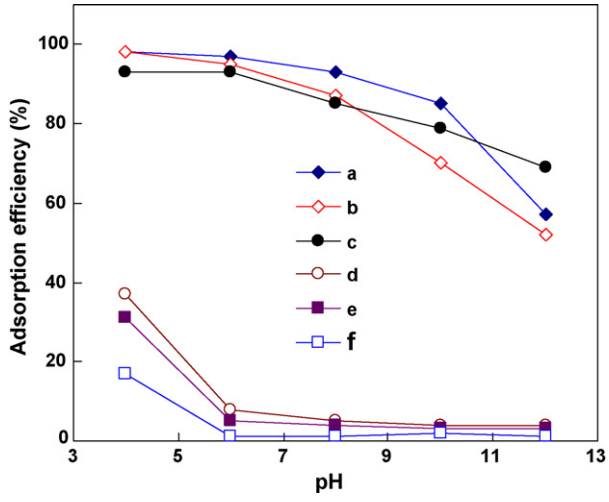


Fig. 2. pH dependence of the adsorption efficiencies of 100 μg mL^{-1} proteins in 3 mL aqueous solution at an ionic strength of 0.1 M by 20 mg NmimCl–PVC hybrid with a bulk grafting ratio of 15.1%. (a) Lys; (b) cyt-c; (c) Hb; (d) IgG; (e) Trf; (f) BSA.

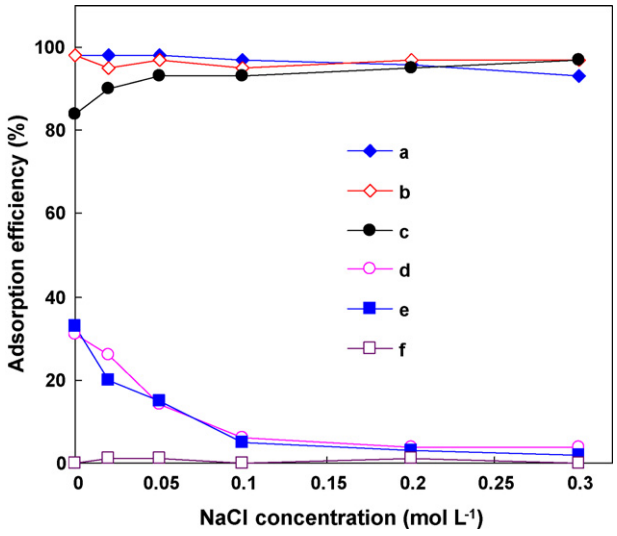


Fig. 3. The ionic strength dependence of adsorption efficiencies of 100 μg mL^{-1} proteins in 3 mL aqueous solution (pH 7.0) by 20 mg NmimCl–PVC hybrid with a bulk grafting ratio of 15.1% for N-methylimidazolium. (a) Lys; (b) cyt-c; (c) Hb; (d) IgG; (e) Trf; (f) BSA.

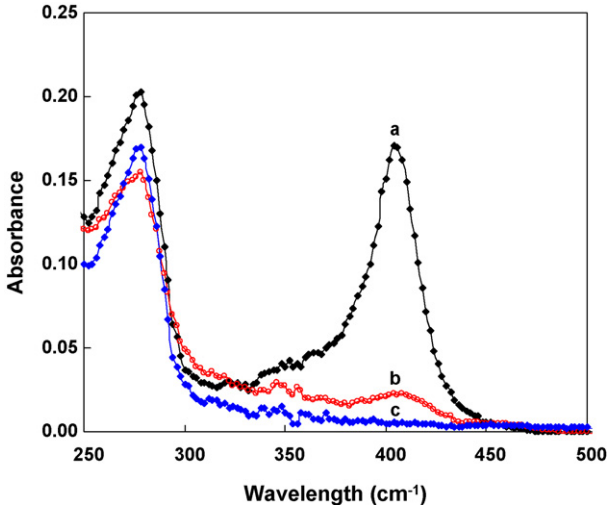


Fig. 4. UV–vis spectra of a mixture of 30 μg mL^{-1} Hb and BSA in 3 mL of Tris–HCl buffer (pH 7.0, 0.1 M) before and after adsorption by 20 mg of the hybrids. (a) Without adsorption; (b) NmimCl–PVC with a surface grafting ratio of 2.8% adsorbs both Hb and BSA; (c) selective adsorption of Hb by NmimCl–PVC with a bulk grafting ratio of 15.1%.

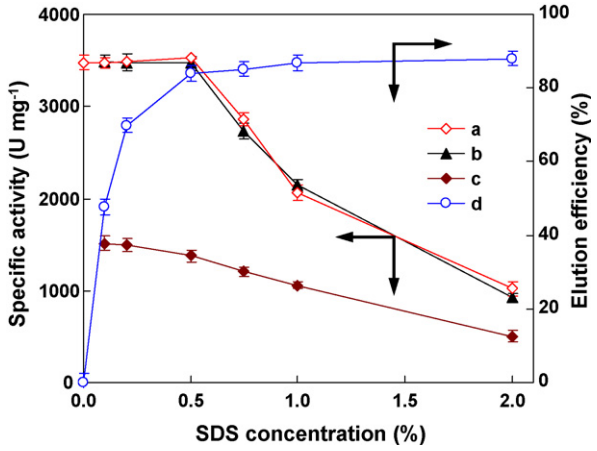


Fig. 5. (a) The specific activity of 30 μg mL^{-1} Hb in pure solution in the presence of SDS; (b) 30 μg mL^{-1} Hb in Tris–HCl buffer (pH 7.0, 0.1 M) was adsorbed by 20 mg hybrid with a bulk grafting ratio of 15.1%, and the specific activity was measured after elution with 500 μL SDS; (c) 30 μg mL^{-1} Hb in Tris–HCl buffer (pH 7.0, 0.1 M) was adsorbed by 20 mg PVC, and the specific activity was measured after elution with 500 μL SDS; (d) the effect of SDS concentration on the elution efficiency of the retained Hb from the hybrid.

peared (Fig. 4c) indicating the retention of ca. 95% of Hb. Meanwhile, the adsorption of BSA was virtually not observed.

Further experiments indicated that the adsorption capacity for Hb by the NmimCl hybrid with an immobilization ratio of 15.1% is ca. 26.5 mg g^{-1}. A significant improvement has been achieved when compared to a capacity of ca. 0.15 mg g^{-1} for Hb by extraction with a hydrophobic ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate [32]. This observation illustrates a distinct advantage of the present hybrid for improving the adsorption capacity of proteins.

The above results indicated that the immobilization of N-methylimidazole significantly suppressed the non-specific adsorption of proteins by PVC and the NmimCl–PVC hybrid provided excellent selectivity for basic proteins. The simultaneous control of pH and ionic strength of the sample solution further enhanced the selectivity.

3.4. The elution of adsorbed proteins from the NmimCl–PVC hybrid

The elution of the adsorbed proteins by the NmimCl–PVC hybrid into aqueous phase is necessary in order to meet the requirements for further biological investigations. The experiments indicated that the retained Lys and cyt-c could be readily recovered by phosphate buffer (pH 11.5, 0.2 M) and carbonate buffer (pH 10.5, 0.2 M), with elution efficiencies of 87% and 89%, respectively. However, the effective elution of Hb could only be achieved by sodium dodecyl sulfate (SDS) which possesses favorable protein solubilization properties, because of the coordination interactions between the iron atom in the heme group of Hb and the cationic moiety of the ionic liquid. Considering that SDS has the tendency to cause denaturing of protein [33], its effect should thus be carefully evaluated. For this purpose, the specific activity (activity unit per milligram of protein) of Hb after adsorption by the NmimCl–PVC hybrid and elution by SDS was measured with comparison to a pure Hb solution. The results were given in Fig. 5 along with the dependence of Hb elution efficiency upon the concentration of SDS solution. It indicated that a SDS concentration of 0.5% (m/v) facilitates a favorable elution efficiency of ca. 84% for Hb, and at the same time the activity of Hb was maintained as compared to that in pure solution, indicating no denaturing of Hb by the NmimCl–PVC hybrid during the adsorption process. Thereafter, although the increase of SDS concentration results in a slight increment of the elution efficiency, a significant drop of the activity of Hb was observed. In practice, a 0.5% (m/v) SDS solution is favorable.

We have previously demonstrated that after extraction into a hydrophobic ionic liquid [32], hemoglobin can only be stripped back into aqueous phase by using a 6% SDS solution. Such a high concentration of SDS tends to significantly denature the Hb. At this point, the use of 0.5% SDS solution further illustrated the biocompatibility of the NmimCl–PVC hybrid.

Fig. 5 illustrates that a much lower specific activity, i.e., 1376 U mg^{-1}, for that part of hemoglobin eluted from the pure PVC particles was achieved with respect to 3463 U mg^{-1} for that by the NmimCl–PVC hybrid with an immobilization ratio of 15.1%. The loss of the majority of Hb activity indicates a significant protein denaturing effect by pure PVC during the adsorption process.

Fig. 6 illustrates the effect of N-mim immobilization ratio on both adsorption and elution efficiency of hemoglobin, with pure PVC particles as a comparison. It can be seen that although pure PVC adsorbed 93% of Hb in the solution, only 24% of that could be recovered by elution with a 0.5% (m/v) SDS solution. When the NmimCl–PVC hybrid with a lower immobilization ratio was used, a lower adsorption efficiency of Hb was observed, which was gradually enhanced up to 94% as the immobilization ratio was increased to 15.1%. At the same time, the elution of the retained Hb became much easier as compared to that by pure PVC, giving rise to a much favorable elution efficiency of up to 84%.

The above observations indicated that the immobilization of N-methylimidazole greatly improved the biocompatibility of PVC as proved by the avoidance of protein denaturing, i.e., the maintenance of the activity of hemoglobin after adsorption.

3.5. A practical use: isolation of hemoglobin from human whole blood

The practical applicability of the NmimCl–PVC hybrid was demonstrated by selective adsorption of hemoglobin from human whole blood. The blood sample was collected from a volunteer and treated as described in the literature [34]. The sample after appropriate dilution was subjected to the adsorption procedure as detailed in Section 2. After elution, the eluate was assayed by SDS-PAGE [35], and the electrophoretogram was illustrated in

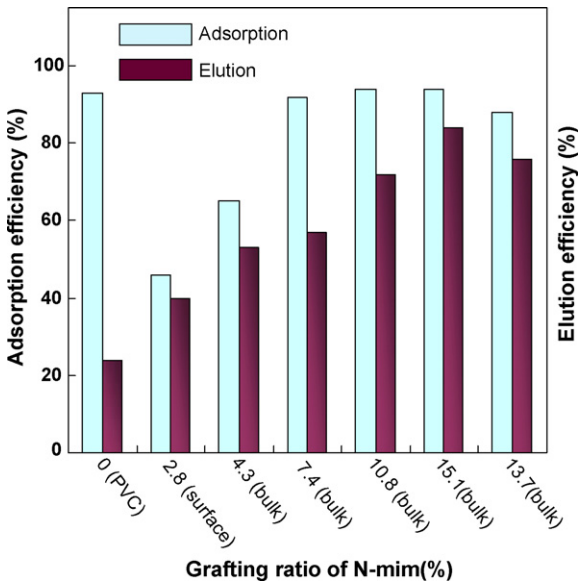


Fig. 6. The effect of grafting ratio on the adsorption and elution of Hb. 30 μg mL^{-1} Hb in 3 mL Tris–HCl buffer (pH 7.0, 0.1 M) was adsorbed by 20 mg of NmimCl–PVC hybrid with various grafting ratios, and then eluted with 500 μL SDS solution (0.5%, m/v).

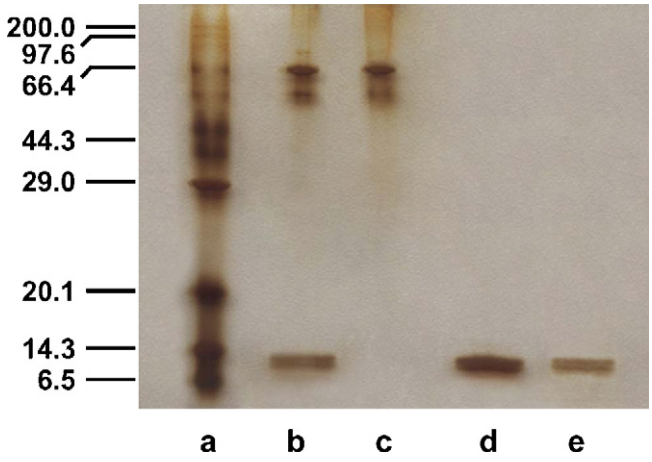


Fig. 7. The SDS-PAGE of proteins. The samples were subjected to electrophoresis on a 15% acrylamide gel at 180 V, followed by silver staining. (a) The molecular weight standards (M_r in kDa); (b) 3000-fold diluted human whole blood; (c) 3000-fold diluted human whole blood after pretreated by the NmimCl–PVC hybrid; (d) hemoglobin isolated from human whole blood with the present procedure; (e) a pure hemoglobin solution of 20 μg mL^{-1}.

Fig. 7. It can be seen that by treating with the present procedure, hemoglobin was effectively isolated from the coexisting protein species in a complex biological sample matrix, e.g., the 66.4 kDa albumin was effectively eliminated. At the same time certain extent of preconcentration of hemoglobin was achieved. This observation well illustrated the practical applicability of the present system for the effective isolation of hemoglobin from biological samples with complex matrix components.

4. Conclusions

The immobilization of N-methylimidazole onto the bulk chain of the polyvinyl chloride substantially suppressed the non-specific adsorption of proteins and meanwhile excellent selectivity for basic protein adsorption is achieved. In addition, the biocompatibility of the grafted hybrid is also improved to a large extent as compared to its PVC precursor, characterized by the maintenance of the protein activity after adsorption/elution process. These observations provide a promising potential for the selective isolation of basic proteins from complex biological samples matrixes. Furthermore, the one-step modification procedure offers a useful alternative for the immobilization of ionic liquid-based entities onto polymeric substrates for the improvement of the overall performance of the materials, i.e., selectivity and biocompatibility.

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