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One-Pot Extraction of keratin from Human Hair via Green Route

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Abstract

Growing global awareness of sustainability concepts has made it more popular in recent years to transformation from biological waste to biological resources using sustainable chemistry also use them to produce goods. This article describes a safe method for simultaneously extracting keratin and extracting individual's hair melanin with an ionic liquid [BMIM]Cl, also known as 1-butyl-3-methylimidazolium chloride. The recovered protein was thoroughly examined through chemical characterisation, secondary structure investigations, and thermal analysis. Blood-contacting biomaterials, including sealants, catheters, haemostats, scaffolds for tissue engineering, and the like, can use keratin since haemolytic potential testing showed that it is haemocompatible. The ellipsoidal form of melanin was preserved during the extraction process, as demonstrated by scanning electron microscopy. 2,2-diphenyl-1-picrylhydrazyl indicative was shown to be reduced by the pigment.

Keywords

Applications, Bioplastics, Biomedical, Extraction, Keratin, and Keratinase

Introduction

There has been a surge in interest recently in using green chemistry to extract resources from trash in order to produce things with a less ecological imprint. As an alternative to the costly methods used today, biowastes can be processed to reduce land contamination and contribute to a greener future. One biowaste that is widely available everywhere in the world is human hair. Human hair is typically disposed of as solid waste and burned, which pollutes the environment. Thus, it is important to highlight advancements in hair upcycling for the creation of high-value materials that are advantageous to both the economy and the environment. Keratin, which makes up the majority of hair, can be isolated and used in a variety of biological applications.

Melanin is another biopolymer that can be taken out of hair. The most prevalent form of melanin, known as eumelanin, is found in black human hair. It is created when tyrosine undergoes oxidative polymerization, producing a derivative that is then transformed into melanin by a series of following processes. Due to its structure, melanin has antimicrobial, robust broadband UV and visible absorption, and the capacity to scavenge free radicals. Because of these characteristics, melanin is a promising option for a range of biological uses.

Using alkaline solutions, boiling acids and cleaning the resulting melanin precipitate with organic solvents are common methods for extracting melanin. Enzyme processes are also utilized. Nevertheless, there are still problems with the previously mentioned methods, such

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as their high cost, insolubility in alkaline environments, and disintegration in boiling acid. Conversely, Chemical methods are commonly used to separate keratin from various resources including saponification, oxidation, reductive reaction, sulfite pulping, and steam hydrolysis. Such techniques, which utilise dangerous substances as well as unregulated, extreme reactingcircumstances, cannot be sustained for the environment. The environmental burden is further increased by the fact that these procedures frequently leave behind hair remnants that may need to be disposed of. In order to tackle the problem of ecological degradation, it is therefore essential to optimize the keratin and melanin extraction process.

Ionic liquids (ILs) are an eco-friendly substitute for traditional biopolymer extraction techniques. They are made up of organic cations and either inorganic or organic anions. Because of their distinct physical and chemical features, like low vapour pressure as well as temperature of melting, ILs are known as green solvents. For particular solutes, these characteristics help with nonvolatility, high thermostability, nonflammability, and high solvation ability. An ideal process is still required to separate the two biopolymers—keratin and melanin—from hair waste in a single, eco-friendly reaction that uses less energy and labour and produces fewer waste materials for landfills. This study shows that the two biopolymers can be extracted from hair in one step using IL and [BMIM]Cl.

Through rupturing the keratin's intermolecular hydrogen bonds, the IL disintegrated the hair. When mild hydrochloric acid was added to the reaction mixture, the melanin precipitated out of it. Keratin was tested for molecular weight, chemical structure, thermal stability, and crystallinity after being extracted by ILs. Additionally, melanin's morphology, surface roughness, functional moieties, UV absorption, and thermal stability were investigated. Additionally, the extracted melanin and keratin showed low free-radical scavenging activity and low haemolytic potential, respectively, demonstrating their potential use as biomaterials in tissue engineering and biomedical applications. Furthermore, the dialysis residue containing ILs showed conductivity and potential applications in bioelectronics following the extraction process. Additionally, the IL during dialysis residue was recovered with the aid of decrease-strain distillation to evaporate water, and it was then again and again used for the extraction methods, demonstrating that ILs may be recycled. In summary, the study offers a waste-loose, environmentally friendly approach for getting rid of biopolymers from resources of renewable strength and using them for various uses in biomedicine.

Objectives of the Study

• Using Ionic Liquids to extract Keratin in a more environmentally friendly manner.

• IL"s are able to be repurposed following every single extraction process.

Literature Review

1. Excellent mammalian cell attachment and proliferation had been tested through keratin extracted using the Shindai approach from numerous keratin resources and processed as movies (Reichl, 2009; Yamauchi et al., 1996) or porous scaffolds (Srinivasan et al., 2010; Xu et al., 2013).

2. By incorporating a plasticizer into combined keratin-polysaccharide movies, the mechanical and biocompatibility characteristics of the films can be superior (Tanabe et al., 2002).

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3. "When combined with synthetic polymers, keratin lower than wool can be a building material for a multilayer film produced by a layer-by-layer approach (Yang et al., 2009).

4. An improved keratin sponge, highly porous and flexible, was prepared from keratin reduced in the presence of calcium alginate beads. The keratin sponge, which had a maximum porosity of about 98.9%, produces satisfactory bacterial lines in dry conditions, even in the. state that it facilitated cell adhesion and spreading (Hamasachi et al., 2008)."

5. Ozaki et al. (2014) reported the synthesis of a porous keratin hydrogel resembling a sponge using extracted kerateine and the denaturing agent guanidine hydrochloride. The gel exhibited remarkable mechanical strength, porosity, and rapid swelling and facilitated the excellent attachment and proliferation of various animal cells.

Materials

Human hair samples, IL [BMIM]Cl, dialysis tubes with cellulose membranes, N, N′ methylene bis-acrylamide SDS-PAGE, TrisHCl, sodium lauryl sulphate, ammonium ersulphate, Brilliant Blue R-250 Coomassie Dye, tetramethyl ethylene diamine, glycine, protein ladder, Trizma base, ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) are used in the antioxidant assay of dimethyl sulfoxide (DMSO), acetone, ethanol, hydrochloric acid, and sodium hydroxide. Human blood is accumulated using citrate vacutainers. For the nuclear magnetic resonance (NMR) investigation, a three-kDa MWCO Amicon Ultra centrifugal clearout and deuterium oxide (D2O) have been hired. All checks used Millipore water.

Thermogravimetric analysis (TGA) was performed on hair and IL in a nitrogen gas atmosphere using the "SDT Q600 V20.9 Build 20" instrument to determine their thermal stability before the dissolution process. The samples were heated from 25 to 800 °C at a rate of 20 °C/min. Hair solutions aimed at removing bio resources using [BMIM]Cl were obtained by modifying previously published methods."

Methods

After giving the hair samples a thorough wash in shampoo combined with warm distilled water (DW), they were cut into thin slices, rinsed with ethanol, and allowed to air dry. To sum up, 10 g of the IL were liquefied in a container with a circular bottom. The container was stored in a magnetic stirrer-equipped oil bath with silicon. Three different levels—135 °C, 155 °C, and 175 °C—were employed to dissolve human hair. One gramme progressively added strands of hair to the dissolved IL after the temperature was raised to the required degree Celsius, and the mixture was constantly stirred at 330 rpm. Once the hair had completely dissolved, the reaction was stopped. The hot combination was immediately added to 50 millilitres of 1 M HCl, and it was centrifuged at 5,500 revolutions per minute to extract the keratin protein-containing supernatant and a dark-brown precipitate of crude melanin granules. Following the collection of the supernatant, DW and 1 M HCl were used to repeatedly wash the pellet. They redispersed the pellet using a cellulose membrane dialysis tube, maintaining a pH of around 9 in the DW and changing it every 12 hours. After dialysis, the resulting aqueous solution was lyophilized to obtain pristine keratin powder. The yield of biopolymers from the IL was calculated according to Ji et al.

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

wherein melanin or keratin may be the biopolymer. To determine the dissolution rate of keratin extract, the concentration of free ions/total dissolved solids in keratin extract was quantified in parts per million (ppm) using a total dissolved solids (TDS) metre, and then this measurement compared dialysis residues, Millipore wastewater (the negative control), and concentrated sodium chloride (the positive). Free ions and products were measured by extracting 3 mL of free ions from each of the three samples (n = 3), placing them in a 6-well plate, and inserting the TDS metre probe into the sample of internal complexity to obtain this comparison.

Figure 1

The attributes of keratin

Using polyacrylamide gel electrophoresis with sodium dodecyl sulphate, the molecular weight of the extracted keratin was ascertained. The corresponding details provide specifics on how pristine, rejuvenated keratin is processed. After the pretreated keratin was prepared as a 20 mg/ml stock solution, it was mixed with the gel loading buffer, which consisted of 250 mM TrisHCl (pH 6.8). The answer comprised 0.5% Coomassie Brilliant Blue, 5% β-mercaptoethanol, 50% glycerol, and 10% SDS**.** This mixture was then heated for 5 minutes at

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95 °C. The sample was recovered in a 12% acrylamide separating gel and 4% stacking gel after being loaded against a pre-stained protein ladder. Coomassie brilliant blue R-250 was used to stain the bands, and a gel documentation system was used to capture the image.Fourier-rework infrared spectroscopy (ATR-FTIR) with attenuated general reflectance was used to investigate the useful companies of keratin and extract the usage of [BMIM]Cl in the 4000−600 cm−1 variety. To confirm thermal stability, the extracted keratin underwent TGA. Samples of human hair with regenerating keratin have been subjected to X-ray powder diffraction (XRD) within the 2θ-variety of 5−110° employing a MiniFlex II desktop X-ray diffractometer from Rigaku.

The hemocompatibility of Keratin

Brief blood was diluted 50× in 0.9% saline and placed into a citrate vacutainer. Ten milligrams of keratin were added to 300 microliters of diluted blood. Blood diluted in deionized (DI) water was employed as the positive control (PC), while an empty microcentrifuge tube was used as the negative control (NC).For two hours, in an incubator shaker, the sample and controls were gently shaken while keeping the temperature at 37 °C (n = 6). To eliminate RBCs and debris, the clear liquid was then gathered and subjected to 15 minutes of 1000g centrifugation. Ultimately, 96-well plates were pipetted with 100 µL aliquots so that a plate reader could read the absorbance at 545 nm.

Research Suggestions

• In the near future, we must find numerous ways to extract Keratin via alternative routes and commercialize them.

Results and Discussion

Dissolution of Hair.

In this instance, human hair was dissolved using IL [BMIM]Cl. The IL works mechanistically by severing the hydrogen bonds that hold molecules of hair keratin together. Studies reveal that because of their high electro- negativity, imidazolium chlorides work better as biopolymer dissolution solvents than other imidazolium halides that have been tested. TGA of [BMIM] and hair was carried out in order to evaluate their thermostability prior to hair dissolution. According to the TGA graph, the IL begins to degrade thermally at about 245 °C and ends at about 325 °C. One decomposition peak can be seen in the IL's derivative, TG (DTG). In contrast, the DTG of hair displays two prominent decomposition peaks. This outcome is consistent with a prior study by Valkov et al. The evaporation of moisture causes the initial significant hair shift, which happens between 70 and 100 °C; actual degradation takes place between 240 and 270 °C. Therefore, in order to prevent any thermal degradation during the bioresource extraction process, the reactions were carried out below 200 °C. We found that hair dissolves completely in [BMIM]Cl at temperatures of 12, 6.5, and 3 hours at 135, 155, and 175 °C, respectively. These findings suggest that temperature affects the solubility of hair.

In the studies that followed, keratin and melanin were extracted at the extraction procedures at the other two temperatures, which required significantly more time, so the dissolution temperature of 175 °C was chosen. To obtain the melanin precipitate, the solution containing the dissolved hair was added to 1 M HCl. One notable benefit of this process is that 1 M HCl

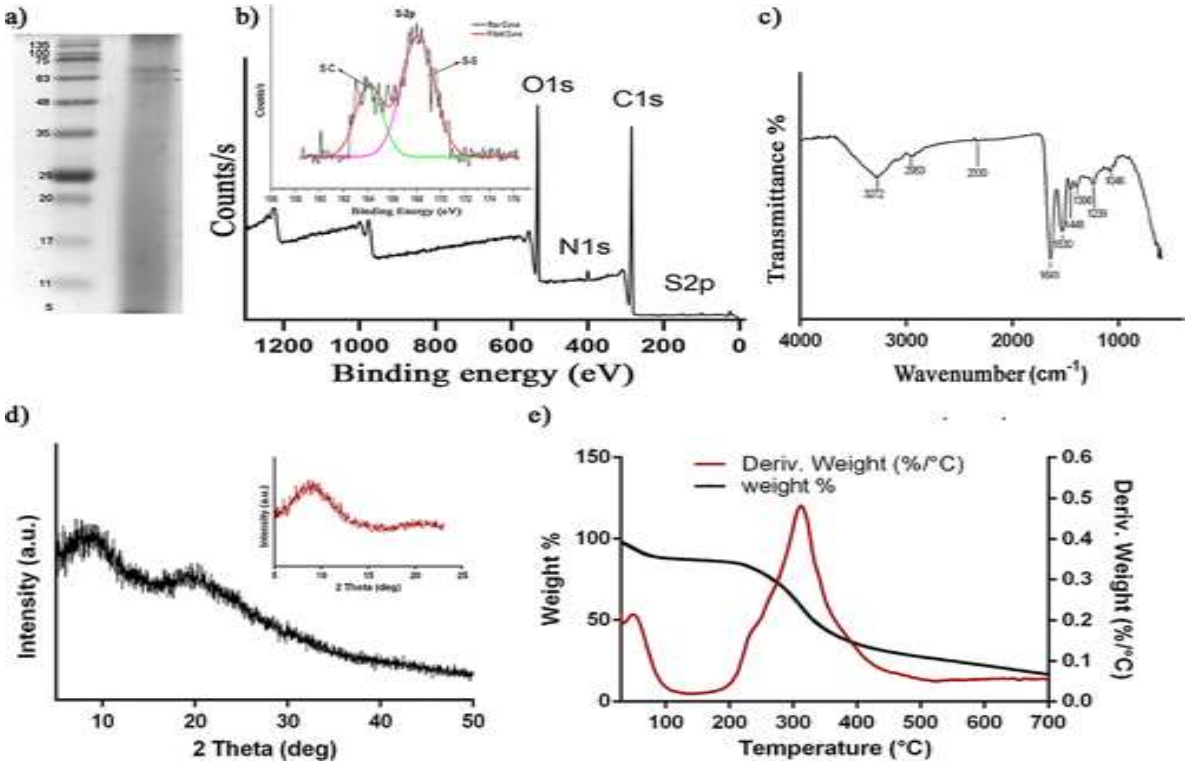
is used for the extraction of melanin rather than heated, strong acids, which can be dangerous and distort pigment morphology. Moreover, keratin is readily retrieved through straightforward supernatant dialysis. Melanin yield was 16.9% ± 0.1 and keratin yield was 43.8% ± 0.3 (n = 3) at 175 °C.

Our study's keratin yield is significantly higher than that of a prior study that extracted keratin using [BMIM]Cl and found that the yield was 18% at 180 °C.25 Furthermore, the extraction of melanin in addition to keratin was not mentioned in the report. Furthermore, it was discovered that the ions present in the dialysis (4870 ppm) and saturated sodium chloride solution, or PC, were greater in concentration ($p < 0.0001$) than the free ion concentration in the keratin (dissolved in Millipore water)., ensuring that the keratin obtained is of high purity. There were no discernible ions present in the Millipore water (NC). This demonstrates that when dialyzed against water, the IL was successfully removed from the supernatant, leaving behind keratin protein that had grown again.

Characterization of Keratin

Characterization of the regenerated keratin extracted from human hair using [BMIM]Cl in order to determine its crystallisation, molecular mass, thermal stability, and group function was done. By using SDS-PAGE analysis, it was found that the keratin's molecular weight was between 63 and 68–70 kDa. Additionally, smears are visible in the gel, most likely as a result of the high-temperature treatment used to dissolve hair in the IL and cause dissociations of particular protein configurations. Using wide-scan XPS, the extracted keratin's elemental composition was determined. The XPS analysis of keratin demonstrates that the extracted keratin contains fundamental elements.

Figure 2



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Characterization of human hair keratin extracted with an IL [BMIM]Cl. SDS-PAGE molecular weight analysis of keratin (a) and XPS analysis of regenerated keratin (b). Sulphur deconvolution data is shown in the inset. (c) Keratin ATR-FTIR peaks; (d) Regenerated Keratin and Hair XRD (inset); and (e) Regenerated Keratin TGA-DTG graph.

Hemocompatibility of Keratin

To determine the extracted keratin's ability to hemocompatibility in general and RBC rupture, a hemolysis assay was conducted. The powdered keratin had a substantially reduced hemolytic potential (1.8 ± 0.5%) in contrast to PC (94.4 ± 1.5%) and DI water ($p < 0.0001$, $n = 3$), based on the hemolysis assay graph (Figure 4). Therefore, it is suggested that keratin in its purest form is a haem compatible biopolymer that can be used to create various scaffold types for biomedical applications.

This work presents detailed physicochemical characterizations of the two biopolymers keratin and keratin as well as a green extraction method done in one step from human hair waste. Interestingly, full use of the response by products was additionally accomplished., confirming the viability of this environmentally approachable process. Although the elevated temperature utilised throughout the extraction process may have contributed to the structural disintegration of biopolymers, the physiochemical and structural integrity of keratin was maintained, as demonstrated by the fact that biological activity is predicated on structural integrity. The regenerated keratin powders can be regarded as hemocompatible because they did not significantly rupture the red blood cells. In the future, we hope to create haemostatic bandages—which can be applied to battlefields and accident scenes—using the regenerated keratin. Additionally, ILs were found and regenerated frequently, which ultimately made the process economical and sustainable. In summary, this research suggests utilising zero-waste green chemistry to extract bioresources from human hair that regenerate, having the capacity to create biomaterials.

Conclusion

In conclusion, our study shows that ionic liquid [BMIM]Cl is a dependable, environmentally pleasant approach for doing away with keratin and melanin from human hair. Keratin's intermolecular connections are broken during the hair disintegration system by means of [BMIM]Cl, which makes it less difficult to eliminate the protein. The procedure ensures little warmth deterioration, retaining the extracted keratin's structural integrity. Concurrent extraction of the vital biopolymer melanin is accomplished without using harsh chemical compounds, keeping the melanin's function and shape. The molecular weight, elemental composition, thermal stability, and organisation capability of the regenerated keratin are found through characterization investigations, which also validate its structural characteristics and purity. Interestingly, the retrieved keratin shows an excessive degree of hemocompatibility with fantastically little RBC rupture compared to controls, indicating that it may be used for numerous biomedical applications, including tissue engineering scaffold development. The one-pot extraction approach used right here demonstrates the opportunity for waste-free operations similarly to the effectiveness of ILs in sustainable biopolymer extraction. This technique has extra benefits for the environment and economy because of its ability to regenerate ILs for future extractions. These effects provide possibilities for extra research into different extraction strategies and the commercialization of biomaterials primarily based on keratin. Our research gives an achievable way to apply human hair debris to provide beneficial biopolymers that have the ability to be used in biomedical applications

via the use of zero-waste green chemistry ideas. This study highlights the capability for ecologically pleasant procedures to manufacture high-fee substances from renewable sources, contributing to the growing landscape of sustainable biopolymer extraction.

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