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Separation of chitin from shrimp shells enabled by transition metal salt aqueous solution and ionic liquid

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ABSTRACT

Chitin is a widely used important industrial polymer mainly from shrimp shells, but its commercial preparation is under the great challenge of serious pollution due to the requirement of HCl and NaOH. Herein, we demonstrated that high purity chitin can be obtained from waste shrimp shells (WSSs) by cascade separation with transition metal salt aqueous solution and ionic liquid (IL). Firstly, calcium carbonate of WSSs was effectively removed in the metal salt aqueous solution driven by the ion exchange interaction. Subsequently, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) had bifunctional abilities to remove residual protein and introduced metal salts simultaneously by hydrogen bonding and coordination interactions. The key experimental factors affecting the separation process were systematically studied, including the type of metal salts, temperature, and [Bmim]Cl loading. After sequential treatment with a 20% (mass) $NiSO_4$ aqueous solution at 130 °C and [Bmim]Cl at 150 °C, the purity of α-chitin can be up to 96.5% (mass) that meets commercial requirements. The use of metal salts with higher coordination ability makes the preparation of chitin no longer depend on the commonly acid-base reaction, which is conducive to the preservation of chitin structure.

1. Introduction

Chitin, poly(β–(1 → 4)-2-acetamido-2-deoxy-D-glucan, is the second largest biopolymer in nature. Due to its properties of non-toxicity, biodegradability, biocompatibility, and chemical stability [1], chitin has been widely used in numerous fields such as the textile industry [2], medicine [3], cosmetic, [4] *etc.* Commercially available chitin is produced mainly from shrimp and crab shells, and these sources contain protein and calcium carbonate except for chitin. Currently, chemical separation, enzymatic treatment [5], and microbial fermentation [6] have been developed to prepare chitin. However, the industrially chemical method usually needs a large amount of HCl and NaOH to remove protein and calcium carbonate, leading to vast water consumption and severe pollution. Although the enzymatic and fermentation methods can solve the pollution problem, the lower removal rate of protein and calcium carbonate and longer treatment time limit their large-scale applications [7].

Recently, extraction of chitin from shrimp or crab shells using ionic liquid (IL) has attracted increasing interest, because IL is the new generation of green solvent with a special hydrogen bond network and unique properties. By designing different functional ILs, chitin can be prepared from shrimp shells using the extraction or pulping method. When shrimp shells were treated with [Emim]OAc, the chitin was dissolved in the used IL and obtained by adding anti-solvent [8]. However, when the cation of the [Emim]OAc was changed to $[NH_3OH]^+$, the $[NH_3OH]$OAc has the ability to remove protein and calcium carbonate other than dissolving chitin. Thus, chitin was obtained in the residue other than regeneration [9]. Therefore, it is reasonable to adjust the interaction between IL and shrimp shells by changing the structure of the ILs. The designable ILs provide alternation for chitin preparation from shrimp shells.

Different from traditional acid removal of calcium carbonate, Feng *et al.* [10] reported an ion-exchange method for removing cal-

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cium carbonate in urea/$Zn(CH_3COO)_2{\cdot}2H_2O$ aqueous solution. Despite the advantages of little damage to the chain of chitin in this method, the final product was chitin/Zn complex instead of chitin due to the strong coordination ability of the introduced metal ions. However, these metal ions are likely to de-coordinate by forming new complexes with another ligand. As previously reported, the $[Bmim]_2[NiCl_4]$ can be formed by mixing [Bmim]Cl and $NiCl_2$ [11], indicating that IL is a potential ligand to remove the introduced metal salt through multiple interactions. Hence, it is reasonable to obtain chitin by the integration of using metal salts aqueous solution and IL sequential treatment. Compared with the traditional method and single IL process, this protocol will provide an opportunity for chitin separation without acid-base reaction and regeneration process. This is not only environmentally friendly but also conducive to keep the original structure of the chitin.

Herein, high purity chitin was prepared in two steps by metal salts aqueous solution and IL treatment. Firstly, the metal salts aqueous solution was used to remove calcium carbonate by ion exchange due to the difference of coordination ability. The 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) was subsequently used to dissolve the residual protein and heterometallic salt by the synergetic effect of hydrogen bonds and coordination interactions. The decalcification abilities of different metal salt aqueous solutions were studied systematically, and the $NiSO_4$ aqueous solution was optimized as the best media. Important experiment conditions including the concentration of $NiSO_4$ aqueous solution and temperature were studied to obtain the intermediate product with the lowest calcium content. Furthermore, the key parameters that affect the efficiencies of deproteinization and residual metal removal were also investigated in the [Bmim]Cl treatment step, including the temperature and mass ratio of [Bmim]Cl and shrimp shells. Characterizations of Fourier transform-infrared (FT-IR), powder X-ray diffraction spectra (XRD), and solid ^{13}C nuclear magnetic resonance (NMR) were conducted to determine the structure of chitin and evaluate the damage of this method to the chain of the chitin. Based on the above results, a mechanism on the high purity chitin preparation was preliminary proposed by the ^1H NMR and FT-IR spectra.

2. Materials and Methods

2.1. Materials

The metal salts including $Zn(NO_3)_2{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $MnCl_2{\cdot}4H_2O$, $NiSO_4{\cdot}6H_2O$, $Ni(NO_3)_2$, $NiCl_2$, LiCl and dimethylacetamide (DMAc) were obtained from Sinopharm Chemical Reagent Co. without further treatment. The 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) was from Linzhou Keneng Technology Co., which was washed with ethyl acetate and dried for 72 h in the oven before use. The red shrimp (*Solenocera crassicornis*) was purchased from Wal-Mart Supermarket, which was peeled to produce shrimp shells. The obtained shrimp shells were washed, dried, ground, and sieved to obtain shrimp shells powder, of which particle size was below 0.125 mm. The used shrimp shells powder consisted of 57.2% (mass) calcium carbonate, 12.3% (mass) protein, and 30.5% (mass) chitin.

2.2. Preparation of chitin process

The chitin was prepared from shrimp shells using metal salts aqueous solution and [Bmim]Cl in two steps. The typical process is as follows. In the first step, shrimp shells powder (0.25 g) was added to the metal salts aqueous solution (5 g, 20% (mass)), and the mixture was stirred and reacted at a fixed temperature for 24 h. After the reaction, the mixture was centrifuged to separate liquid and solid. The solid was decalcified shrimp shells powder, which was lyophilized for 24 h. During the step, different inorganic salts were explored, which included $Zn(NO_3)_2{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $MnCl_2{\cdot}4H_2O$, $NiSO_4{\cdot}6H_2O$, $Ni(NO_3)_2$, and $NiCl_2$. Additionally, the experiment temperatures were also studied varying from 60 °C to 130 °C. In the second step, the decalcified shrimp shells powder was mixed with [Bmim]Cl with different mass ratios (1:10–1:80, shrimp shells powder: [Bmim]Cl) and reacted at temperature changed from 90 °C to 150 °C for 24 h. After the reaction, the mixture was added 5 ml dimethyl sulfoxide (DMSO) and separated by centrifugation. The DMSO was used to reduce the viscosity of the mixture. The obtained solid was washed with deionized water (DI water) five times and dried in a freeze dryer. The upper liquid was added DI water as an anti-solvent to produce the regenerated product. The regenerated product was also washed with DI water five times and lyophilized. All the experiments were carried out three times. The chitin was in the solid, and the regenerated product was collected to explore the dissolution behavior of shrimp shells in the [Bmim]Cl.

2.3. Content analysis of the sample

The calcium carbonate and protein content of the obtained product was analyzed by the ICPE-9000 and Kieldahl method as reported before [12]. The $CaCO_3$ content of the sample was calculated by the following formula:

$$CaCO_3\ \ \text{content} = \frac{C_{Ca} \times V \times \text{Dilution}}{\text{Conversion factor} \times \text{ODW}_{\text{sample}}} \times 100\% \quad (1)$$

where C_{Ca} is concentration of calcium ion of sample HCl solution determined by ICPE-9000, $mg{\cdot}L^{-1}$; V is volume of tested solution, L; Dilution is 10; ODW_{sample} is the dry mass of the sample, mg; Conversion factor is the mass fraction of calcium in $CaCO_3$, 0.4.

The Ni content of the sample was calculated by the following formula:

$$\text{Ni content} = \frac{C_{Ni} \times V \times \text{Dilution}}{\text{ODW}_{\text{sample}}} \times 100\% \quad (2)$$

where C_{Ni} is concentration of nickel ion of sample HCl solution determined by ICPE-9000, $mg{\cdot}L^{-1}$; V is volume of tested solution, L; Dilution is 10; ODW_{sample} is the dry mass of the sample, mg.

The protein content of the sample was calculated by the following formula, and the sample was pretreated using NaOH aqueous solution (5% (mass)) before measurement as reported before [12]:

$$\text{Protein content} = \frac{1.401 \times C \times (V_{\text{titration}} - V_{\text{blank}})}{\text{ODW}_{\text{sample}}} \times 6.25 \quad (3)$$

where C is the concentration of titrated acid, 0.02 $mol{\cdot}L^{-1}$; $V_{titration}$ is titration volume of sample, ml; V_{blank} is titration volume of blank sample, ml; ODW_{sample} is the dry mass of the sample, g.

In addition, the ash content of the chitin with the lowest nickel, calcium carbonate, and protein content was measured using the weighing method [13]. The obtaiend chitin was placed in the crucible and heated by a muffle furnace at 600 °C for 4 h. After heating, the residue was cooled down to room temperature and weighed, which was the mass of the ash.

Based on the above resutls, the Purity (% (mass)) and Yield (% (mass)) of the chitin was calculated as follows:

$$\text{Purity} = 100\% - \text{Protein content} - \text{Ash content} \quad (4)$$

$$\text{Yield} = \frac{m_R \times \text{Purity}}{m_s \times \text{Chitin content}} \times 100\% \quad (5)$$

where, m_R is the mass of the residue, g; m_s is the mass of the shrimp shells, g; chitin content of the shirmp shells, % (mass).

2.4. Characterization of chitin

The obtained highest purity chitin was characterized by several methods to determine its structure. FT-IR (Nicolet 380 Spectrometer, Thermo Fisher Scientific, America) was used to characterize the functional groups with KBr as the blank in the range 4000–400 cm^{-1}. Solid ^{13}C NMR (Avance III HD 125 MHz, Bruker, Switzerland) was carried out to determine the carbon skeleton. XRD (SmartLab X-ray Diffractometer, Rigaku Corporation, Japan) were conducted in the range 5°–90° with a scanning rate of 10 (°)/min to determine the crystallinity. The X-ray photoelectron spectroscopy (XPS spectra) were recorded by X-ray photoelectron spectroscopy (Thermo Fisher 250xi, USA), which were divided peaks using Avantage software. Elemental analysis was carried out on a Vario EL Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Germany).

The viscosity-average molecular weight of the obtained chitin was measured by the viscosity of the chitin in a LiCl/DMAc solvent according to the previous method [14]. The commercial and obtained chitin were dissolved in a LiCl/DMAc solvent (5% (mass)) for 5 days at room temperature to prepare the solution with a concentration between 0.01 to 0.05 $g{\cdot}dl^{-1}$, respectively. The viscosity of the obtained solution was analyzed by a Lovis 2000 M/ME Rolling-ball viscometer (Anton Paar GmbH, Germany). The viscosity-average molecular weight (M_w) was calculated as follows.

$$\eta = 7.6 \times 10^{-5} M_w^{0.95} \quad (6)$$

where, η was the intrinsic viscosity of the obtained solution.

3. Results and Discussion

Different from the traditional method of chitin extraction, acid and base were completely saved in the current protocol for the decalcification and deproteinization of shrimp shells. As an alternative way, non-volatile metal salt aqueous solution and [Bmim]Cl were employed, respectively. Based on the ion exchange strategy, various metal salts were used to figure out the effect of metal ions and acid radicals on decalcification. Moreover, the influence of the experiment temperature on calcium removal was also studied because of its favorable effect. Then, the [Bmim]Cl was selected to remove protein and induced metal due to its excellent dissolution ability. The experiment temperature and mass ratio were explored to prepare the higher purity chitin.

3.1. Removal calcium carbonate of shrimp shells with metal salts aqueous solution

According to the ion exchange rule, the copper, manganese, zinc and, nickel salts were employed due to their higher coordination ability compared with that of calcium carbonate. Shrimp shells was treated with different metal aqueous solutions (20% (mass)) at room temperature for 24 h, respectively, which included $Cu(NO_3)_2{\cdot}3H_2O$, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $NiSO_4{\cdot}6H_2O$, $NiNO_3$, and $NiCl_2$. After the reaction, the precipitates were collected to determine the calcium carbonate and protein content. As shown in Table 1, the calcium carbonate contents of the obtained precipitates are all lower than that of shrimp shells, but the used metal salt aqueous solutions performed different decalcification abilities. When the acid radical was fixed as NO_3^- or Cl^-, the Ni^{2+} exhibited higher decalcification ability than Cu^{2+}, Mn^{2+}, and Zn^{2+}. The metal salt aqueous solution had a very close ability to remove calcium with the same metal ion. This indicated that the metal ion type is the key factor in calcium carbonate removal.

Table 1
The content of decalcified shrimp shells with different metal salt aqueous solution treatment

Type of used metal salt	$CaCO_3$ content/% (mass)	Protein content/% (mass)
$Cu(NO_3)_2{\cdot}3H_2O$	24.0 ± 1.3	14.2 ± 1.2
$MnCl_2{\cdot}4H_2O$	41.3 ± 1.4	13.8 ± 1.5
$ZnSO_4{\cdot}7H_2O$	47.6 ± 1.0	14.3 ± 1.4
$Zn(NO_3)_2{\cdot}6H_2O$	45.8 ± 1.2	15.3 ± 1.1
$NiSO_4{\cdot}6H_2O$	10.1 ± 0.8	15.1 ± 0.8
$Ni(NO_3)_2$	11.7 ± 0.5	14.2 ± 1.2
$NiCl_2$	12.2 ± 1.4	15.0 ± 1.1

Although the used metal salts aqueous solutions were weakly acidic with the pH values below 7 (as shown in Table S1, Supplementary Material), the order of acidity was different from the calcium removal ability. It indicates that the acidity of the metal salt aqueous solution is not the main reason for the calcium carbonate removal. The $NiSO_4$ aqueous solution has the highest ability of decalcification among the used metal aqueous solution. The colors of the precipitates obtained from copper, manganese, and nickel salts aqueous solutions were blue, pink, and green, respectively. This indicated that the corresponding metal ion was exchanged to the residual shrimp shells, which was in accordance with the previous studies [10,12]. However, the protein content of these precipitates was all about 14% (mass).

As studied before [10,12], the temperature is the most important factor affecting the decalcification ability of the metal salts aqueous solution. Thereafter, the $NiSO_4$ aqueous solution was selected as the optimal solvent to explore the temperature factor. The $NiSO_4$ aqueous solution (20% (mass), 5.0 g) was mixed with shrimp shells powder (0.25 g), which was reacted at 20 °C (room temperature), 60 °C, 90 °C, 110 °C, and 130 °C for 24 h, respectively. As shown in Fig. 1, the calcium carbonate of the obtained precipitate decreased from 10.1% (mass) to 1.4% (mass) with the temperature rising. At the same time, the nickel content of the obtained precipitate increased from 10.3% (mass) to 45.4% (mass) correspondingly. These results further proved that the calcium carbonate was replaced with nickel, then it was removed. However, the rate of the nickel content increase is higher than that of the calcium content decrease. This may be because of the coordinate ability of the chitin, which also can absorb the nickel ion. Previous work showed that the chitin nanofilm exhibited high adsorption

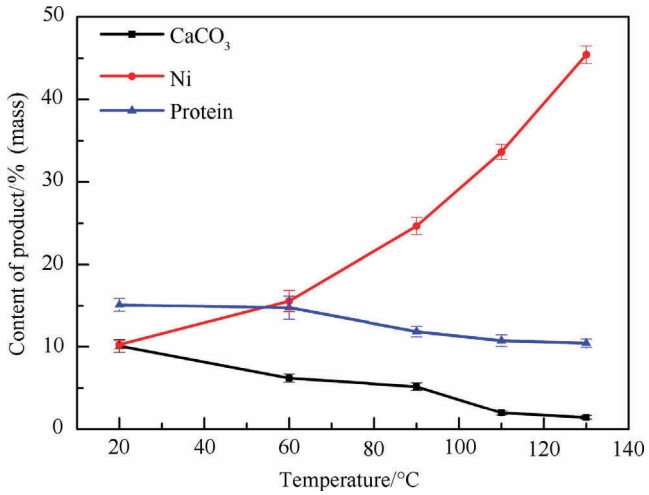

Fig. 1. The calcium carbonate, protein, and nickel element content of precipitates obtained from $NiSO_4$ aqueous solution treatment at different temperatures. Other conditions: experimental time: 24 h; a mass ratio of shrimp shells to $NiSO_4$ aqueous solution = 1:20.

capacity for the Ni^{2+} [15], confirming the above speculation. Furthermore, the protein content of the precipitate was slightly reduced because of protein dissolving in the water at a higher temperature. Yan *et al.* [16] have found that the protein of the shrimp shells can be completely hydrolyzed and removed with water treatment above 180 °C. This proved the hot water can remove the protein, which is consistent with the above results.

3.2. Removal of nickel salt and protein with [Bmim]Cl

As originally assumed, the calcium carbonate in shrimp shells was removed by the $NiSO_4$ aqueous solution treatment. The previous study has shown that the normal IL [Bmim]Cl is an efficient solvent for dissolving various macromolecules such as protein [17,18], cellulose [19,20], and chitin [21]. Moreover, the ILs are also alternative solvents to extract metal ions due to their special hydrogen bonding and electrostatic interaction [22,23]. Therefore, [Bmim]Cl was selected as a solvent to treat the obtained decalcified shrimp shells to remove the remaining protein and introduced nickel salts. The temperature and mass ratio of shrimp shells to [Bmim]Cl were explored, because these factors have a greater impact on the deproteinization process [12].

The [Bmim]Cl (5.0 g) and decalcified shrimp shells powder (0.25 g) were mixed and reacted for 24 h at different temperatures including 90 °C, 110 °C, 130 °C, and 150 °C. Then, the mixture was added DMSO and separated to collect the precipitate and supernatant. After adding water or ethanol to the supernatant, a light green floccule was formed, which was named the regenerated product. During the reaction process, the [Bmim]Cl gradually turned green, which initially proved that [Bmim]Cl can remove nickel salts. To further determine the role of [Bmim]Cl in the process, the precipitates and regenerated products were analyzed with FT-IR and ICPE.

As literature reported, [Bmim]Cl can dissolve not only neat chitin with a solubility of 10% (mass) [24] or 24.4% (mass) [8], but also keratin protein with a solubility of about 50% (mass) [18]. There will be a competition between protein and chitin dissolution, when [Bmim]Cl dissolves shrimp shells. Thus, the main content of the precipitate and regenerated product was determined by FT-IR firstly to figure out the dissolution behavior. As shown in Fig. 2(a), the spectra of precipitates were similar to that of the chitin. With temperature increase, the characteristic peaks of chitin at 3268 cm^{-1}, 3106 $cm^{-1}$and 1660 cm^{-1} belong to —OH and amide I bond gradually became apparent, and the peak at 663 cm^{-1} may be attributed to the nickel salts gradually disappears. The FT-IR results preliminarily indicated that the [Bmim]Cl can remove protein and nickel.

On the other side, the FT-IR spectra of the regenerated products from water and ethanol involved the protein characteristic peaks of —OH, amide bond, which was similar to the bovine serum albumin (Fig. 2(b)). Additionally, the appearance of regenerated products was green, which was reasonable to speculate that the regenerated products also contain nickel salts. This indicated that part of nickel salts can be removed from the [Bmim]Cl by adding anti-solvent. Above all, the FT-IR spectra showed that the main content of precipitates and regenerated products were chitin and protein, respectively, proving that [Bmim]Cl preferentially dissolved protein and nickel salt compared with chitin. Because the chitin was mainly in precipitates, the subsequent content analysis was focused on them.

Content analysis of the precipitates showed that the protein and nickel content of the precipitates decreased from 3.7% (mass) to 1.3% (mass) and 28.5% (mass) to 6.8% (mass), respectively (Fig. 3(a)) with temperature raise. This result was consistent with the above FT-IR spectra (Fig. 2), proving that the higher temperature was a benefit for protein and nickel removal. The change in calcium

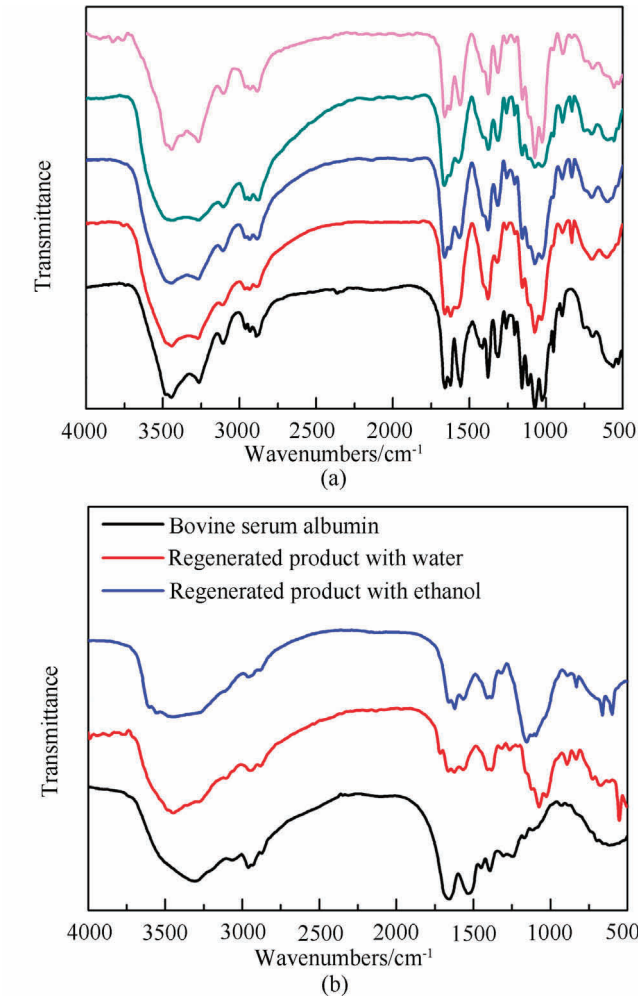

Fig. 2. The FT-IR spectra of (a) chitin (black line) and the precipitates obtained from [Bmim]Cl treatment at 90 °C (red line), 110 °C (blue line), 130 °C (green line), and 150 °C (pink line), and (b) Bovine serum albumin (black line), the regenerated product with water (red line) and regenerated product with ethanol (blue line) under the temperature of 130 °C. Other conditions: experimental time: 24 h; a mass ratio of shrimp shells to [Bmim]Cl = 1:20.

carbonate content was small, because there were no hydrogen bond sites in the calcium carbonate. These results also confirm our above assumptions that after replacing calcium ions with metal ions with stronger coordination ability, it is easier to the subsequent removal with [Bmim]Cl. As above results, the experiment temperature was the key factor for [Bmim]Cl to remove protein and nickel salts.

The above experiments exhibited that [Bmim]Cl can effectively remove protein and nickel salt, but the purity of the obtained precipitate has not yet met the practical requirement. The simple method to improve purity is to improve the amount of [Bmim]Cl with more hydrogen bond sites. As shown in Fig. 3(b), the content of the precipitate showed that the method was effective. With adding more [Bmim]Cl, the content of protein, nickel, and calcium carbonate all decreased significantly. Although the calcium carbonate is difficult to form a hydrogen bond with [Bmim]Cl, it can be peeling of with protein removal as calcium carbonate is attached to the protein surface [25]. When the mass ratio of decalcified shrimp shells and [Bmim]Cl was up to 1:80, the precipitate containing 0.3% (mass) calcium carbonate, 0.6% (mass) protein, and 0.3% (mass) nickel. The ash content of the obtained chitin was 2.9% (mass). Thus, the purity of the obtained chitin is 96.5% (mass)

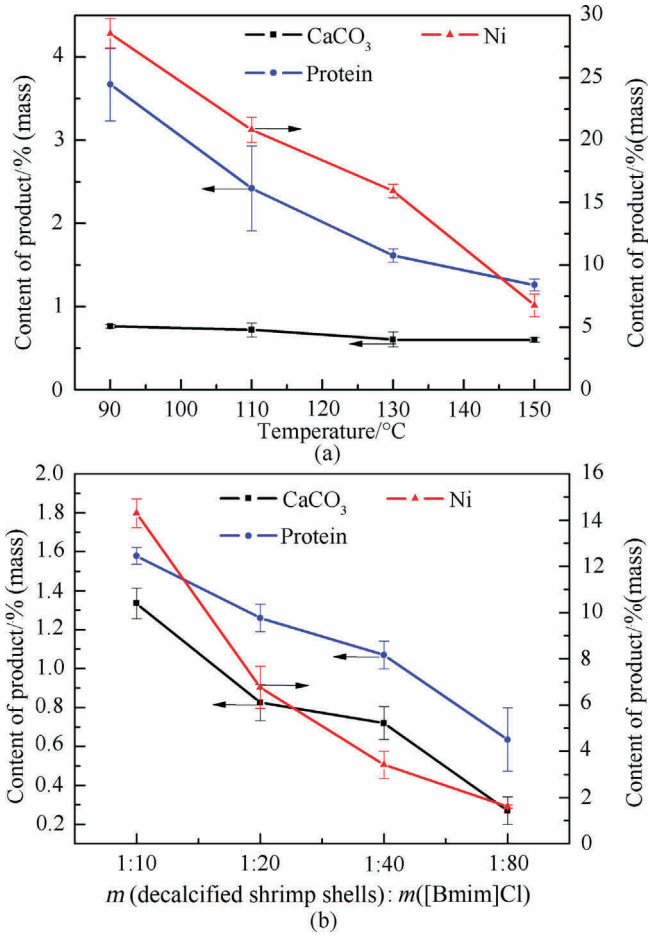

Fig. 3. The calcium carbonate, protein, and nickel element content of precipitates obtained from (a) [Bmim]Cl treatment at 90 °C, 110 °C, 130 °C and 150 °C for 24 h with a mass ratio of 1:20 (decalcified shrimp shells: [Bmim]Cl), and (b) obtained from [Bmim]Cl treatment at 150 °C for 24 h with a mass ratio of 1:10, 1:20, 1:40 and 1:80 (decalcified shrimp shells: [Bmim]Cl).

with a yield of 72.5% (mass), which was comparable to the commercial chitin with 97.4% (mass) purity containing 2.4% (mass) protein and 0.3% (mass) ash content. In addition, the viscosity-average molecular weight of commercial and obtained chitin were 120998 g·mol^{-1} and 125010 g·mol^{-1}, respectively.

3.3. Characterization of obtained chitin

FT-IR, XRD, and solid ^{13}C NMR were carried out to characterize the highest purity chitin to explore the effect of the process on the structure of chitin, as literature has pointed out that the treatment process can change the structure of the chitin [26].

As shown in (Fig. 4(a)), the FT-IR spectrum of the decalcified shrimp shells was close to the chitin. The peaks at 871 cm^{-1} and 1420 cm^{-1} ascribed to calcium carbonate have not been found, showing that it has been removed. However, the peaks at 3268 cm^{-1} and 3106 cm^{-1} were not clear, which may be owing to the interaction between Ni^{2+} and —NH, —NHC═O— of the chitin. The characteristic peak of the amide bond at 1660 cm^{-1} was also not obvious due to the residual protein. In addition, new peaks at 663 cm^{-1} and 596 cm^{-1} were observed, which may belong to the nickel salts. After [Bmim]Cl retreatment, the FT-IR spectra of the obtained chitin were the same as the chitin, proving its high purity. Furthermore, it contained the peaks at 1660 cm^{-1} and 1621 cm^{-1} attributed to the amide I bond of α-form chitin, exhibiting that the crystal configuration was not changed.

Additionally, the XRD patterns (Fig. 4(b)) exhibit that the decalcified shrimp shells have additional peaks at 14.8°, 25.7°, 29.7°, and 31.9° compared with chitin, which was ascribed to the $NiSO_4·6H_2O$ (International Centre for Diffraction Data (ICDD) card No. 33-0955), $NiSO_4·H_2O$ (ICCD card No. 21-9974) and $NiSO_4·7H_2O$ (ICDD card No. 01-0403), and $NiCO_3·Ni(OH)_2$ (ICCD card No. 35-0501) respectively. Although the XRD results indicated that the main introduced nickel salt in shrimp shells was $NiSO_4$, high-resolution XPS was further employed to study element state and the interactions between nickel ion and chitin. As shown in Fig. 5(a), the C 1s spectrum involves four peaks at 284.8 eV, 286.4 eV, 288.0 eV, and 291.1 eV. The first three peaks were ascribed to C8—H/C—C, C5—C6/C2—N/C3—OH/C6—OH/C—O—C4, and C7═O/O—C1—O in chitin, respectively. The last peak was assigned to the CO_3^{2-}, which was due to the residual $CaCO_3$ in the shrimp shells [10,12]. In the O 1s spectrum (Fig. 5b), peaks at 531.1 eV and 532.5 eV were respectively attributed to the C—O—C/—OH and N—C═O in the chitin [27]. The additional peak at 534.1 eV was from SO_4^{2-} [28]. The N 1s spectrum showed that there were located in 399.3 eV and 402.0 eV (Fig. 5c), which belonged to —C2—NH_2/—C2—NH—C7═O in the chitin, and R—NH···Ni^{2+} complex. A pair of electrons in the N atom was shifted to the bond between —NH and Ni^{2+}, leading to the increase of N energy [10].

The high-resolution XPS (Fig. 5(d)) of Ni atom contained two spin–orbital components of 2p 1/2 and 2p 2/3. In detail, the peaks at 878.9 eV and the 860.4 eV were the satellite peaks. The peaks at 873.0 eV and 855.7 eV were the characteristic peaks of the Ni^{2+} [29]. Combined with the O 1s high XPS spectrum, the peak at 855.4 eV was due to the formation of the R-NH···Ni^{2+}. The XRD and XPS results indicated that the introduced nickel salts were absorbed and formed interaction with chitin, simultaneously.

After [Bmim]Cl treatment, the obtained chitin included characteristic peaks of chitin at 9.4° and 19.2° ascribed to (020) and (040)/(110) (Fig. 4(b)). Additionally, the peak at 12.6° assigned to the amorphous region decreased significantly. According to the CI=$(I_{110}-I_{am})/I_{110} \times 100\%$), the CI of the obtained chitin was 95%, which was higher than that of Sigma chitin with 92%. The results showed that the metal salt-IL method had less effect on the crystalline region of the chitin.

Moreover, the solid ^{13}C NMR spectrum (Fig. 4(c)) included all the characteristic peaks of the chitin, showing that the main carbon skeleton was not destroyed during the process. Specifically, the double peaks assigned to the C3 and C5 also indicated that the product was the α-chitin. According to the above results, after the metal salt and ionic liquid treatment, the major functional group, crystal form, and carbon skeleton of chitin have not changed significantly compared with the raw form in shrimp shells.

3.4. The mechanism of chitin preparation

According to a previous study, the mechanism of metal salt aqueous solution remove calcium carbonate in shrimp shells was due to the ion exchange between the metal ion and calcium ion [10]. In this study, since the used nickel salt also has higher coordination ability compared with the calcium carbonate, it is still reasonable to use the ion exchange mechanism to explain the process of calcium removal. The previous study about chitin preparation using urea/$Zn(OAc)_2$ aqueous solution has indicated that calcium ions were exchanged with zinc ions to produce $ZnCO_3$. Then, $ZnCO_3$ was hydrolyzed to basic zinc carbonate [10]. In addition, as shown in Fig. 4(b), XRD patterns of the decalcified shrimp shells include peaks at 34.8°, which was ascribed to $NiCO_3·Ni(OH)_2$. According to the literature and the XRD results, the calcium ion was exchanged with nickel ion and $NiCO_3$ was formed. $NiCO_3$ was further hydrolyzed to basic nickel carbonate.

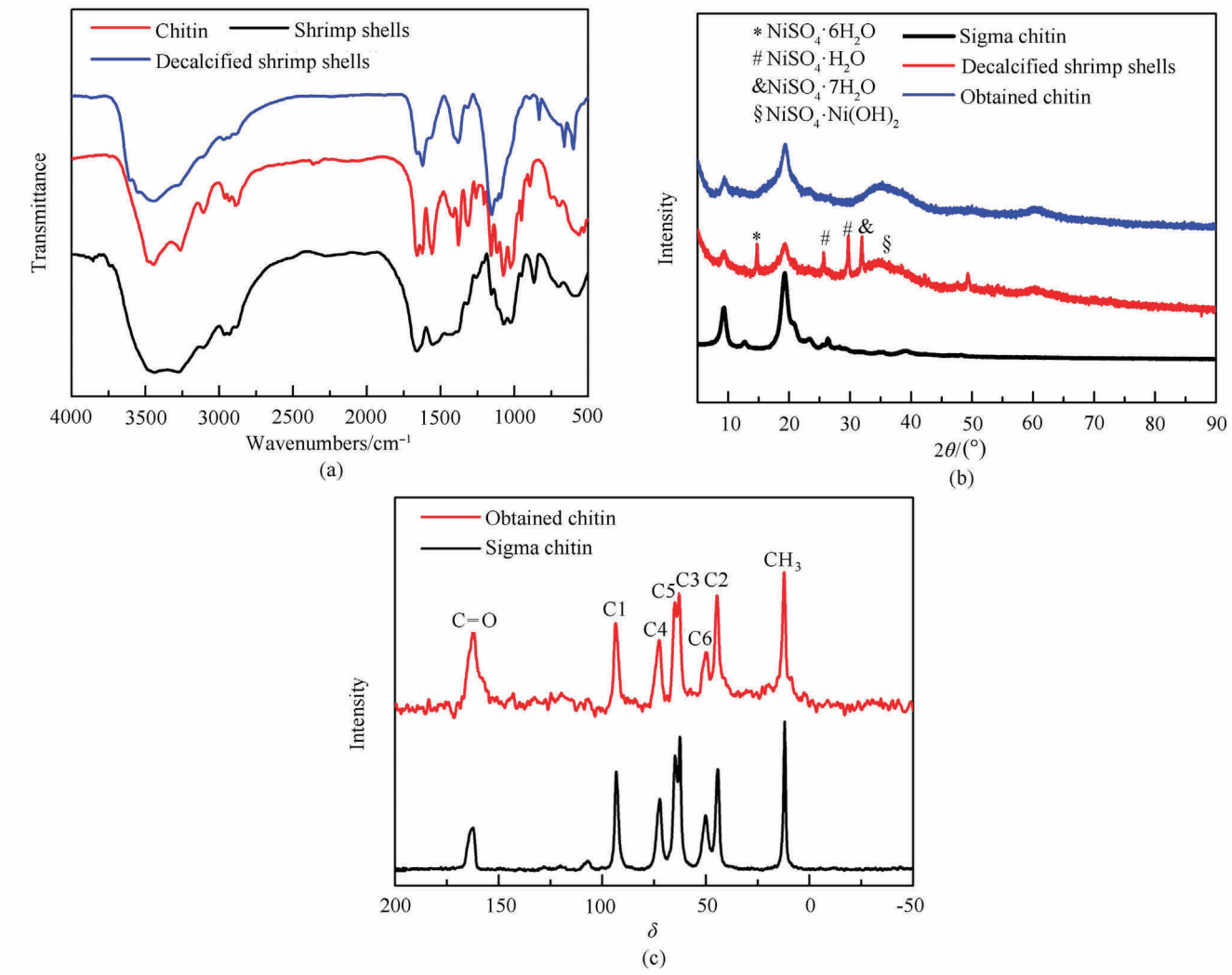

Fig. 4. (a) FT-IR spectra and (b) XRD patterns of shrimp shells, decalcified shrimp shells, and obtained chitin. (c) Solid ^{13}C NMR spectra of Sigma chitin and obtained chitin. The conditions of the decalcification process: 130 °C, 24 h, *m* (shrimp shells): *m* ($NiSO_4$ aqueous solution) = 1:20 The conditions of the deproteinization process: 150 °C, 24 h, *m* (shrimp shells): *m* ($NiSO_4$ aqueous solution) = 1:80.

However, the structure of shrimp shells is complex, in which chitin fibers are wrapped by protein to form glycoprotein fibers, which are embedded in the calcium carbonate matrix [30]. This complicated structure hinders the study of the mechanism of chitin preparation. In addition, the interactions between ILs and polymers are also complicated because of the numerous hydrogen bonding sites. So, it needs much work to figure out the mechanism of protein and nickel removal in this process. Hence, the preliminary work was carried out by NMR and FT-IR to explore the interactions between decalcified shrimp shells and [Bmim]Cl. Further work about deproteinization and nickel removal is still under study.

Firstly, the raw [Bmim]Cl and reacted [Bmim]Cl were analyzed with ^1H NMR to explore the changes in the density of electron clouds around hydrogen of the [Bmim]Cl. After decalcified shrimp shells and [Bmim]Cl reaction, the supernatant 1 was collected and characterized with ^1H NMR, which was the blue line in Fig. 6(a). The supernatant 1 was added to anti-solvent to precipitate the protein, and the obtained supernatant 2 was also characterized using ^1H NMR as shown in Fig. 6(a) (red line). According to the above results, supernatant 1 and 2 were composed of [Bmim]Cl, nickel salt and protein, and [Bmim]Cl and nickel salt, respectively. In the supernatant 1, compared with the neat [Bmim]Cl, the chemical shift of H1, H2, and H3 from the imidazolium ring notably moved towards the higher field by 0.08, 0.05, and 0.04, respectively. Interestingly, in the supernatant 2, the signals of H1, H2, and H3 atoms changed to the lower field by 0.08, 0.07, and 0.06 in chemical shift. Moreover, a small shoulder signal after the H3 signal was observed in the H2 of supernatant 1 and 2. These results showed that the interactions between [Bmim]Cl and nickel salts, protein were mainly from the imidazolium ring, because the N atom with lone pair electrons is recognized to form hydrogen bonding and coordinate bond easily. In the [Bmim]Cl/nickel salts solvent, the Ni^{2+} can form interaction with N, leading to electronic cloud density of H decrease. Additionally, as Wang *et al.* [11] reported, the [Bmim]Cl can be reacted with $NiCl_2$ to form $[Bmim]_2[NiCl_4]$, proving that coordination interaction can be formed between [Bmim]Cl and nickel salts. To study the formation of new IL in this process, the element of [Bmim]Cl/nickel salts were measured. As shown in Table 2, the contents of carbon, hydrogen and nitrogen of [Bmim]Cl/nickel salts were between [Bmim]Cl and $[Bmim]_2[NiCl_4]$. In addition, the sulfur content of [Bmim]Cl/nickel was zero. These results indicated that [Bmim]Cl reacted with the nickel salts in the decalcified shrimp shells to form new IL. However, the

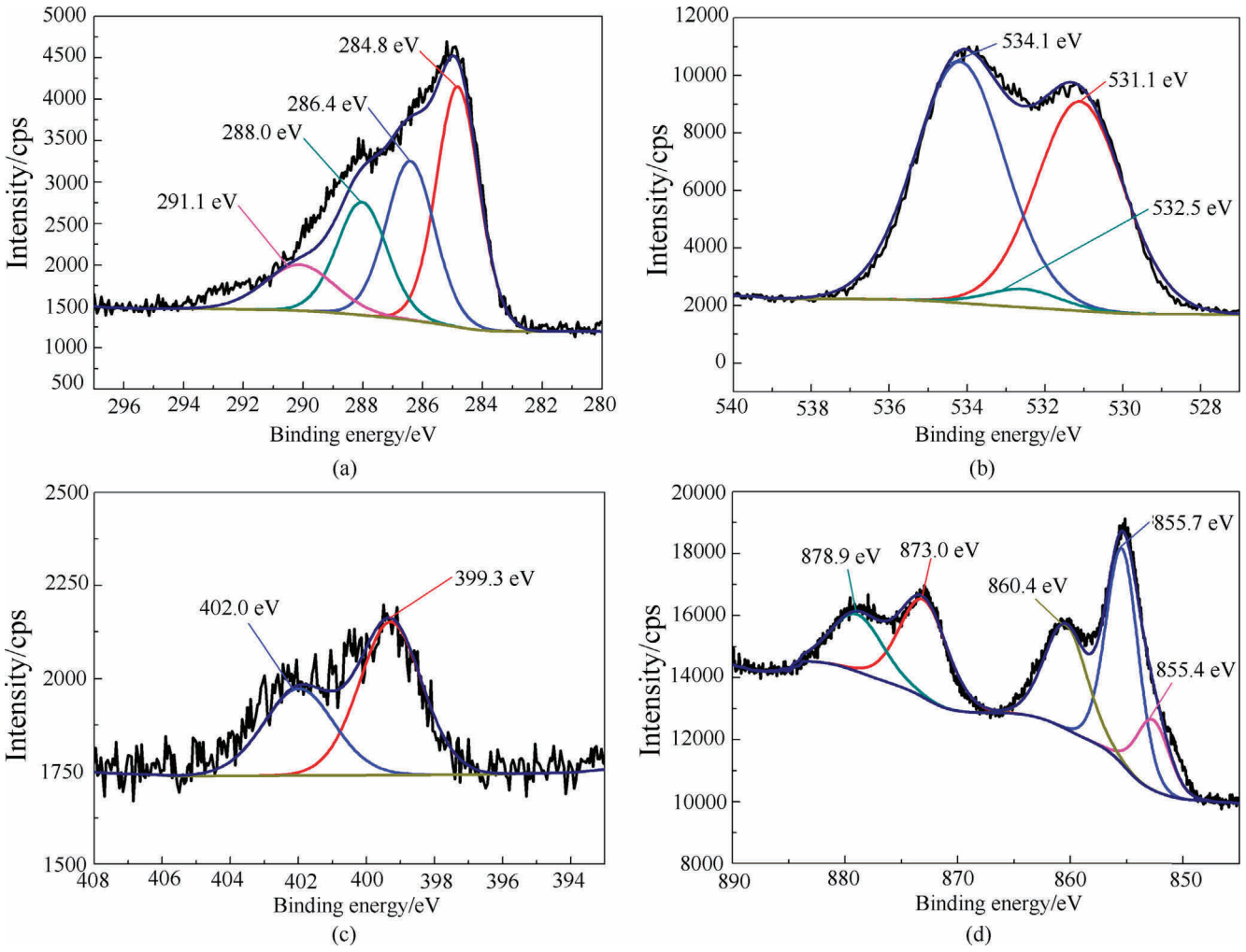

Fig. 5. High-resolution XPS spectra of (a) C 1s, (b) O 1s, (c) N1s, and (d) Ni of decalcified shrimp shells. Experimental condtions: 130 °C, 24 h, 1:20 (mass ratio, shrimp shells: $NiSO_4$ aqueous solution).

obtained ionic liquids were a mixture of [Bmim]Cl, $[Bmim]_2[NiCl_4]$, and other [Bmim]Cl/nickel salts, because the molar ratio between [Bmim]Cl and Ni^{2+} was different from 1:1 in preparation of $[Bmim]_2[NiCl_4]$. Hence, according to the above ^1H NMR and elements content results, the nickel salts removal by [Bmim]Cl was due to coordination interaction in two forms. On the one hand, nickel salts in the decalcified shrimp shells were formed in coordination interaction with imidazolium ring of [Bmim]Cl. On the other hand, the nickel salts can react with [Bmim]Cl to form the new metal IL acting as part of the complex anion. However, the [Bmim]Cl, protein and nickel salt interact with each other, which was more complicated. It is difficult to figure out the specific interaction sites by ^1H NMR, which requires further research using additional experiments and simulation calculations.

Furthermore, the FT-IR was used to study the change of [Bmim]Cl functional group during the process as shown in Fig. 6(b). However, the FT-IR spectra of the treated [Bmim]Cl were almost the same as that of neat [Bmim]Cl, suggesting that the amount of dissolved protein and nickel salts was not enough to affect the vibration of the functional group of the [Bmim]Cl. It also indicated that the main groups of the [Bmim]Cl were almost not destroyed by the dissolved protein and nickel salts, which was conducive to recycle [Bmim]Cl.

4. Conclusions

In this work, chitin with a 96.5% (mass) purity was obtained from shrimp shells using $NiSO_4$ aqueous solution and [Bmim]Cl in two-step treatment. In the first step, the calcium carbonate can be removed by transition metal salt aqueous solution with higher coordination ability. The results showed that the type of metal salts and experiment temperature had a significant effect on calcium carbonate removal. In the second step, the [Bmim]Cl can remove the protein and nickel salts from the first step mainly due to the aromatic imidazole ring according to the ^1H NMR results. According to the FT-IR, XRD, and solid ^{13}C NMR results, the treatment process had little effect on the structure of chitin. The structure and crystal form of the obtained high purity chitin was the same as the natural chitin in the shrimp shells, which was beneficial for subsequent applications. In this process, the use of metal salts aqueous solution and [Bmim]Cl makes the preparation chitin without acid and base. Importantly, this work provides a new greener method for chitin preparation from shrimp shells in principle. Furthermore, it expands the application of transition metal salts to the natural product separation process and ionic liquids to the inorganic metal salts dissolution.

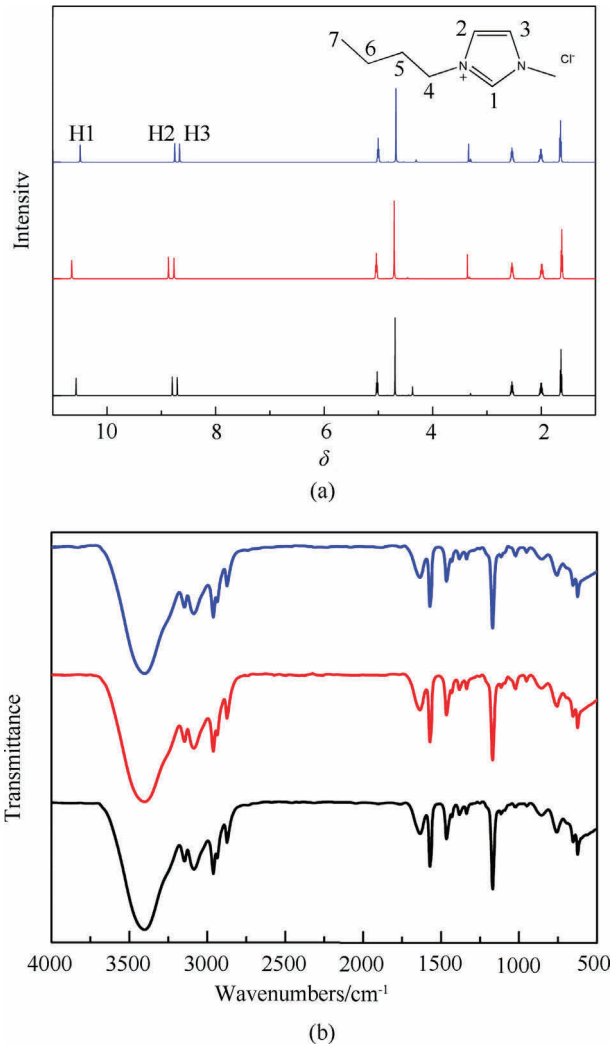

Fig. 6. (a) The ^{1}H NMR spectra of [Bmim]Cl (black line), [Bmim]Cl, and nickel salts (red line) and [Bmim]Cl, nickel salts, and protein (blue line). (b) The FT-IR spectra of [Bmim]Cl (black line); [Bmim]Cl and nickel salts (red line); [Bmim]Cl, nickel salts, and protein (blue line).

Table 2
The elements value of ILs

Sample	C/%	H/%	N/%	S/%
[Bmim]Cl	54.96	8.59	16.03	0
$[Bmim]_2[NiCl_4]$	40.11	6.27	11.70	0
[Bmim]Cl/nickel salts①	52.89	8.05	15.67	0

①[Bmim]Cl/nickel salts was obtained by the following method. After [Bmim]Cl reacting with decalcified shrimp shells, the supernatant 1 was obtained. Then, the ethanol was added to the supernatant 1 to regenerate protein and part of nickel salts. After separation, supernatant 2 was obtained and was dried using rotary evaporation and oven to remove ethanol. The obtained liquid was [Bmim]Cl/nickel salts.

Data Availability

No data was used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cjche.2022.01.031.

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