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Article

One-Pot Extraction of Bioresources from Human Hair via a Zero-Waste Green Route

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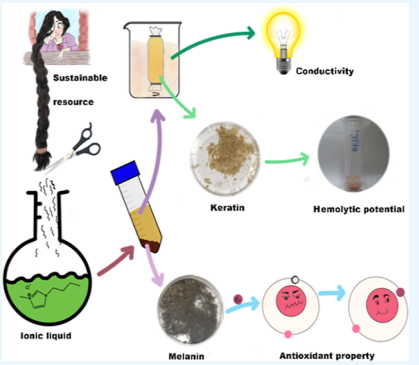
ABSTRACT: In recent years, the extraction of bioresources from biowaste via green chemistry and their utilization for the production of materials has gained global momentum due to growing awareness of the concepts of sustainability. Herein, we report a benign process using an ionic liquid (IL), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), for the simultaneous extraction of keratin and melanin from human hair. Chemical characterization, secondary structure studies, and thermal analysis of the regenerated protein were performed thoroughly. Hemolytic potential assays demonstrated hemocompatibility of the keratin, and thus, it can be used in blood-contacting biomaterials such as sealants, catheters, hemostats, tissue engineering scaffolds, and so on. Scanning electron microscopy showed retention of the ellipsoidal morphology of melanin after the extraction procedure. The pigment demonstrated the ability to reduce 2,2-diphenyl-1-picrylhydrazyl indicative of its free-radical scavenging activity. Notably, the IL could be recovered and recycled from the dialysis remains which also exhibited conductivity and can be potentially used for bioelectronics. Altogether, this work investigates an extraction process of biopolymers using green chemistry from abundantly available biowaste for the production of biomaterials and does not produce any noxious waste matter.



INTRODUCTION

The extraction of resources from waste via green chemistry for the production of materials that have a smaller ecological footprint has witnessed a spiraling interest in recent times.[1,2] Biowastes can be processed to cut down land pollution as alternatives to current expensive techniques for a greener tomorrow.[3−7] Human hair is a biowaste that is abundantly available worldwide. Traditionally, human hair is either incinerated or dumped as solid waste which causes environmental pollution.[8,9] Therefore, innovations in upcycling of hair for the production of high-value materials that benefit the environment as well as the economy should be emphasized. Hair is composed fundamentally of keratin that can be extracted and utilized for various biomedical applications.[10−13] Another biopolymer that can be extracted from hair is melanin.[14,15] Of the various types of melanin, eumelanin found in black human hair is the most common and is produced by oxidative polymerization of tyrosine to a derivative, which in subsequent multiple steps is converted to melanin.[16] The structure of melanin gives it properties such as strong broadband UV and visible absorption, free-radical scavenging ability, and antibacterial properties.[15,17] These properties make melanin a valuable candidate for various biomedical applications.[14,18−20] The common methods of melanin extraction are alkaline treatment, the use of boiling acids followed by washing the melanin precipitate with organic solvents, and enzymatic procedures.[21] However, problems such as insolubility in alkaline conditions, decomposition in boiling acid, and affordability persist with the above-said methods. Keratin, on the other hand, is commonly extracted from different sources by chemical processes such as alkali hydrolysis, reduction, oxidation, sulfitolysis, and steam explosion.[22,23] These processes use hazardous chemicals and uncontrolled drastic reaction conditions which are not environmentally viable.[10,13,23] Moreover, these processes often leave behind hair residues that may need to be discarded, leading to a further increase in environmental burden. It is thus imperative to optimize the extraction process of keratin and melanin to address the issue of environmental pollution and to make the overall process cost-effective. An alternative to conventional methods for biopolymer extraction in an environmentally friendly way is using ionic liquids (ILs), which are composed of organic cations and organic or inorganic anions. ILs are known as green solvents due to their unique physicochemical properties such as low melting temperature and low vapor pressure contributing to nonvolatility, nonflammability, high thermostability, and high solvation ability for specific solutes.[24,25] Ji et al. reported the

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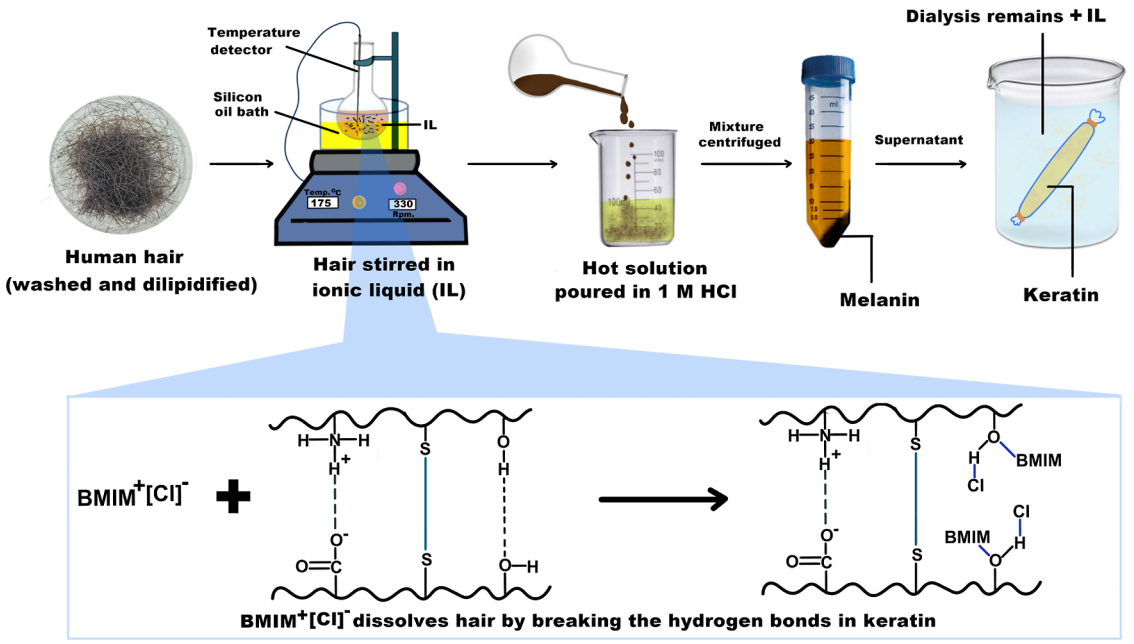

Figure 1. Schematic representation of extraction of melanin and keratin from human hair using the IL [BMIM]Cl.

extraction of keratin from duck feathers using 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) with sodium sulfite.[26] Liang et al. extracted melanin from alpaca fibers using ILs.[27] Although extraction of keratin or melanin from natural resources using ILs is documented in the literature,[26−29] there is only one report that mentioned simultaneous extraction of keratin and melanin using a hydrated IL to date.[28] This study reports a complex procedure that involves harsh chemicals such as chloroform and methanol in the preprocessing steps of extraction, thus defeating the purpose of the use of green chemistry in the subsequent steps. Also, neither a detailed characterization of melanin was studied, nor possible applications of the biopolymers were proposed. Therefore, there is still a need for an optimized procedure to extract both the biopolymers—keratin and melanin—from hair waste in an environmentally benign, single reaction simultaneously saving energy and labor costs as well as reducing the landfill materials. Our study reports one-pot extraction of the two biopolymers in a sustainable way from hair using the IL, [BMIM]Cl, in a single reaction.

The IL dissolved the hair by breaking the intermolecular hydrogen bonds of keratin. The melanin was precipitated from the reaction mixture upon treatment with mild hydrochloric acid. Keratin was characterized for molecular weight, chemical structure, thermal stability, and crystallinity after extraction by ILs. Melanin was also characterized for morphology, surface roughness, functional moieties, UV absorption, and thermal stability. Furthermore, the extracted keratin and melanin demonstrated low hemolytic potential and free-radical scavenging activity, respectively, showcasing their utility as biomaterials in biomedical and tissue engineering applications. Furthermore, the dialysis remains comprising ILs after the extraction procedure showed conductivity and could be used for bioelectronic applications. Moreover, the IL in the dialysis remains was also recovered by evaporating water by the reduced pressure distillation technique and reused multiple times for the extraction processes, thus proving the recyclability of ILs. Altogether, the work provides a zero-waste green approach to extracting biopolymers from renewable resources and their uses in different biomedical applications.

EXPERIMENTAL SECTION

Materials. Human hair samples were collected from local hair salons near Jadavpur, Kolkata, India. Hair was dissolved in the IL [BMIM]Cl (Sigma). Cellulose membrane dialysis tubes (MWCO 14 KDa) were purchased from Sigma-Aldrich. SDS-PAGE was performed by using *N*,*N*′ methylene bis-acrylamide (ultra-pure, ICM), Tris HCl (Merck), sodium lauryl sulfate (SLS, SRL), ammonium persulfate (APS, SRL), Coomassie Brilliant Blue R-250 dye (Thermo Fisher), tetramethylethylenediamine (TEMED, SRL), glycine (Merck), Trizma base (Sigma), and protein ladder (PUREGENE). For the antioxidant assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) and ascorbic acid were purchased from SRL; dimethyl sulfoxide (DMSO) was purchased from Merck. Other chemicals used were acetone (Rankem), ethanol (HiMedia), hydrochloric acid (HCl, Rankem), sodium hydroxide (NaOH, Qualigen), and citrate vacutainers (BD vacutainer) were used to collect human blood. Deuterium oxide (D_2O) and 3 kDa MWCO Amicon ultracentrifugal filter were purchased from Merck for the nuclear magnetic resonance (NMR) study. Millipore water was used throughout the experiments.

Dissolution of Hair Using the IL. Thermogravimetric analysis (TGA) of hair and the IL was done on an "SDT Q600 V20.9 Build 20" machine under a nitrogen gas atmosphere to determine their thermal stability prior to the dissolution process. The samples were analyzed from 25 to 800 °C with a heating rate of 20 °C/min. The dissolution of hair for bioresource extraction was done using [BMIM]Cl by modifying previously published methods.[28,30] A typical experimental procedure for hair dissolution is summarized in Figure 1. The hair samples were at first thoroughly washed with warm distilled water (DW) and shampoo followed by cutting them into small slices, rinsing them with ethanol, and then air drying. In brief, 10 g of the IL was melted in a round-

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bottom flask. The flask was kept in a silicon oil bath fitted with a magnetic stirrer (Tarsons, India). Three different temperatures, 135, 155, and 175 °C, were used for the dissolution of human hair. After raising the temperature to the desired degree Celsius, 1 g of hair was progressively added to the melted IL and stirred continuously at 330 rpm. The reaction was stopped after the complete dissolution of hair was observed. The hot mixture was directly poured in 50 mL of 1 M HCl and centrifuged at 5500 rpm to get a dark-brown precipitate of crude melanin granules and the supernatant containing keratin protein. After collecting the supernatant, the pellet was washed repeatedly with DW and 1 M HCl. The pellet was redispersed in water and freeze-dried to obtain powdered melanin. The supernatant containing protein was dialyzed for 72 h at 4 °C, using a cellulose membrane dialysis tube maintaining a pH around 9 of the DW, changing it every 12 h. After dialysis, the resulting aqueous solution was lyophilized to obtain pristine keratin powder. The yield of biopolymers derived from the IL was calculated according to Ji et al.[26]

$$\text{Yield } \% = \frac{\text{weight of biopolymer}}{\text{weight of hair taken}} \times 100\%$$

where the biopolymer may either be keratin or melanin.

To determine the purity of extracted keratin, the concentration of free ions/total dissolved solids present in the keratin extract was measured using a total dissolved solids (TDS) meter in parts per million (ppm) and compared with dialysis remains, Millipore water (negative control), and a saturated sodium chloride solution (positive control). Toward this end, 3 mL of each of the samples (n = 3) was taken in 6-well plates and the probe of the TDS meter was dipped in the samples to check the free ion/solid content.

Characterization of Keratin. The molecular weight of the extracted keratin was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The processing of pristine regenerated keratin is detailed in the Supporting Information. A stock sample of 20 mg/mL was made from the pretreated keratin and was mixed with the gel loading buffer comprising 0.5% Coomassie brilliant blue, 5% β-mercaptoethanol, 50% glycerol, and 10% SDS in 250 mM Tris HCl (pH 6.8) followed by heating at 95 °C for 5 min. The sample was loaded against a prestained protein ladder and resolved in 12% acrylamide separating gel and 4% stacking gel. The bands were stained with Coomassie brilliant blue R-250, and the image was obtained in a gel documentation system (Bio-Rad).

The functional groups of keratin extracted using [BMIM]Cl were analyzed by attenuated total reflectance−Fourier transform infrared spectroscopy (ATR-FTIR) (TENSOR 27, BRUKER) in the range of 4000−600 cm^{-1}. The basic elemental analysis of extracted keratin was determined by wide-scan X-ray photoelectron spectroscopy (XPS) (ESCALAB Xi+, Thermo Fisher Scientific Pvt. Ltd., UK) using dual-anode Mg Kα/Al Kα microfocused monochromated Al Kα X-ray sources (900 μm beam) and dual-anode Al/Mg Kα sources. TGA of the extracted keratin was performed to determine thermal stability. X-ray powder diffraction (XRD) of human hair and regenerated keratin samples was done using a Rigaku, MiniFlex II, DESKTOP X-ray diffractometer in the 2θ-range of 5−110°.

Hemocompatibility of Keratin. The hemolytic potential or the rupture of red blood cells (RBCs) due to interaction with the extracted keratin powders was determined following a previously published report.[31] In brief, blood drawn into a citrate vacutainer was diluted 50× in 0.9% saline. 300 μL of diluted blood was incubated with 10 mg of keratin. An empty microcentrifuge tube was used as a negative control (NC), and blood diluted in deionized (DI) water was considered as the positive control (PC). The sample and controls were mildly agitated in an incubator shaker for 2 h maintaining the temperature of 37 °C (n = 6). Thereafter, the supernatant was collected and centrifuged at 1000g for 15 min to remove RBC/debris. Finally, 100 μL aliquots were pipetted in a 96-well plate for absorbance reading at 545 nm in a plate reader (Thermo Scientific). Hemolysis percentages were determined using the formula

$$[(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{NC}})/\text{Abs}_{\text{PC}}]$$

where NC and PC are negative and positive controls, respectively.

Characterization of Melanin. The solubility study of the precipitate was done in different solvents such as water, acetone, concentrated HCl, chloroform, and 0.5 M NaOH.[32] The surface morphology of melanin was analyzed using FE-SEM by ZEISS, SIGMA. Topographic analysis of melanin samples was determined by AFM (Keysight, Holmarc) in contact mode. Melanin sample dispersed in water was dropwise added on freshly cleaved mica and air-dried in the absence of light. AFM images were processed in PicoView software.[33]

The functional groups' characterization of extracted melanin was done using ATR-FTIR in the range of 4000−600 cm^{-1}. Melanin powder at a concentration of 0.5 mg/mL was dissolved in 0.5 M NaOH with continuous shaking at 50 °C, and the absorbance was determined using UV−visible spectroscopy (Jasco UV−vis spectrophotometer) by scanning from 200 to 800 nm.[32] For blank, 0.5 M NaOH was used. The thermal degradation behavior of melanin was evaluated using TGA.

Antioxidant Activity of Melanin. The antioxidant activity of the extracted pigment was determined on the basis of the free-radical scavenging effect of stable DPPH following a published report with some modifications.[34] Prior to UV measurements, a 0.2 mM DPPH solution was freshly prepared in 95% ethanol. Thereafter, 1 mL of melanin at various concentrations (200−1000 μg/mL) was pipetted into 2 mL of the prepared DPPH solution and 2 mL of 95% ethanol and mixed well. The mixture was agitated and kept in a dark room for a duration of 30 min. The absorbance of the resulting solution was read at the wavelength of 517 nm in a plate reader (Thermo Scientific, Multiskan GO) against an NC (2 mL DPPH + 3 mL 95% ethanol). 1 mg/mL ascorbic acid was considered the PC. The ability to scavenge the DPPH radical was calculated using the following equation

$$I\% = [1 - (A_T - A_j)/A_c] \times 100\%$$

where A_c represents NC, A_T represents the absorbance of the test sample, and A_j represents the absorbance of 1 mL of sample + 4 mL of 95% ethanol.

Demonstration of Conductivity of Dialysis Remains. The dialysis remains from the hair processing were analyzed for conductivity. In brief, the solution of dialysis remains after evaporation of water using rotavapor was inserted into a disconnected circuit to repair or act as a component to light up a light-emitting diode (LED). The ability of the dialysis

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remains to glow an LED light was evaluated by applying 2.75 V through it with a known resistance of 1000 Ω. The keratin solution was used as a control.

Recycling of the IL. The recycling of the IL for the extraction of biopolymers—keratin and melanin—was done by evaporating dialysis remains, mainly consisting of water and the IL by the reduced pressure distillation technique. ^{1}H NMR was recorded at 400 MHz (JEOL) in D_2O using tetramethylsilane as the internal standard to evaluate the purity of the recycled IL and compared it with the pure form of the IL. The recycled IL was ultracentrifuged in a 3 kDa MWCO Amicon ultracentrifugal filter at 16*g* for 40 min for NMR sample preparation. The yield of the extracted biopolymers was also evaluated following the report by Ji et al.[26]

Statistical Analysis. Data were expressed as the mean ± standard deviation of triplicate samples and statistically evaluated using GraphPad Prism software. There were significant differences between the experimental groups when the *p*-value was less than 0.05.

RESULTS AND DISCUSSION

Dissolution of Hair. Herein, the IL [BMIM]Cl was used for the dissolution of human hair. Mechanistically, the IL acts by disrupting the intermolecular hydrogen bonds present in hair keratin.[35] Studies show that imidazolium chlorides are more effective as solvents for biopolymer dissolution compared to other tested imidazolium halides owing to high electronegativity.[36] Prior to hair dissolution, TGA of [BMIM]Cl (Figure S1) and hair (Figure S2) was performed to assess their thermostability. The thermal degradation of the IL as observed from the TGA graph starts at ∼245 °C and completes at ∼325 °C. The derivative TG (DTG) of the IL indicates the presence of a single decomposition peak. DTG of hair, on the other hand, shows two major decomposition peaks. This result is similar to a previous report by Valkov et al.[37] The first mass change for hair around 70−100 °C is due to the evaporation of moisture, and actual degradation occurs within the range of 240−270 °C. The reactions were therefore performed below 200 °C to avoid possible thermal degradations while extracting bioresources. In our study, a complete dissolution of hair in [BMIM]Cl at the temperature of 135, 155, and 175 °C was observed around 12, 6.5, and 3 h, respectively, indicating that temperature influences the solubility of hair.

The dissolution temperature of 175 °C was used to extract keratin and melanin in the subsequent studies as the time of extraction processes for the other two temperatures was quite prolonged. The solution containing dissolved hair was poured in 1 M hydrochloric acid (HCl) to obtain the melanin precipitate. The use of 1 M HCl for melanin extraction instead of hazardous hot concentrated acid that distorts the morphology of the pigment is a noteworthy advantage of this process. Also, keratin can easily be recovered by simple dialysis of the supernatant. At 175 °C, the yield of melanin and keratin was 16.9% ± 0.1 and 43.8% ± 0.3 (n = 3), respectively. The keratin yield in our study is substantially higher than a previous report which also used [BMIM]Cl to extract keratin and reported a yield of 18% at 180 °C.[25] Moreover, the report did not mention the extraction of melanin along with keratin.

Furthermore, to ensure that the keratin obtained is of high purity, free ion concentration present in the keratin (dissolved in Millipore water) and the dialysis remains were determined using a TDS meter (Figure 2). It is evident that the ions present in regenerated keratin extract (9 ppm) are significantly less (p < 0.0001) than the ions present in the dialysis remains (4870 ppm) and saturated sodium chloride solution, i.e., PC. The Millipore water (NC) displayed no detectable ions. This proves the successful removal of the IL from the supernatant when dialyzed against water, leaving behind regenerated keratin protein.

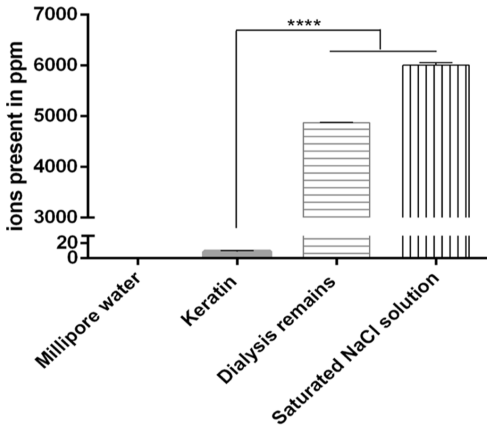

Figure 2. Total ions present in keratin, dialysis remains, Millipore water (NC), and saturated sodium chloride solution (PC) were measured using a TDS meter in ppm. Statistical significant differences between groups (n = 3) at ****p < 0.0001.

Characterization of Keratin. The regenerated keratin extracted from human hair using [BMIM]Cl was thoroughly characterized for the determination of molecular weight, functional groups, crystallinity, and thermal stability. The molecular weight of the keratin was found to be 63 and 68−70 kDa by SDS-PAGE analysis (Figure 3a). Smear can also be observed in the gel probably because of high-temperature treatment for dissolving hair in the IL which caused dissociations of some protein structures.

The elemental composition of the extracted keratin was determined by wide-scan XPS. The XPS of keratin shows the presence of basic elements in the extracted keratin: overall carbon (C 1s signal at ∼284 eV), oxygen (O 1s signal at ∼531 eV), nitrogen (N 1s signal at ∼400 eV), and sulfur as C−S (S 2p signal at ∼163.5 eV) was detected (Figure 3b). The presence of intact disulfide bonds can be deduced from the deconvolution data (inset of Figure 3b). The narrow scan XPS spectra of C 1s, O 1s, N 1s, and S 2p are presented in Figure S3. Furthermore, the atomic wt % of each of the elements present in keratin is provided in the Supporting Information (Table S1). Figure 3c shows the FTIR spectra of regenerated keratin. The peak 3272 cm^{-1} for keratin represents N−H and O−H stretching vibrations for amide A. The absorption peaks at 2953 and 2330 cm^{-1} may be attributed to C−H stretching. The characteristic peaks at 1641 and 1530 cm^{-1} correspond to amide I (C═O) and amide II (vibration due to C−N and N−H bending), respectively. The keratin peak at 1239 cm^{-1} corresponds to amide III due to C−N, C−O stretching, and N−H and O═C−N bending vibrations. The peaks of the regenerated protein are similar to the IR peaks of hair, indicating preservation of the functional groups after IL-mediated extraction (Figure S4).[30]

The crystallinity of the regenerated keratin was determined by XRD studies. From Figure 3d, two distinct peaks at 9 and 20° can be seen. The peak observed around 9° indicates the presence of an α-helix in the proteins, whereas the peak around

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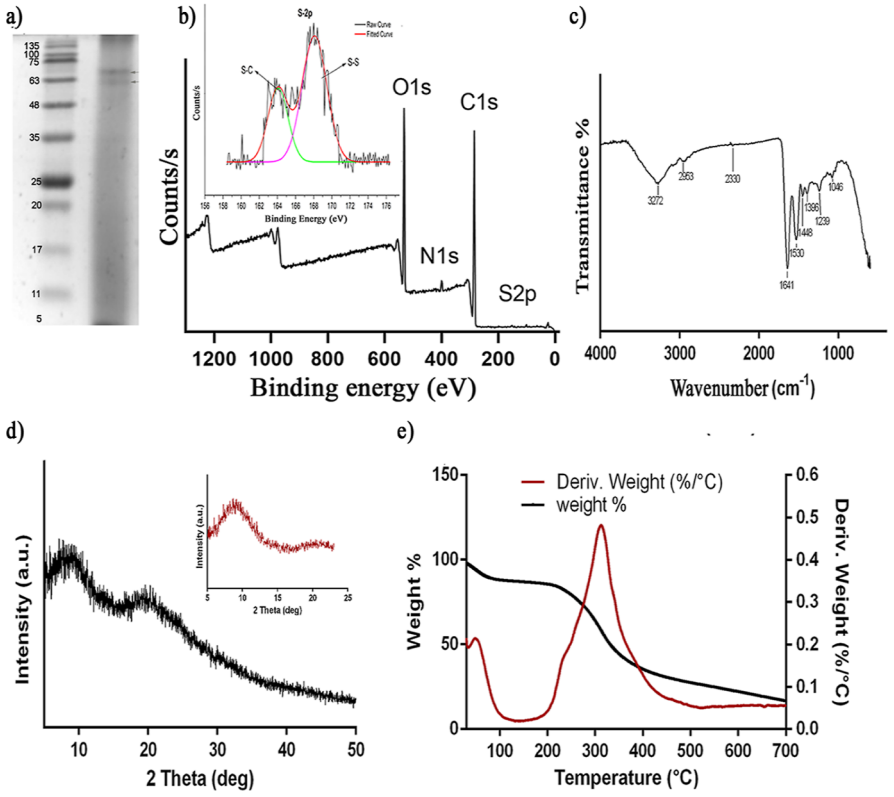

Figure 3. Characterization of keratin extracted from human hair using an IL [BMIM]Cl. (a) Molecular weight determination of keratin by SDS-PAGE and (b) XPS of regenerated keratin. Inset shows deconvolution data of sulfur. (c) ATR-FTIR peaks of keratin, (d) XRD of regenerated keratin and hair (inset), and (e) TGA−DTG graph of regenerated keratin.

20° confirms the presence of β-sheet structures.[28,38] Interestingly, XRD of hair revealed the presence of only the α-helix (inset of Figure 3d). The presence of both secondary structures in keratin may be due to the destruction of the original crystal domain in hair and reconstruction during the regeneration process. Evidently, the peak intensity of the regenerated protein indicates greater content of the α-helix compared to the β-sheet. The thermal stability of keratin extracted using the IL was evaluated by TGA−DTG (Figure 3e). Similar to hair, the weight loss took place in two steps for keratin, as shown by the derivative TG (DTG) analysis curves. The TGA graph shows that the water molecules bound to the keratin structure are first removed at a temperature range of 60−100 °C. This was immediately followed by thermal degradation of the proteins starting at approximately 220 °C, and more than 50% mass loss was observed at around 330 °C. This result is similar to a previously published work by Arslan et al.[39] Some residues can be seen in the graph which could be due to hair dying and other impurities as the hair wastes were randomly collected.

Hemocompatibility of Keratin. A hemolysis assay of the extracted keratin was performed to determine its potential to rupture the RBCs and overall hemocompatibility. The hemolysis assay graph (Figure 4) showed that the keratin powder had a significantly lower hemolytic potential (1.8 ± 0.5%) compared to DI water, PC (94.4 ± 1.5%) ($p < 0.0001$, $n = 3$). Thus, it can be proposed that the pristine form of keratin is a hemocompatible biopolymer that can be utilized in fabricating different forms of scaffolds for biomedical applications.

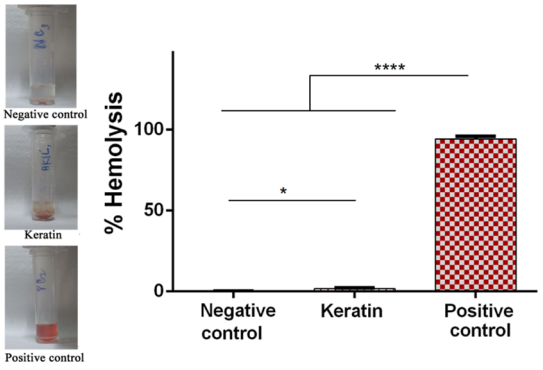

Figure 4. Images and graph showing the hemolytic potential of keratin powder in comparison with DI water (PC) and empty microcentrifuge tube (NC). Significant differences between groups at *$p < 0.05$ and ****$p < 0.0001$.

Characterization of Melanin. Solubility analysis of the precipitate, melanin, from the hair dissolution demonstrated that the pigment was insoluble in H_2O, acids, and organic solvents while dissolving only in an alkali aqueous solution (Table S2 and Figure S5). The results are in agreement with a previous study.[32]

The AFM evaluation was done for the topographic analysis of melanin. Figure 5a−d shows a representative 2D AFM height image and phase image along with a profile line and 3D image of melanin extracted from human hair, respectively. The height image shows an uneven surface of melanin, while the phase image suggests the presence of substructures along with grooves and ridges. The average surface roughness value of

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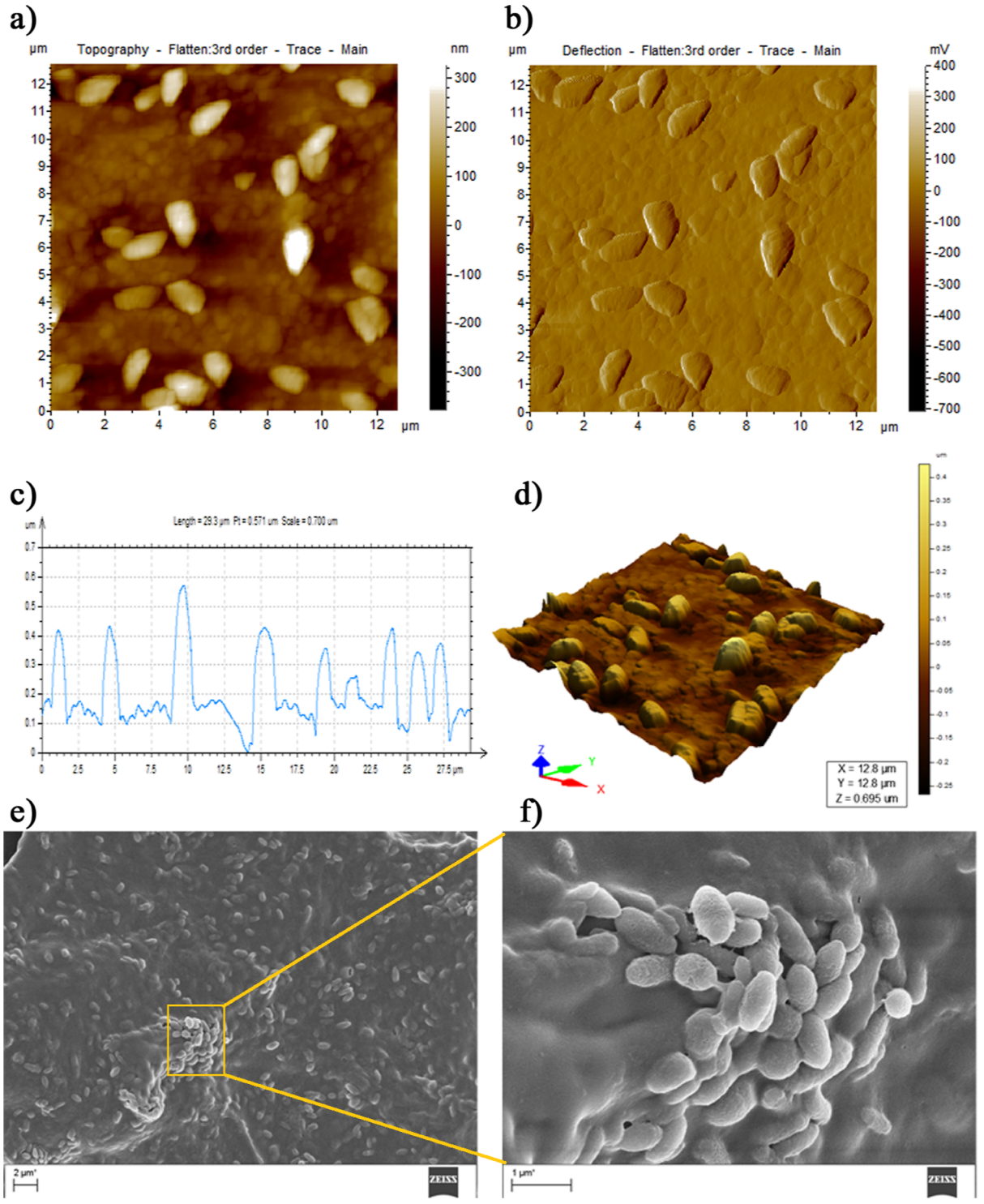

Figure 5. (a) 2D AFM height image of melanin, (b) 2D AFM phase image of melanin, (c) height profile curve of melanin, (d) 3D AFM images of the top surface of melanin together with a height profile along the line, (e) representative SEM image of melanin, scale bar, 2 μm, and (f) high-resolution SEM image of melanin, scale bar, 1 μm.

melanin was found to be 0.09 μm. This value is comparable to the values reported by previous publications.[33,40]

FE-SEM depicts the ellipsoidal morphology of melanin (Figure 5e,f). Previous studies reveal that enzymatic extraction only yields ellipsoidal-shaped granules,[41] while alkaline and acid-based isolation results in distortion of the morphology.[33] Notably, in this study, the extraction procedure assisted by [BMIM]Cl preserves the granule morphology. The longitudinal length is found to be 1.14 ± 0.08 μm, and the transverse length is 0.56 ± 0.07 μm. The aspect ratio of melanin is approximately 2.04 which is almost similar to the data reported by Liu et al.[33]

The FTIR spectrum of melanin is shown in Figure 6a. The peak at 3271 cm^{-1} represents N−H and O−H stretching vibrations. The peaks at 2961 and 2869 cm^{-1} represent CH_3 symmetrical stretching. The peak at 2924 cm^{-1} probably

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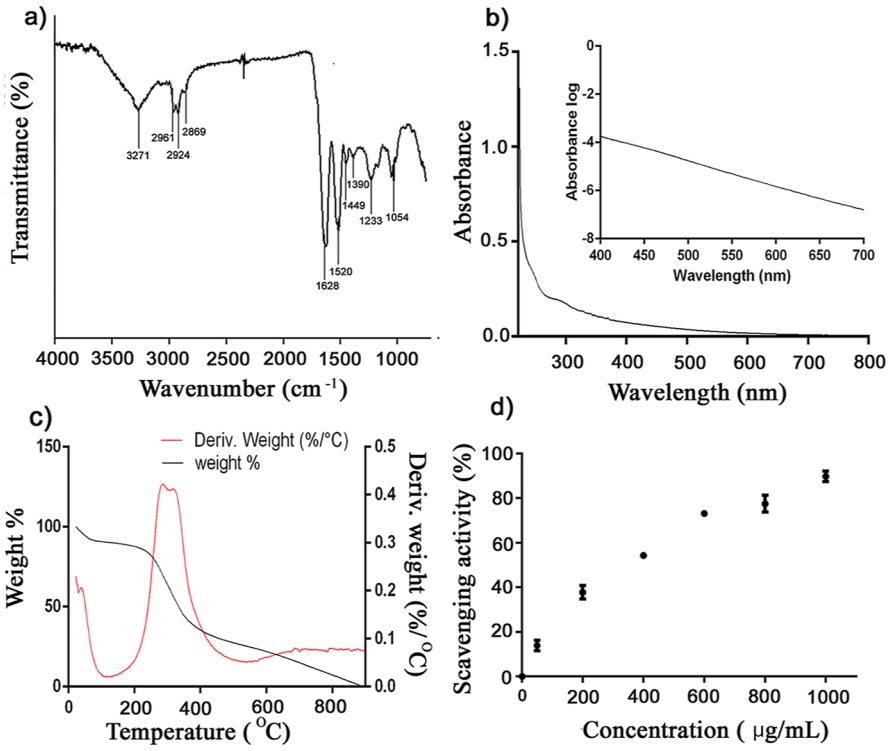

Figure 6. Characterization of melanin extracted from human hair. (a) FTIR, (b) UV−vis spectroscopy, (c) TGA−DTG of melanin, and (d) scavenging activity of extracted hair melanin on the DPPH radical.

represents the oscillation of CH_3, CH_2, or aliphatic CH groups, and that at 1628 cm^{-1} corresponds to the vibration of ample aromatic groups (C═O and C═C). The peaks at 1449, 1390, 1233, and 1054 cm^{-1} may be due to CH_2CH_3 bending, C−N stretching, phenolic C−O−H stretching, and asymmetric C−O−C stretching, respectively. The two peaks at 1520 and 1390 cm^{-1} strongly imply the presence of pyrrole and indole groups in melanin.[42−44] According to the chemical disorder model, melanin consists of a diverse ensemble of chromophores that would lead to monotonic and broadband absorption spectra.[17] Indeed, from Figure 6b, it can be observed that the UV absorbance in melanin is significant and progressively reduces as the wavelength increases (visible-light range). The spectrum however did not show any distinct peak. The results are comparable to those found in previous studies of human hair eumelanin.[45]

TGA of human hair melanin (Figure 6c) illustrates two steps of thermal degradation. The first mass loss appeared at around 70 °C, which can be mainly attributed to the evaporation of weakly bound water. The second mass loss started around 260 °C. This may be due to the decomposition of aliphatic components followed by the decomposition of aromatic components above 400 °C.

The DPPH assay was performed to evaluate the free-radical scavenging property of the melanin pigment. Melanin is known to be a very good antioxidant in the biological system.[46] Melanin interacts with the DPPH radical in the ethanolic solution and donates a hydrogen atom to form a colorless compound. The amount of DPPH reduced was detected by noting the decrease in absorbance at 517 nm using a UV−visible spectrophotometer.[47] Figure 6d shows that the scavenging effect of hair melanin is concentration-dependent.

Conductivity of Dialysis Remains. A conductivity study of the dialysis remains was done (Figure 7). The dialysis

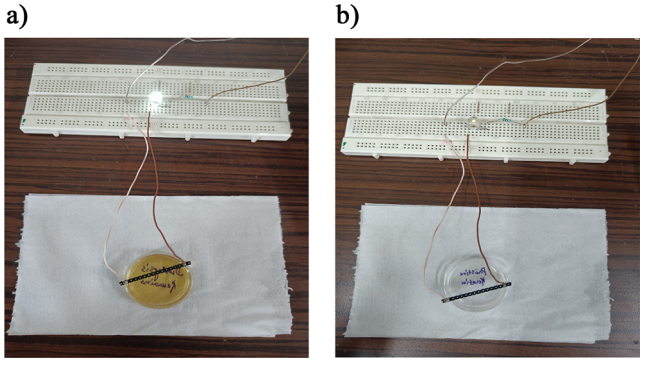

Figure 7. Optical images showing the (a) electrical conductivity of the dialysis remains at 2.75 V and the (b) insignificant electrical conductivity of the keratin solution at 2.75 V.

remains could brightly glow an LED light when 2.75 V was applied across it with a 1000 Ω resistance (Figure 7a). In contrast, the keratin solution inserted into a disconnected circuit did not show visible conductivity at a similar voltage because of a significantly lower quantity of free ions (Figure 7b). Actually, the presence of the IL in the solution of dialysis remains contributed principally to the conductivity of the solution. Usually, positive and negative ions reside together in neutral pairs in ILs, forming a neutral substance that cannot conduct electricity. Occasionally, these "paired" neutral states become free-charged particles, making the IL conductive.[48] In addition, the presence of ions from keratin and melanin may also have contributed to the overall conductivity of the dialysis remains. In the future, the solution of dialysis remains after sufficient processing may be reused in different innovative ways for bioelectronic applications. The feasibility of reusing the

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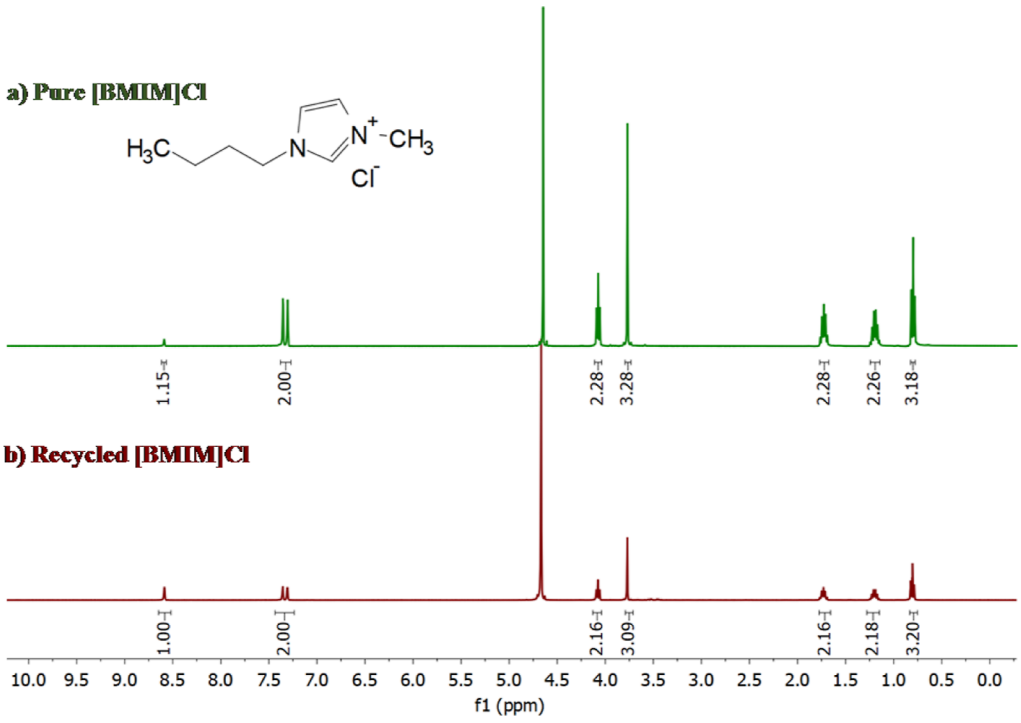

Figure 8. ^{1}H NMR (400 MHz, D_2O) of (a) pure [BMIM]Cl and (b) recycled [BMIM]Cl.

dialysis remains provides sustainability to the present process of extraction.

Recycling of the IL. After the dialysis of the extracted keratin from human hair waste, the technique of reduced pressure distillation was used to recover the IL from the dialysis remains.

^{1}H NMR was performed to check the purity of the recycled IL. Figure 8 presents that the number of protons and position of each peak of the recycled IL are the same as those for the pure IL form, confirming the purity of the recycled IL.

Followed by the recovery of the IL, it was directed to reuse for the extraction of keratin and melanin. Table 1 depicts the efficiency of the IL with reuse times and the yield of the biopolymers extracted. It is evident that the efficiency of the IL for the keratin extraction process significantly reduced, lowering the yield with the IL reuse times, while the yield of melanin remained almost the same.

Table 1. Result of Reuse Times of the IL (175 °C, 330 rpm, 10% Human Hair in the IL)

times	1	2	3
yield of keratin (%)	15.2	13.8	11.2
yield of melanin (%)	17	17.8	17.4

CONCLUSIONS

This work documents a single-step green extraction process of the two biopolymers—keratin and melanin—from human hair waste along with their elaborate physicochemical characterizations. Notably, complete utilization of the reaction leftovers was also accomplished, asserting the sustainability of this eco-friendly procedure. The high temperature used in the extraction process could contribute to the structural disintegration of the biopolymers; nevertheless, the physiochemical and structural integrity of keratin was maintained as evidenced by SDS-PAGE, XPS, XRD, FTIR spectroscopy, and TGA. Also, the ellipsoidal shape with grooves and ridges on the surface of melanin granules was preserved in this process compared to the distorted morphologies caused by the traditional acidic/alkaline extraction processes, signifying the benign nature of the extraction procedure used in this study. Since structural integrity is the basis of biological activity, the regenerated keratin powders were found not to rupture the RBCs significantly and hence can be considered hemocompatible. In the future, we want to use the regenerated keratin for developing hemostatic bandages to quickly halt bleeding for use in accident sites and battlefields. Furthermore, the significant antioxidative ability with strong UV absorption of melanin suggests their utilization for biomedical applications. Melanin can be coated onto thin films or fused into dermatological products to be utilized as sun-protective materials. Moreover, in this work, we have demonstrated the conductivity of the dialysis remains, showcasing a possible application in bioelectronics. Also, ILs were recovered and recycled multiple times, making the overall process sustainable and economical in the long run. Altogether, this study proposes the derivation of bioresources using zero-waste green chemistry from renewable human hair and their potential for biomaterial production.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01428.

Detailed experimental procedure, pretreatment of pristine keratin with 8 M urea for SDS-PAGE analysis, TGA and DTG graph of the IL and human hair, XPS spectra of keratin, ATR-FTIR spectra of human hair, and solubility study of melanin (PDF)

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Notes

The authors declare no competing financial interest.

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