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The Extraction of Keratin from Human Hair Waste for Copolymerization with Nitrile Butadiene Rubber

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ABSTRACT

Human hair contains a rich source of a fibrous protein called α-keratin. Due to keratin's intrinsic ability to self-assemble and polymerize into porous, fibrous scaffolds and reproducible architecture, dimensionality, and porosity, its biomedical application has become widespread. However, the extraction of keratin from hair proves challenging and ineffective, hindered by its disulfide bonds. The purpose of this research is to explore a relatively simple procedure to extract keratin from hair waste and fuse it with NBR in hopes of enhancing its mechanical properties and improving its biodegradation. Keratin's non-covalent bonds were broken using mechanical disruption by a grinder. Hair keratin is then extracted via solubilization by utilizing sodium chloride. The alkaline hydrolysis method, a simple, cheap, and time-affordable method, is explored with different concentrations of NaOH. Then, the recovered keratin from each solution is mixed with dissolved NBR (Nitrile Butadiene Rubber), a well-known rubber used for a variety of purposes, such as the construction of hoses, belts, sealing parts, oil seals, oil wells, and shoe soles. A series of tests on the mechanical properties of the keratin-filled NBR samples were then tested, such as tensile strength, elongation at break, hardness, and elasticity. 10g NaOH/100mL solution yielded the highest amount of keratin, with the addition of keratin from the same solution in NBR enhancing the mechanical properties the most, although only modest. Additionally, the inclusion of keratin in NBR improves its biodegradability.

Introduction

Hair is thought to hold no use and is a common waste product throughout the world. It is treated and disposed of like waste in many hair salons and barber shops, as hundreds and thousands of hairs are thrown away annually. As hair decomposes slowly, it can also cause many environmental problems, such as choking drainage systems, emitting toxic gasses when burnt, espousing hair dust that can lead to respiratory problems, and becoming a breeding ground for pathogens (Reichl, 2009).

Human hair, though, contains a rich source of a fibrous protein called keratin, more specifically, α-keratin. α-keratin, commonly found in mammals, serves as the primary component of wool, hair, nails, hooves, and the stratum corneum (outermost layer of the skin). With its abundance and excellent physical properties, keratin holds the potential for producing biomaterials. However, because of its structure, the extraction and solubilization of keratin from hair waste are generally ineffective and very harsh (Chilakamarry et al., 2021).

Keratin's impressive durability, biocompatibility, and biodegradability have been explored greatly in biomedical, agricultural, pharmaceutical, cosmetic, and industrial applications. Examples include nerve conduit filler for peripheral nerve regeneration, hydrogels or films for wound healing, hemostatic agents, and scaffolds for tissue regeneration. Keratin's intrinsic ability to self-assemble and polymerize into porous, fibrous scaffolds and reproducible architecture, dimensionality, and porosity are crucial for cell growth. Additionally, since keratin solutions can be electrospinning to form three-dimensional fibrous scaffolds, keratin is structurally tunable at a macroscopic level (Donato et al., 2020).

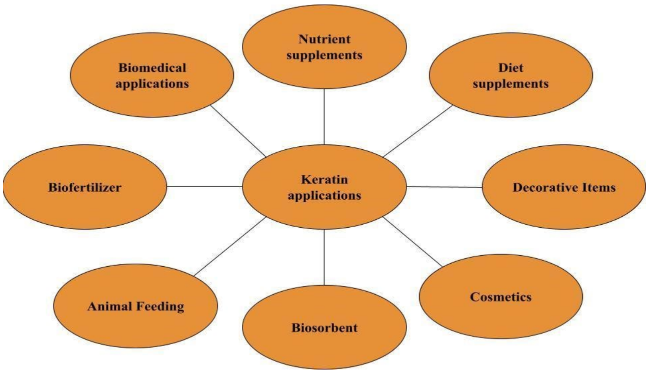


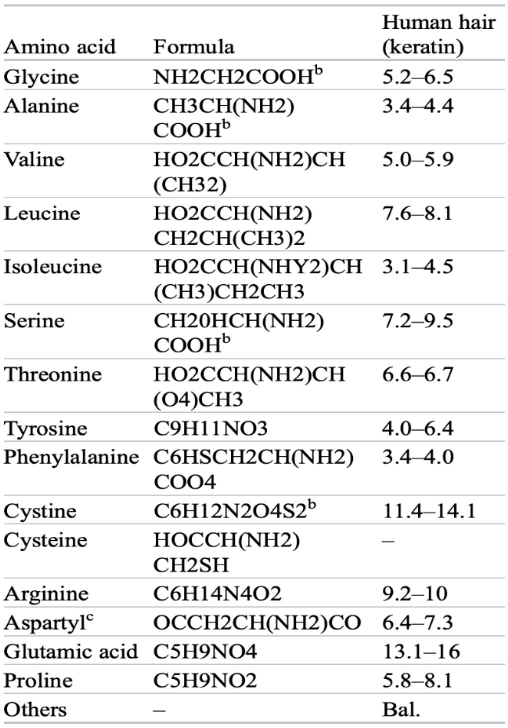
Figure 1. Keratin-based biomaterials

Hair is keratinized elongated fibers whose primary constituent are heavily crosslinked hard keratins. Each fiber is composed of three principal sections: the cuticle, cortex, and medulla. The thin, scaly, outermost layer of the fiber, the cuticle, mostly contains B-keratins that protect the follicle from physical and chemical damage. The main body of the hair fiber, the medulla, contains spindle-shaped cells rich with keratin filaments. Sometimes, the medulla is at the center of the hair fiber, which contains a column of loosely connected keratinized cells (Rouse & Van Dyke, 2010).

The hair fiber is made up of 50 to 60% α-keratin and 20-30% matrix proteins. The α-keratins fabricate together, forming micro-fibrous structures called keratin intermediate filaments (KIFs) that contribute to hair's toughness. The α-Keratins are rich in amino acids and include water-soluble and insoluble amino acids, such as alanine, phenylalanine, valine, isoleucine, and methionine. But α-Keratins are insoluble in water and organic solvents. Due to containing both acidic and basic groups, the protein is an amphoteric molecule (Horvath, 2009).

Amino acid residues are present in keratin. The acidic amino acid groups are aspartic acid and glutamic acid. However, more basic than acidic groups are present: arginine, histidine, and lysine. Reactive groups such as free carboxyl, amide, aliphatic and phenolic hydroxyls, and sulfhydryl groups can be found as well.

Cystine is the most reactive amino acid residue in keratin. Half cystine, the content of cysteine plus half cystine in original keratin, is a principal amino acid in α-keratin. The high content of half cystine provides disulfide bonds in α-keratin, distinguishes it as a high-sulfur protein from other biopolymers, and gives it its three-dimensional structure. Due to the abundance of keratin in hair containing a high content of cysteine (unlike other proteins), hair is durable, strong, and unreactive to the natural environment. Furthermore, intramolecular disulfide linkage and the intertidal peptidyl linkages of the α-keratin create rigid bonds that resist chemical reactions with insoluble properties.



Amino acid	Formula	Human hair (keratin)
Glycine	NH2CH2COOH^{b}	5.2–6.5
Alanine	CH3CH(NH2) COOH^{b}	3.4–4.4
Valine	HO2CCH(NH2)CH (CH32)	5.0–5.9
Leucine	HO2CCH(NH2) CH2CH(CH3)2	7.6–8.1
Isoleucine	HO2CCH(NHY2)CH (CH3)CH2CH3	3.1–4.5
Serine	CH20HCH(NH2) COOH^{b}	7.2–9.5
Threonine	HO2CCH(NH2)CH (O4)CH3	6.6–6.7
Tyrosine	C9H11NO3	4.0–6.4
Phenylalanine	C6HSCH2CH(NH2) COO4	3.4–4.0
Cystine	C6H12N2O4S2^{b}	11.4–14.1
Cysteine	HOCCH(NH2) CH2SH	–
Arginine	C6H14N4O2	9.2–10
Aspartyl^{c}	OCCH2CH(NH2)CO	6.4–7.3
Glutamic acid	C5H9NO4	13.1–16
Proline	C5H9NO2	5.8–8.1
Others	–	Bal.

Figure 2. Amino acid content for human hair (keratin), note high Cystine, which is associated with disulfide bridge formation (Murr, 2014).

The alkaline hydrolysis method for the extraction of keratin from hair is composed of a strong alkali solution catalyzed by heat (Reddy et al., 2020). A simple and conventional method, this process involves alkali hydrolysis at a high temperature.

NBR is a synthetic rubber made from acrylonitrile and butadiene. NBR's resistance to oils, fuels, and other chemicals singularize it from other natural rubbers. In addition to its resilience, NBR has superior strength but inferior flexibility (Threading Ham et al., 2011). Its stability in high temperatures as well has found applications in aeronautical engineering. Besides aeronautical engineering, NBR also has uses in nitrile gloves, hoses, seals, grommets, and seals, grommets, and self-sealing fuel tanks.

This study presents the findings of keratin derived from human hair waste collected from local barber/salon shops fused with NBR, along with its mechanical properties. Overall, the new polymer is a biodegradable, sustaining material suited for many potential applications.

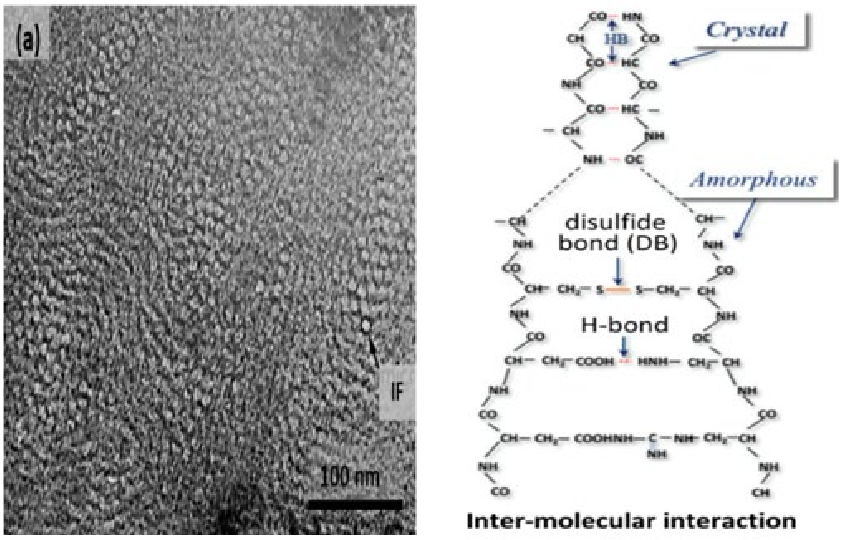


Figure 3. Cross section of human hair (α-keratin) stained with osmium tetroxide (to the left). Hierarchical structure, intermolecular bonds, and crystals formed within hair keratin (to the right)

Materials

Extraction and Purification

- Hair waste: Retrieved from barber hair salons/barber shops in the local area
- NaOH (sodium hydroxide): drain cleaner: Retrieved from PETAA group
- NaCl: Retrieved from MS Fine Chem
- HCl (hydrochloric acid): retrieved from ThermoFisher Scientific
- Mortar Grinder: Retrieved from home
- Scissors
- Funnel
- Graduated cylinder
- 250mL Erlenmeyer flasks Glassware
- Litmus blue pH paper
- Pot
- Stove
- Stainless-Steel Steamer

Polymerization

- MATNIKS Nitrile Rubber Sheet (NBR)
- Klean-Strip Acetone 95% Pure Low VOC
- 3 Mason jars
- Pot
- Stove
- Stainless-Steel Steamer
- Thermometer

Properties

- PCE-MTS500
- Active compost soil
- Large container
- Humidifier
- Thermometer

Methodology

Human hair samples were retrieved from hair salons/barber shops in the local area. We categorized our experiment into two procedures: extraction, purification, and polymerization. The total amount of hair amassed was 5.08 grams.

Extraction and Purification

First, we cut the hair with scissors into a fine powder to maximize the surface area for dissolution. Then, we broke non-covalent bonds in keratin from the powder hair with 10.00g NaCl by mechanical disruption using a mortar grinder. In essence, using sodium chloride, the solubilization of keratin from the hair occurred. Mortar grinding led to a homogenous mixture of fine hair powder and NaCl, with 14.98g being retrieved.

Three different solutions were created with different concentrations of NaOH and distilled water: 20.00g/100mL; 15.00g/100mL; and 10.00g/100mL, respectively. Three equivalent masses from the 14.98g of powder hair and NaCl were divided into three separate solutions. A pot of water was heated to 70 degrees Celsius for 3 hours, and a stainless-steel steamer was placed at the bottom for the three separate solutions.

The disulfide bridges in keratin were broken by the reduction of cysteine by NaOH. The change in colorization from black hair in a clear, milky white solution into a dissolved, completely black solution is evidence of the effectiveness of the solubilization. The percent solubilization was calculated by:

$$100 - [(mass\ of\ precipitate / mass\ of\ solution) \times 100]$$

Figure 4. Fine-powder hair and NaCl before and after grinding with a mortar grinder

Once the keratin had been dissolved in the NaOH solutions, 77.16 mL, 115.74 mL, and 154.32 mL of 3.24 molar concentration HCl were added to reach a pH of 7 for the 10g/100mL, 15g/100mL, and 20g/100mL NaOH solutions, respectively. This neutralization was proven with the use of a universal litmus pH paper. The following solutions were dried to result in white powder. The powders were then washed with ice cold water following ice cold IPA to result in purified keratin powder to remove any other chemicals. The final masses were collected to calculate the percent yield, which was calculated by:

$$(mass\ of\ solute\ /\ original\ mass\ of\ hair) \times 100$$

Figure 5. NaOH solutions after heating to 70o C for 3 hours. From left to right: 10g NaOH /100mL, 15g/100mL, 20g/100mL

Polymerization

6.40g of NBR was amassed and was then shredded almost into a fine powder using an Anymill Plus KA-2400 Grinder. 250mL of Klean-Strip acetone was then measured. In total, 3 samples were created for the three different recovered keratin. Both the shredded NBR and acetone were placed in a sealed mason jar and left for 2 days at room temperature.

To speed up the dissolution process, a pot of water was heated to 50 degrees Celsius, and a stainless-steel steamer was placed at the bottom for the mason jar to sit. During this process, equal masses of recovered keratin were added. After 1 hour, the solids were dried again at 25 degrees Celsius (room temperature) for 2 days.

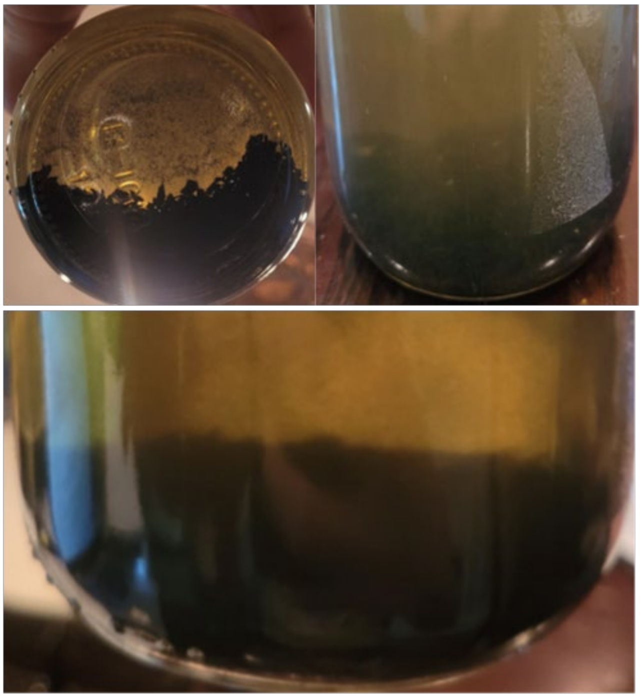


Figure 6. One solution of 6.40g of NBR and 250mL of acetone a. first day: clear, pickle-green solution b. second day: murky, dark green solution

Mechanical Properties

Mechanical properties were tested according to ISO 37:2017 with a PCE-MTS500. Samples shaped in dumbbells with a width of 4mm wide were tested for tensile strength and elongation at break.

Shore's hardness was determined according to ISO7619-1, and Zwick/Roell's ZwickRoell Shore Type A with 12,5 N was used. Schob's method, testing resilience or elasticity, was carried out according to ISO standard 4662.

Biodegradation

For biodegradation, the samples were placed in active compost soil for 30 days, kept at 30 degrees Celsius, and 75% Relative Humidity. Afterward, the samples were taken out, and changes to the samples were observed. Furthermore, the soil-incubated samples were tested for tensile strength and elongation at break.

Results and Discussion

Extraction and Purification

The 10g/100mL NaOH solution solubilized the hair the greatest, yielding the most keratin. The lower the concentration of NaOH, an alkaline, the higher the percent solubilization. Additionally, the 10g/100mL NaOH solution had the lowest pH due to having the lowest concentration of NaOH. Generally, the yields of each concentration are considerably low. According to Shavandi et. al, the extensive hydrolysis of the protein polypeptides and the degradation of amino acids, including cysteine residues, contribute to the loss of a large amount of protein content (Shavandi, 2016).

Table 1. The yield of keratin during extraction

NaOH	**10g/100ml**	**15g/100ml**	**20g/100ml**
Solubilization (%)	98.22	76.82	84.67
Yield (%)	12.82	8.68	10.54
pH	11	12	13

The pH indicates the acidity or alkalinity of a solution, measuring the H+ (H3O+) concentration in a solution. A specific amount of acid has to be added to reach neutrality (pH = 7) from a basic solution. In addition to the potential loss in keratin yield mentioned above, the high NaOH and HCl concentration used is likely correlated with the low yield of keratin due to the increase in ionic concentration within the sample solution overall. The separator ions formed from the neutralization process (Na+ and Cl-) could disrupt the secondary and tertiary bonds between amino acids resulting in unstable folding of amino acid a-helix structure.

Mechanical Properties

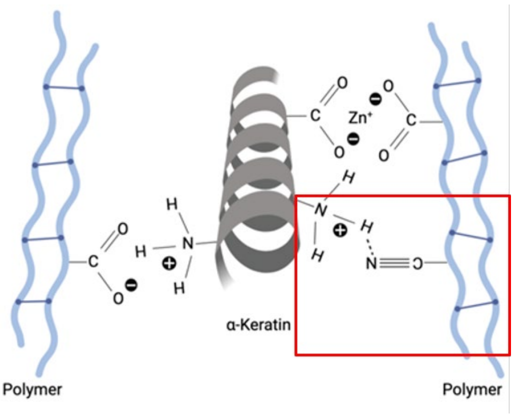


Figure 7. Possible interactions between α-Keratin and nitrile butadiene polymers (Prochoń, 2013) (Das et al., 2020)

Both Prochoń et al. and Das et al. depict possible coordination between ZnO and proteins as fillers for XNBR (carboxylated nitrile rubber). However, this study excludes ZnO and XNBR and rather focuses on Keratin (α-Keratin) and NBR. Carboxyl groups shown in the figure above are absent in NBR, but cyanide groups (CN^-) are present. The positively charged amine group (NH_3^+) in α-Keratin interacts with the negatively charged cyanide group to form an ionic bond, potentially producing crystal lattice structures.

Table 2. The effect of keratin on the mechanical properties of NBR

Parameters	**NBR**	**I**	**II**	**III**
Tensile Strength (MPa)	2.20	2.24	2.22	2.21
Elongation at Break (%)	348	352	351	349
Hardness (IHRD)	46.2	47.5	47.3	47.1
Elasticity (%)	26	23	24	24

- Sample I: Keratin extracted from 10g/100ml NaOH Solution with NBR mix
- Sample II: Keratin extracted from 15g/100ml NaOH Solution with NBR mix
- Sample III: Keratin extracted from 20g/100ml NaOH Solution with NBR mix

For tensile strength and elongation at break, Samples I, II, and III generally had higher values than NBR. However, the difference is not significant, especially considering the largest difference (.04) between Sample I and NBR in tensile strength and again for elongation at break. But the addition of keratin in NBR composites does improve their mechanical properties.

Samples I, II, and III all have higher hardness values and lower elasticity values than those of NBR. In terms of hardness and elasticity, the addition of keratin clearly hardens and stiffens the NBR polymer.

The influential ionic interactions between the amine groups of α-Keratin and cyanide groups of NBR demonstrated in Figure 7 could explain the increase in NBR's mechanical properties. The ionic bonds between α-Keratins and the NBR would strengthen the molecular structure throughout the rubber.

All three Keratin-filled NBR samples retain attain higher mechanical property values than NBR alone, but a trend is noticed where Samples II and III hold lower values than Sample I. All three samples were treated with equivalent amounts of recovered Keratin, and a variable that differs between the three is the NaOH concentration that the Keratins were extracted from. The hydrolysis of the protein polypeptides and the degradation of amino acids could disrupt the molecular structure of α-Keratin. So, when the concentration of an alkaline (in this case, NaOH) in a solution increase, it is more likely the α-Keratin's structure deforms. This concept also impacts and is manifested in the lower degree of enhancement in values for Samples II and III, III's values only deviating the least from NBR's. The Keratin in Sample III, therefore, made the least significant change in the mechanical properties of NBR. Furthermore, Keratin's structure in Sample III is most likely the most damaged due to being solubilized in the highest (strongest or most basic) alkaline concentration (20g NaOH/100mL), the Keratin's polypeptides being extensively hydrolyzed.

Biodegradation

Table 3. The effect of keratin on the mechanical properties of NBR after soil incubation test

Parameters	**NBR**		**Sample I**	
	Before	After	Before	After
Tensile Strength (MPa)	2.20	2.16	2.22	1.97
Elongation at Break (%)	348	323	352	330

After soil incubation, the mechanical properties such as tensile strength and elongation at break weakened. The tensile strength of the NBR sample did not change much, but Sample I underwent a significant change (≈11%) in tensile strength. Therefore, the addition of keratin increases the degree of biodegradation in NBR.

Additionally, after soil incubation, Sample II was seen with more blisters and holes, unlike NBR, which mostly remained unchanged except for a color change.

As previously mentioned in section 4.2, the ionic bonds formed between the amine groups in α-Keratin and the cyanide groups in NBR could potentially arrange in a crystal lattice structure. Although α-Keratin and NBR may be water-insoluble, the ionic bonds that form between them increase the water adsorption of the polymer, as ionic bonds are water-soluble. According to Prochoń et al., the water adsorption of the protein increases the biodegradation.

Conclusion

Through this study, we proved that hair can be used to extract keratin through the alkaline method. Keratin would then be utilized as an additive with Nitrile Butadiene Rubber (NBR). Adding keratin to the nitrile butadiene rubber slightly improved the mechanical properties of NBR. To some extent, the pollution of hair waste can potentially decrease because of using keratin in biopolymers to some extent. The biodegradation of NBR with keratin is boosted under earthly conditions due to water adsorption made possible by the proteins in α-Keratin, further indicating that it can naturally deteriorate in the environment safely.

The alkaline hydrolysis method of extracting keratin is relatively simple but rather inefficient. The procedure did not demand many materials but yielded low amounts of keratin. Large amounts of a strong acid were also required to neutralize and solutions. Despite these drawbacks, the alkaline method is fast and relatively cheap compared to other methods.

Our study has proven that keratin, an additive to NBR, has enhanced the mechanical properties of the standard NBR. This combination has a wide variety of applications in the production of oil-resistant products. It would also be environmentally friendly because of its biodegradability. However, further research and tests should be done to explore keratin + NBR's properties and its cost-benefit analysis.

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