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Extraction of keratin with ionic liquids from poultry feather

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ABSTRACT

Ionic liquid (IL) is a new eco-friendly solvent to accelerate dissolution of natural polymer and several common water-soluble imidazole ionic liquids (ILs) have been selected to dissolve poultry feather and extract keratin. [Bmim]Cl has shown the best solubility, thus it has been studied to extract the keratin. Because Na_2SO_3 can unfold disulfide bond of polypeptide chains in different keratin molecules in feather by forming R-SSO_3Na and the addition of water can increase solubility of Na_2SO_3 and decrease viscosity of solution, Na_2SO_3 and water have been used to improve the extraction process. During the solubilization process, SEM pictures show that the smooth structure of the feather was destroyed and followed by the dissolution of the keratin into the liquid phase. The keratin was easily separated from the liquid phase as a solid precipitate after adding some more water owing to the miscibility of the IL with water and the immiscibility of the keratin with water. The keratin precipitate was filtrated and the liquid was distilled to remove water, then the IL and Na_2SO_3 can be recycled. The optimum extraction conditions for keratin are: 20 wt.% of water in IL–water, 10 wt.% Na_2SO_3 in liquid phase, the weight ratio of liquid/feather = 20, extraction temperature at 90 °C, and extraction time of 60 min; under these conditions, the dissolution rate of feather is 96.7% and the yield of keratin is 75.1%.

1. Introduction

The poultry industry produces a great amount of feather each year especially with popular fast food such KFC and only a little part of them is used as filler, adornment and forage. Although the feather can be biodegraded, it not only needs large dumping ground but also discharges the landfill leachate and pollution gas. Thus if not treated in time it causes an environmentally disposal problem [1]. Recently it is very common to hydrolyze keratin in poultry feather with strong acid to produce amino acid in China, but the process results in greatly acid pollution. Enzymatic catalysis hydrolysis is a potentially alternative method, but up to date owing to the outer protective film and compact structure, it is far from industrial application. Therefore, it is very urgent to recycle feather with eco-friendly way.

It is known that keratin is rich in feathers, usually more than 70 wt.%, owing to its unique molecular structure and crystal arrangement [2], keratin has important applications in biomaterials [3], biomedical [4], flocculants [5] and adhesive [6]. In addition, the hydrolysis to produce amino acid is indeed the hydrolysis of keratin in feather. If the pure keratin is extracted from feather, the hydrolysis is no doubt easier because there is no protective film and compact structure and the eco-friendly enzymatic hydrolysis can be industrial application. Therefore, from both environmental and economic point of views, it is quite desirable to develop effective processes to extract keratin from poultry feather.

Feather keratin is a structural protein, characterized by high cystine content and a significant amount of hydroxyl amino acids, especially serine. Feather keratin involves a range of non-covalent interactions (electrostatic forces, hydrogen bonds, hydrophobic forces) and covalent interactions (disulfide bonds) [7]. In addition, its molecular chains also have complex structures of α-helix and β-sheet [2], and these structures must be destroyed during the extraction of keratin. Therefore, it is difficult to dissolve feather to extract keratin. The traditional methods of keratin extraction from feather usually used strong acid, alkali or high concentration of salt solutions [7,8]. These processes were multi-steps and could result in degradation of protein. Furthermore, owing to the consumption of a large quantity of reagents that could not be recovered, they polluted our environment. Although the use of superheated water has been shown as an eco-friendly processing method [9], it could break down the peptide bonds in keratin molecules and result in degradation of protein. Thus, it is urgent to develop a new simple and eco-friendly method to extract feather keratin.

Recently, ionic liquids (ILs), a group of salts existing as liquids at relatively low temperatures, have drawn intense attention as a type of eco-friendly and safe solvents with their advantages of non-volatility (i.e., no discharge comparing with traditional organic solvent),

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non-flammability, chemical and thermal stability and easy recycling. They have remarkable solubility ascribed to their own ionic structure comparing with traditional solvents. In 2002, Swatloski et al. [10] reported that cellulose could be dissolved in ILs and the regenerated cellulose was tested with no significant changes in the degree of polymerization and dispersibility. This discovery has received great attention for the study of ILs as solvents for the natural polymer materials. Owing to the strong polar ILs can destroy the usually strong inter-molecule effect, such as hydrogen bonding in natural polymers, and enhance the dissolution of polymers, so that ILs have been developed to a new class of eco-friendly solvents for natural polymers and the structures of the anions and cations had great influence on the dissolution [11–14].

Up to date, the type of ILs with imidazole cation has been studied to show excellent dissolution for pretreatment of lignocellulose [15–19], wool keratin [20–23], and extraction of proteins [24]. In addition, the imidazole ILs are very common and relatively inexpensive, which is great advantage to decrease the obstacle of the high price for industrial applications. Thus in this study, several water-soluble imidazole ILs were tested to dissolve feather and extract keratin.

In order to improve extracting efficiency, some additives could be used. For example, the imidazole ILs with a little NaOH can mutual enhance pretreatment of corn stover [25]. As for the extraction of keratin from feather, the disulfide bonds of cystine in keratin are the main obstacle. Na_2SO_3 can unfold the disulfide bonds according to the following reaction to accelerate the dissolution of keratin.

$$RSSR' + SO_3^{2-} \rightarrow RSSO_3^- + R'S^-$$

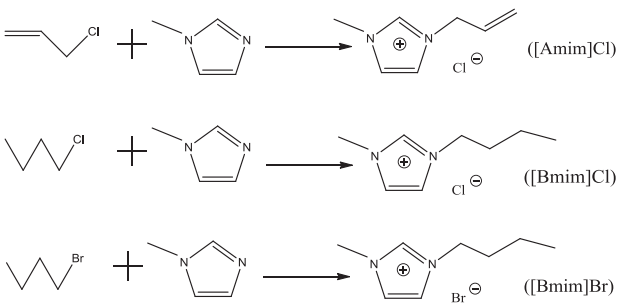
The Na_2SO_3 is a weak alkaline salt, therefore, it cannot destroy the keratin and can be used as an accelerant to extract keratin from feather.

2. Experimental

2.1. Material

Duck feather is a typical poultry feather and the sample of duck feather was provided by Xinyi Hanling Biological Engineering Co., Ltd. (Xuzhou, China). N-methylimidazolium, 3-chloropropene, 1-chlorobutane, 1-bromobutane, Na_2SO_3, iodine, $NaHCO_3$, $Na_2S_2O_3$, and starch were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

ILs [Amim]Cl, [Bmim]Cl and [Bmim]Br were prepared following the three one-step reactions:



[Bmim]NO_3, [Hmim]CF_3SO_3 and [Bsmim]HSO_4 (1-sulfobutyl-3-methylimidazolium hydrogen sulfate) were purchased from Shanghai Cheng Jie Chemical Co. Ltd. (China).

2.2. Keratin dissolution from feather and precipitation

Duck feathers were washed with water, dried and cut into small pieces. The different weight ratios of IL, duck feathers, Na_2SO_3, and water were put into beakers and heated to fixed temperature under magnetic stirring condition. After desired time, the residual solids were removed by suction filtration immediately under the high temperature because the increase of viscosity with decreasing temperature will cause difficulty in filtration. Then, the keratin solution was added with some water at room temperature to precipitate keratin. The solid keratin and liquid phase were separated by suction filtration. IL was recovered from the liquid IL–water phase by evaporating water, and it can be used repeatedly.

The dissolution rate of feather and yield of keratin were used to evaluate the optimal extraction condition. The dissolution rate of feather is expressed as $[(M_0 - m_t)/M_0] \times 100\%$, where M_0 is the initial weight of feather and m_t is the weight of residual feather. The yield of keratin is expressed as $[M_t/M_0] \times 100\%$, where M_t is the weight of keratin.

2.3. Analysis of the feather and extracted keratin

The content of protein in the feather and the obtained keratin were determined by hydrolysis with 6 mol/L HCl solution to produce amino acids at the reflux temperature of about 105 °C during 24 h. Then the total amino acids were analyzed by amino acid analyzer (AAA-Direct, DIONEX) and the measuring condition is the same as that described in the previous paper [26]. The FTIR spectrum of keratin was measured on an Avater370 FTIR spectrograph (Nicolet Co., USA) by KBr method. The molecular weight of extracted keratin was determined by gel permeation chromatography (GPC) (HP-1100) system, which consists of a Phenogel mixed column and a RID (G1362A) detector. THF was used as a mobile phase at a flow rate of 1.0 mL/min and the injection volume was 20 μL. Calibration of GPC was carried out with a standard polystyrene sample.

2.4. Content of Na_2SO_3 in recovered IL

Although Na_2SO_3 benefits the dissolution of keratin by opening the disulfide bonds, the process of keratin precipitation should cause the recovery of these opened disulfide bonds. If the disulfide bonds are recovered with the precipitation of keratin, the Na_2SO_3 should dissolve in IL–water solution and the content of Na_2SO_3 gives the information about the precipitation and extraction of keratin. This is a chemical titration measurement based on the following two equations:

$$SO_3^{2-} + I_2 + 2HCO_3^- = SO_4^{2-} + 2I^- + 2CO_2 \uparrow + H_2O \quad (1)$$

$$2S_2O_3^{2-} + I_2 = 2I^- + S_4O_6^{2-} \quad (2)$$

First, a certain amount of solution with excess iodine and sodium bicarbonate was added to the liquid after filtration of keratin, such that SO_3^{2-} was reduced by I_2 according to Eq. (1). Then, according to Eq. (2), sodium thiosulfate solution was added to oxidize the excess I_2 in the above solution with starch solution as indicator. By subtracting the excess I_2 derived from Eq. (2) from the original total I_2, the amount Na_2SO_3 in the recovered IL was determined.

3. Results and discussions

3.1. In situ change of feather and precipitation of keratin

Some photographs showing dissolving process of the feathers in the IL system are given in Fig. 1 under the temperature of 90 °C, with 20 wt.% of water in IL–water, weight ratio of liquid/feather = 20, and 10 wt.% Na_2SO_3. The branches of feathers dissolved very quickly and at about 20 min these parts were mainly dissolved. At 40 min, the main residual feathers were the stalks and usually they were totally dissolved at 60 min.

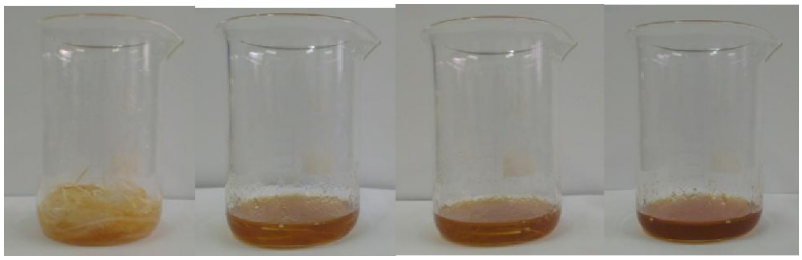


Fig. 1. Selected photographs showing the dissolving process of the feathers with increasing time.

Fig. 2 is the SEM images of the residual feathers at different time, showing the microscopic structures. As for the untreated feather (i.e., 0 min), the surface is very smooth and this is the reason that the hydrolysis is difficult. With increasing time, the smooth surface was destroyed. At 20 min, the outline of feather could be seen, whereas, at 40 min, the structure of residual feather was almost completely destroyed.

As for the keratin–IL solution, the keratin precipitated out when adding some water (Fig. 3). The SEM image of extracted keratin is also shown in Fig. 3 and the keratin does not have the compact structure of the feathers. Therefore, besides the direct application of keratin, because the pure keratin with loose structure is greatly easier hydrolysis than feather, the hydrolysis of keratin to produce amino acid with eco-friendly method such as enzymatic catalysis hydrolysis is more feasible.

3.2. Characterization of the extracted keratin

As for the extracted keratin, it was firstly analyzed by FTIR and the spectrum is given in Fig. 4. The wide band around 3295 cm^{-1} resulted from the peptide bonds (–CO–NH–) and the result indicates that the extracted keratin has the same polypeptide belonging to proteins. The peaks at 1646, 1524 and 1233 cm^{-1} are the vibrations of the peptide bonds known as amides I, II and III, resulting from the secondary structure of β-sheets [27,28], thus the extracted keratin still forms the secondary structure similar with feather fibers.

In order to know the possible change of protein during extracting process, the feathers and extracted keratin were hydrolyzed with 6 mol/L HCl to analyze amino acids of the hydrolysates. Fig. 5 is the chromatograms of standard amino acids and the hydrolysates. The main amino acids of keratin and feather are almost the same, indicating that the proteins of extracted keratin and the feathers should be the same. According to the standard amino acids, the main amino acids of keratin and feather are arginine, threonine, serine, glutamic acid, and cystine. The content of protein of the extracted keratin and feather are determined to 95.3% and 71.6% based on the sum of the five main amino acids mentioned above.

In addition, the extracted keratin was measured by GPC to determine its average molecular weights. The number-average molecular weight and weight-average molecular weight are 8829

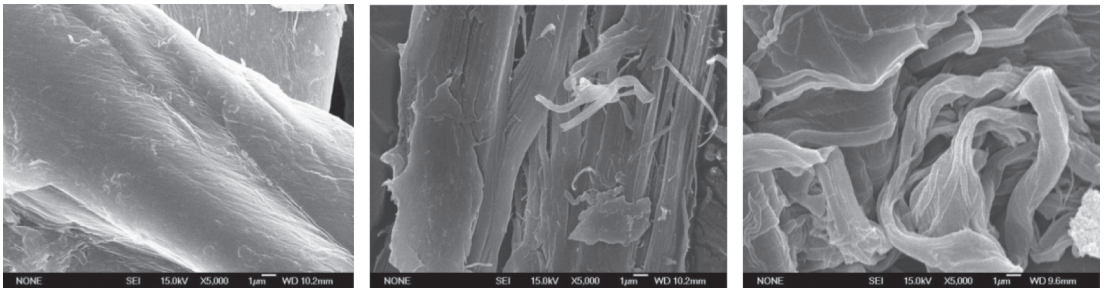


Fig. 2. The SEM images of residual feathers at different time.

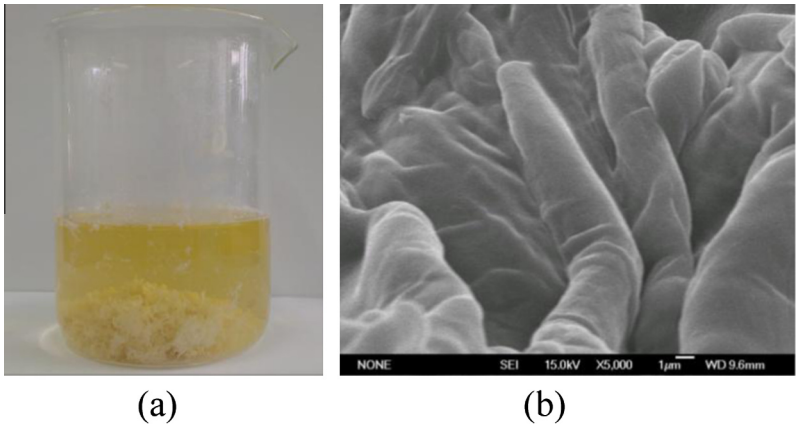


Fig. 3. The photograph of precipitation of keratin and SEM image, (a) precipitation of keratin; (b) SEM image of keratin.

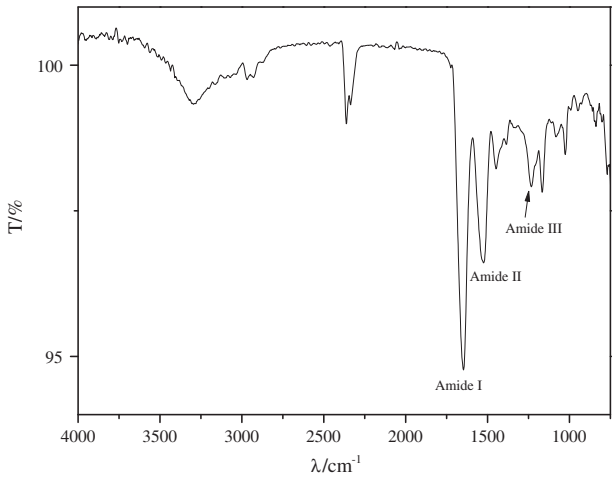


Fig. 4. The IR spectrum of the extracted keratin.

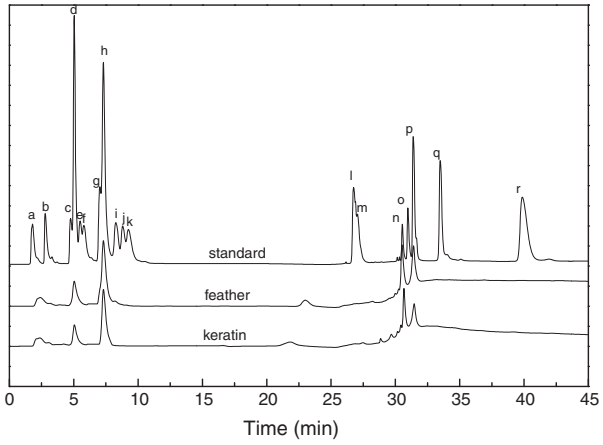


Fig. 5. Chromatogram of standard amino acids, hydrolysate of feather and extracted keratin with 6 mol/L HCl of 24 h; a. arginine; b. lysine; c. alanine; d. threonine; e. glycine; f. valine; g. proline; h. serine; i. isoleucine; j. leucine; k. methionine; l. histidine; m. phenylalanine; n. glutamic acid; o. aspartate; p. cystine; q. tyrosine; r. tryptophan.

and 9736, respectively. The result almost agrees with the published data of about 10,000 of feather keratin [7] and the polydispersion degree of 1.103 shows that the extracted keratin is uniform in its molecular weight. Therefore, the results of composition of amino acids and molecular weight indicate that the extracted keratin remain the same protein ingredient and molecular size of the feather.

3.3. Dissolution of feather in different imidazole ILs

Based on recent studies about ILs in lignocellulose and wool keratin, several common imidazole ILs were selected to dissolve feather keratin. Under the same temperature of 120 °C and time of 60 min, the dissolution rate of 0.5 g feather in 10 g ILs was given in Table 1.

The [Amim]Cl and [Bmim]Cl have the better dissolution rates and [Hmim]CF_3SO_3 has the worst result. Owing to the complex intermolecular effects of keratin, such as hydrogen bonds and disulfide bonds, the destruction of these effects will facilitate the dissolution of keratin. As for the several anions of ILs, the Cl^-, Br^-, NO_3^- and HSO_4^- are better hydrogen ion acceptors than $CF_3SO_3^-$, thus they are easy to form new hydrogen bonds with keratin to open the

Table 1
The dissolution rate of feathers in different ILs.

IL	Dissolution rate (%)
[Amim]Cl	96.05
[Bmim]Cl	96.13
[Bmim]Br	84.18
[Bmim]NO_3	85.11
[Hmim]CF_3SO_3	5.2
[Bsmim]HSO_4	82.57

original hydrogen bonds in feathers, similar with dissolution of cellulose in ILs [29]. Cl^- has strongest electronegativity and thus the ILs of [Amim]Cl and [Bmim]Cl have obviously strongest hydrogen bond effect to increase the dissolution rates. The higher dissolution rates should have higher efficiency for keratin extracting, thus, [Amim]Cl and [Bmim]Cl are the priority to all other ILs.

As for the ILs of [Amim]Cl and [Bmim]Cl, the [Bmim]Cl has a little increase of dissolution and the result is consistent with the dissolution of wool keratin [20]. In addition, as for the preparing materials of 3-chloropropene and 1-chlorobutane for [Amim]Cl and [Bmim]Cl, respectively, 1-chlorobutane is cheaper. Therefore, [Bmim]Cl should be selected to the extracting process.

3.4. Appropriate amount of Na_2SO_3

The disulfide bond of inter-molecule is an important resistance to dissolution of keratin from feather. Because Na_2SO_3 can unfold disulfide bonds of cystine in keratin, its addition to the extracting system should accelerate the dispersion of keratin from feather into liquid phase. Fig. 6 gives the effects of different amounts of Na_2SO_3 on dissolution rate of feather and yield of keratin. It shows that the addition of Na_2SO_3 increases the dissolution rate and yield of keratin. With increasing amount, firstly the rate and yield increase quickly, but when the amount increase to 10 wt.%, the rate and yield are almost no change. Because the effect of Na_2SO_3 is mainly on disulfide bonds, when the main disulfide bonds are opened, the increase amount will not have a further effect. Based on the results, the appropriate amount of Na_2SO_3 is about 10 and the following extracting process is 10 wt.% Na_2SO_3.

Na_2SO_3 opens the disulfide bonds as described by the reaction $RSSR' + SO_3^{2-} \rightarrow RSSO_3^- + R'S^-$. Whereas, when keratin precipitates from liquid phase, the opened disulfide bonds should recover to benefit precipitation of keratin. As for the filtrate of IL–water solution after separation of the precipitation of keratin, the residual Na_2SO_3 was about 81.68% of its initial weight. The result indicates that the most Na_2SO_3 is recovered to remain in solution after keratin precipitation.

3.5. Keratin extraction from feathers in dependency on different conditions

3.5.1. Weight content of water in IL–water

Although water cannot increase protein solubility, it has three main effects to improve the extraction process. First it increases the dissolution of Na_2SO_3 in the IL system. Second it has influence on IL such as the change of structure and decrease of viscosity [30]. As for the IL–water system, besides the decrease of viscosity, the high dielectric constant of water will cause it to form hydrogen bonds with Cl^- to promote the dissociation of the IL, so that the electrostatic interactions and hydrogen bonds between feather keratin and ionic liquid enhance [12,24], which benefit to destroy keratin's structure. Third it acts on the hydrophilic group of keratin to destroy the original structure itself [31] and feather keratin has

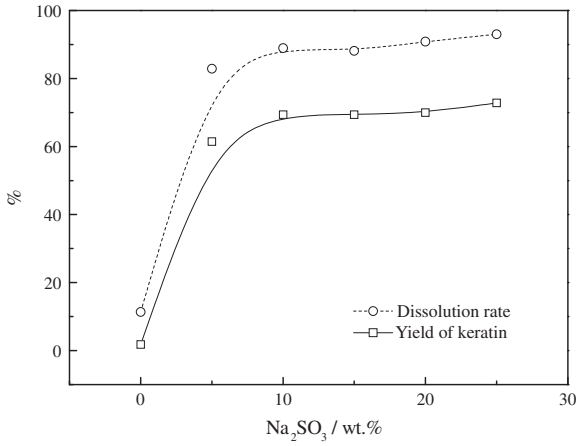


Fig. 6. The result of amounts of Na_2SO_3 on dissolution rate of feather and yield of keratin (80 °C, 120 min, weight content of water in IL–water 20 wt.%, and weight ratio of liquid/feather = 20).

about 40% hydrophilic groups [32], thus some water has effect on the parts and accelerates its dissolution.

Fig. 7 shows the dissolution rates of feather and yields of keratin with different weight content of water in IL–water and some water indeed improves the extracting keratin. The 20 wt.% content of water has the optimization result and the yield of keratin is about 68.5% which is close to the protein content of 71.6% in feather obtained by hydrolysis, indicating that most keratin is extracted. Therefore, some amount of water is advantageous and the appropriate weight content of water in IL–water system should be about 20 wt.% according to the result shown in Fig.7.

3.5.2. Weight ratio of liquid/feather

The weight ratio of total liquid of IL–water system/feather should influence the dissolution rate, separation of keratin, and cost of the process, thus, it is very important to determine an optimal value. Fig. 8 gives the dissolution rate of feather and yield of keratin with different weight ratios. With increasing weight ratio from 10 to 20, the dissolution rate of feather greatly increases and attains to about 95% at 20. Whereas the dissolution rate is nearly no change with the further increase of the ratio from 20 to 40. As for the yield of keratin, it decreases when the ratio increases to 40, which may be resulted from breakdown of peptide bonds by excessive ILs, thus, the weight ratios of 10 and 40 are not fit.

Comparing weight ratios of 20 and 30, both the dissolution rates are more than 95% and the yields of keratin are 68.5% and 70.3%, respectively. The increase of yield from 20 to 30 is very little. Owing to the high price of ILs, the processing capacity of feather with 20 obviously greatly increases than 30, thus, the appropriate ratio should be 20.

3.5.3. Extraction temperature and time

Extraction temperature should pay an important role on the extracting rate and dissolution capacity of feather. The temperature is no more than 100 °C to avoid slow evaporation of water and breakdown of keratin. Fig. 9 gives the dissolution rates of feather and yields of keratin at different temperatures. With increasing temperature, the dissolution rate of feather increases, but as for the temperature of 90 °C, the value is 96.7% and it almost remains no change at 100 °C, indicating that almost all keratin already dissolves at the two temperatures. Whereas, comparing the yields of keratin of 90 and 100 °C, the value decreases from 75.1% to 69.3%.

The 75.1% yield of keratin of 90 °C indicates that the keratin should almost totally be extracted based on the protein content 71.6% of feather obtained from hydrolysis. At higher temperature of 100 °C the keratin should be decomposed to decrease its yield. Therefore, the appropriate temperature of extraction should be 90 °C and the temperature will not only maintain fast dissolution rate of feather but also avoid decomposition of keratin.

Fig. 10 gives the dissolution rates of feather and yields of keratin with different time. At the time of 60 min the dissolution rate of feather is 96.7% and the yield of keratin is 75.1%. With increasing time longer than 60 min, the dissolution is nearly no change, but the yield of keratin decreases, indicating the dissolved keratin will decompose. Thus, as for the temperature of 90 °C, the optimal extraction time to obtain keratin should be 60 min.

3.6. Recycle of ionic liquid

Owing to high price, non-volatility and thermal stability of ILs, the IL should be recycled. After the filtration of keratin, the liquid phase mainly containing water, IL and Na_2SO_3 were reduced pressure distillation to evaporate water and residual volatile impurity. Then the recovered IL containing Na_2SO_3 was directed reused and

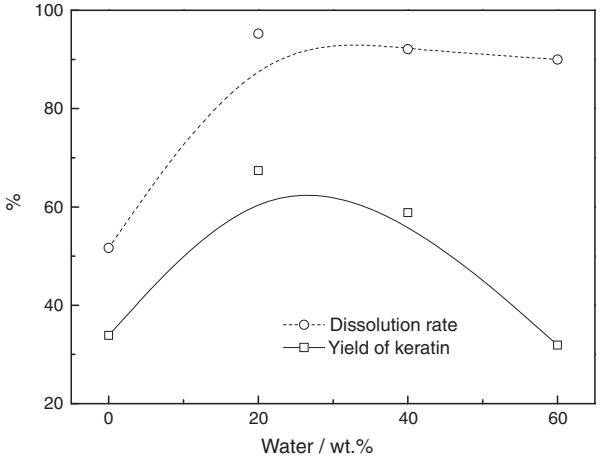


Fig. 7. The dissolution rates of feather and yields of keratin with different weight content of water in IL–water system. (80 °C, 120 min, weight ratio of liquid/feather = 20, and 10 wt.% Na_2SO_3).

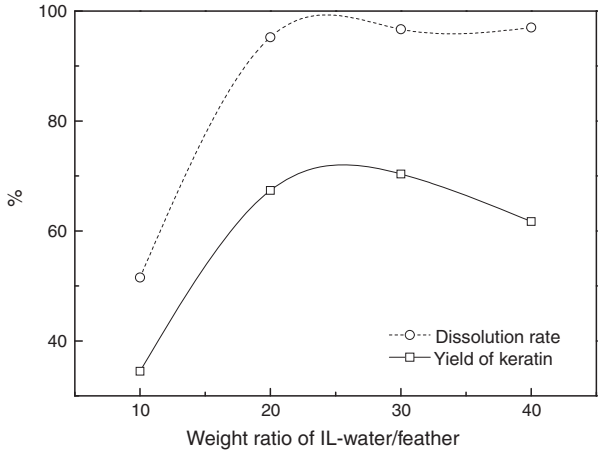


Fig. 8. The effect of the weight ratio of liquid/feather on the extraction of keratin from feather. (80 °C, 120 min, weight content of water in IL–water 20 wt.%, and 10 wt.% Na_2SO_3).

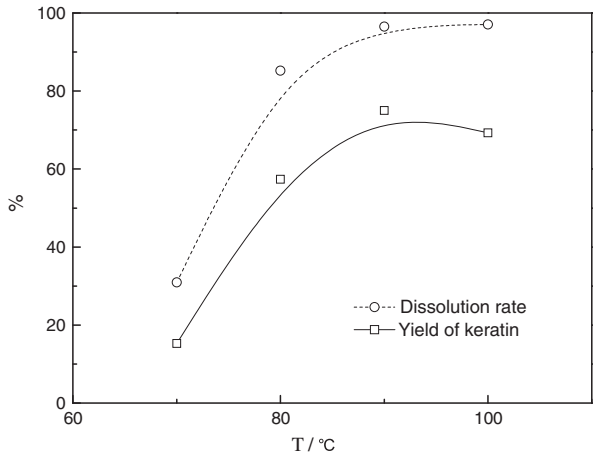


Fig. 9. The effect of temperature on the extraction of keratin from feather. (60 min, weight content of water in IL–water 20 wt.%, weight ratio of liquid/feather = 20, 10 wt.% Na_2SO_3).

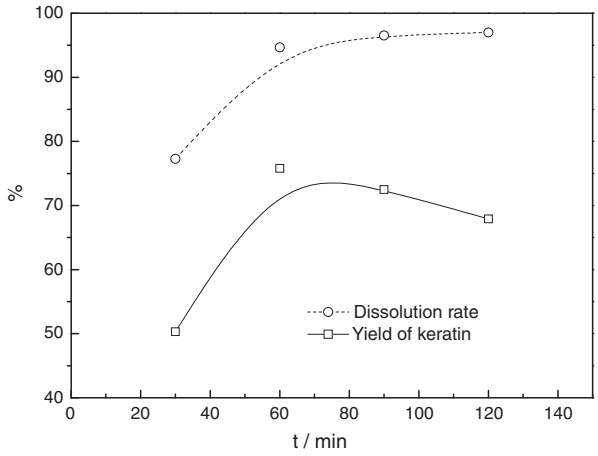


Fig. 10. The effect of time on the extraction of keratin from feather. (90 °C, weight content of water in IL–water 20 wt.%, weight ratio of liquid/feather = 20, 10 wt.% Na_2SO_3).

the result of reuse times was shown in Table. 2. It shows that the efficiency of the extraction almost remains the same as for the use of three times.

Owing to the higher of the dissolution rates of feather than the precipitation of keratin, the result indicates that some other dissolved materials from feathers remain in the liquid phase. In order to understand if these materials will remain in the IL after evaporation of water to decrease purity of IL, the recovery IL was analyzed by FTIR after filtration to separate insoluble solid Na_2SO_3.

The IR spectral of no use and the recovery are given in Fig. 11. The result shows that the two spectral are almost the same, indicating that the recovery IL is stability after the extraction of keratin from feather and its purity remains after evaporation of water and filtration of solid Na_2SO_3. As for the reason why some dissolved materials from the feathers cannot have effect on the purity, it

Table 2
The result of reuse times of the IL (60 min, 90 °C, weight content of water in IL–water 20 wt.%, weight ratio of liquid/feather = 20, and 10 wt.% Na_2SO_3).

Times	1	2	3
Dissolution rate (%)	96.7	96.9	95.5
Yield of keratin (%)	75.1	74.8	75.0

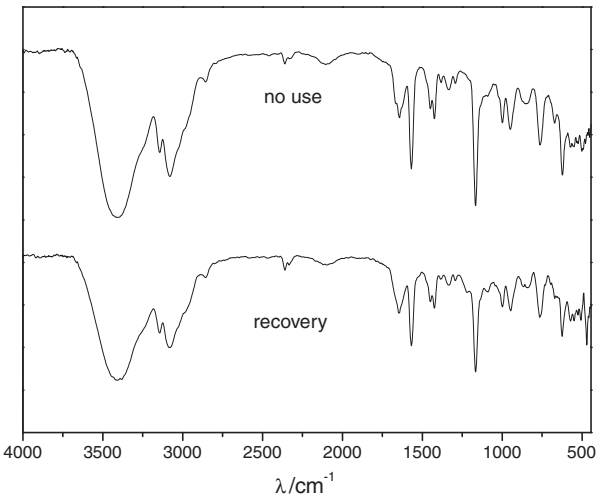


Fig. 11. The IR spectral of the IL of no use and recovery.

can be explained that the feathers contain a little low molecular organic and inorganic components and these materials are separated during evaporation and filtration processes.

4. Conclusion

Imidazole ionic liquid [Bmim]Cl can quickly extract keratin from poultry feathers and the method is high efficiency not only for treatment of waste feathers but also for recovery of high value keratin. Comparing with the traditional method with strong acid or alkali resulting in new pollutants, the solvent of IL is non-volatility and easy recycling, thus there is no discharge of pollutants, which accords with atom economy and is eco-friendly process. Furthermore, the extracted keratin remains the same protein ingredient and molecular size as those in the feather.

Na_2SO_3 can unfold disulfide bonds of cystine in keratin to improve the process of dissolution of keratin from feather and it recovers during keratin precipitation. Therefore, it is easily separated from keratin, which not only benefits the recycling of Na_2SO_3 but also no effect on the quality of the extracted keratin. Water is also beneficial to the extraction process owing to its main effects on the dissolution of Na_2SO_3 and the decrease of viscosity. The yield of keratin can attain 75.1% under extraction time of 60 min at 90 °C, with 20 wt.% of water in IL–water, weight ratio of liquid/feather of 20, and 10 wt.% Na_2SO_3.

Acknowledgements

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