



## Review Article

# Preparation methods keratin and nanoparticles keratin from wool: a review

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### ABSTRACT

Keratins are a group of fibrous proteins. These proteins are abundantly present in nature and constitute the major part of the hair, wool, horns, nails, feathers, and the stratum corneum of the skin. Among others, an ecological and economic point of view, recovery of many organic substances discharged into the effluent of textile mills is of prime importance. This thesis review certain physical and chemical characteristics of the keratin derived utilizing five processes of chemical extraction: reduction, hydrolysis, sulfitolysis, oxidation, and production. Since the keratin is a group of fibrous protein and has many useful applications. For example: In pharmaceutical industries, plastic production, biosorption, and tissue engineering. Considering this advantage, this work mainly concentrates on the nanoparticle keratin synthesis through different methods.

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## 1. Introduction

Environmental concern and growing demand for healthy and sustainable bio-based materials are prompting the search for better and "green" ways to utilize natural by-products available [1].

Keratin is one of the most plentiful and underexploited sources of protein, as the main component of fur, hair, nails, hooves, feathers, and horns [2]. On her head, keratin is present in various sources in nature, over 5,000,000 tons/year, and is accessible from the clothing, butchery, and breeding industries [3, 4].

The bird chicken feathers consist of about 90 % of the structural protein, keratin, which is not a single material, but a complex protein mixture [5].

The two most abundant forms of keratins are called  $\alpha$ -keratins (contains  $\alpha$ -helical structure) and b-keratins (contain a mixture of  $\alpha$ -helical and  $\beta$ -sheet structures) [6].

$\alpha$ -Keratin is found most commonly in mammals. Such type

of structure is structured as a coiled-coil and thus participates in the elongated polypeptide chain in the compact structural arrangement. On the other side, the  $\beta$ -structure holds intermolecular bonding of hydrogen between the amino and carbonyl groups [7]. Keratins are characteristically highly stable and low solubility due to the –S – S– crosslinking of residues of cysteine amino acids [8, 9].

The most distinguishing characteristic of keratin at the molecular level is the high concentration of half-cystine residues (7%-20% of total amino acid residues), most of which are found in the protein terminal regions [10].

Moreover, keratin extracted using different extraction processes can find applications in numerous sectors, the denaturing extraction methods are classified into reductive or oxidative methods, sulphites- or phosphines-mediated extraction[11], enzymatic hydrolysis[3] and ionic liquids

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[12, 13]. This is to prepare the keratin nanoparticles that have important applications in biotechnology.

And on this, a solution might be to synthesize magnetic keratin nanoparticles (MKNPs). As such, MKNPs were prepared via  $\text{Fe}_3\text{O}_4$  in situ synthesis using the co-precipitation method to overcome the handling and separation disadvantage of KNP in the environmental applications [14]. The extracted keratin was further treated with silver nitrate and zinc oxide for the synthesis of nanoparticles (KNPs) [15].

Among the preparation of Ru-Qr dual drug-loaded keratin nanoparticles for biological applications by (SDS or Sodium lauryl sulfate or Tween-20) under magnetic stirrer. Nanoparticles thus formed turned the solution turbid. Recombinant keratin nanoparticles (RKNPs) were therefore prepared using an ultrasonic dispersion method to determine their wound-healing effect [16, 17].

Too, keratin particles have also been prepared with this in mind the effect of important electrospaying conditions such as polymer concentration, feed rate, voltage, and nozzle-collector distance on the average particle size of the electrospayed nanoparticles [18].

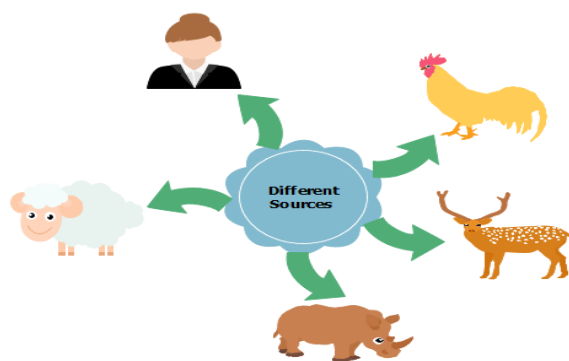


Fig 1. Different sources of keratin.

## 2. Extraction Methods

Keratin can be obtained by different extraction methods (Fig. 2).

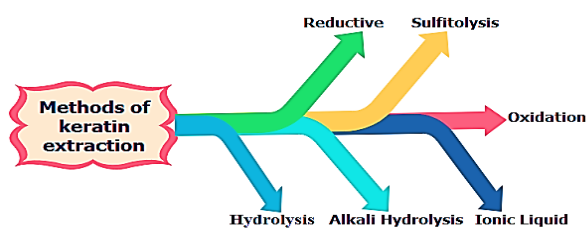


Fig. 2. Methods of keratin extraction.

### 2.1. Reductive Extraction

Some of the benchmark reduction processes for obtaining semi-native keratins appropriate for biomedical applications include wool solubilization with 2-mercaptoethanol (2-ME), as a chaotropic agent in a solution buffered at pH= 9.5, and 8 M urea; the alkali media induces partial unfolding of the tightly packed keratin composition and favors the reduction reaction [19]. More extraction includes extracting reducing agent and denaturant from either the reaction mixture through dialysis where there is an undesirable association of keratin peptides. And it was done analyzed the molecular structure of keratin derived from wool through reduction: extracted from SDS-PAGE electrophoresis, molecular weight proteins homologs as well as 40–60 kDa.

### 2.2. Sulfitolysis Method

At 65 °C, 4 g of wool fibers were handled with 100 mL of an aqueous solution comprising urea (8M), sodium metabisulfite (0.5 M), modified to pH=7 with NaOH (5 M) under shaking using a 2.5 h Linitest unit. The mixture was purified through a 5 mm pore-size filter and dialyzed against sterile water in a circulation system with the cellulose dialysis tube (molecular weight cut off 12–14 kDa) [20].

### 2.3. Oxidation Method

The process by which keratin was extracted from wool was modified as follows. Fibers in 2 % peracetic acid are oxidized overnight at 37 rpm and 180 rpm, then drained and washed with water. The oxidized fibers were then removed 2 rpm with 100 mM Tris-base (pH 10.5) at 37 rpm for 3 h and 180 rpm, and 1 rpm with water. Samples were collected from 0.5 M HCl to precipitate protein and modified to pH 4. It has required precipitated protein to settle overnight. The liquid phase was decanted and the precipitate was washed at pH 4 of 100 mM Tris-HCl and then redissolved at Tris-base of 100 mM, pH ~ 7.5 [21].

### 2.4. Hydrolysis Method

Keratin hydrolysate was processed using a 400 mL ceramic autoclave put in a microwave oven, Landmark Ethos 1600 (Milestones S.p. A., Bergamo, Italy), to handle 40 g of wool in 150 mL superheated water for 30 minutes at 170 °C. At the end of the cycle, the autoclave was cooled, and a 120 mesh stainless steel sieve filtered the resulting liquid. Further filtering of the liquid process was performed using a tangential flow filter (Millipore Pellicon XL) of 0.65 mm pores [20].

Table 1. keratin extraction by different methods [11].

Methods	Extraction solution	Operating Conditions	Yield (%)	References
Reduction	Urea=8 M; Thioglycolic acid=0.2 M; pH=11	Temperature (°C) = 50°C ; Time(h)=3h	70	[1]
	Urea=8 M; DTT=0.2 M EDTA=3 mM; 0.2 M Tris-HCl pH= 9	Temperature (°C) = 20°C ; Time(h)=16h ; Ratio (solid:liquid) (w/w) = ///	60	[23]
	Urea=8 M; DTT= 0.14 M; 0.05 M Tris-HCl pH= 8.6	Temperature (°C) = 25°C ; Time(h)=4h ; Ratio (solid:liquid) (w/w) = 1/50	50	[4]
	Urea=8 M; L-cysteine =0.165 M; NaOH up to pH= 10.5	Temperature (°C) = 75°C ; Time(h)=12h ; Ratio (solid:liquid) (w/w) = 1/10	72	[24]
	TCEP/Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub>	Temperature (°C) = 80°C ; Time(h)=5h	80	[25]
Alkaline Hydrolysis	Wool (10 g) treated with 2 wt% NaOH with HCl to pH = 7	Temperature (°C) = 80°C ; Time(h)=3h	25	[1]
	0.5 N NaOH (pH 13.9)	Temperature (°C) = 62-65°C ; Time(h)=3h ; Ratio (solid:liquid) (w/w) = 1/20	/	[26]
	Wool mixed with an alkali the solution of KOH: NaOH (14:1) at 0.5 to 3% (w/v)	ultrasonic irradiation for 30 min using a 20 kHz sonicator (40% amplitude of 750 W)	100	[27]
	NaOH solution (10 g/L)	Temperature (°C) = 120°C ; Time(min)= 10min ; Ratio (solid:liquid) (w/w) = 1/10	53.5	[28]
Ionic Liquids (ILs)	[Bmim][Cl]	Temperature (°C) = 120°C ; Time(h)= 0.5 h ; Ratio (solid:liquid) (w/w) = 1/6	57	[11]
	[Bmim][Cl]	Temperature (°C) = 120°C ; Time(h)= 0.5 h ; Ratio (solid:liquid) (w/w) = 1/10	78.5	
	[Bmim][OAc]	Temperature (°C) = 120°C ; Time(h)= 0.5 h ; Ratio (solid:liquid) (w/w) = 1/10	16.8	

	[Emim][DEP]	Temperature (°C) = 120°C ; Time(h)= 0.5 h ; Ratio (solid:liquid) (w/w) = 1/10	70.2	
	Urea=8 M; Sodium metabisulphite= 0.5 M; NaOH up to pH=6.5; SDS (g)/wool = 0.6	Temperature (°C) = 60°C ; Time(h)=5h ; Ratio (solid:liquid) (w/w) = 1/15	41	[1]
Sulphitolysis	Urea=8 M; Sodium metabisulphite=0.5 M; NaOH up to pH = 6.5	Temperature (°C) = 100°C ; Time(h)=5h ; Ratio (solid:liquid) (w/w) = 1/30	22	[29]
	Urea=8 M; LiBr=0.1 M; SDS= 0.02; NaOH pH=12	Temperature (°C) = 90°C ; Time(h)= 4 h	50	[30]

### 2.5. Alkali Hydrolysis

Alkali hydrolysis was conducted at 80 °C with 2 % NaOH (wt.) until the fibers were fully solubilized in 3 h. The sample was then neutralized, centrifuged, dialyzed, and lyophilized with HCl. This process has the lowest keratin extraction yield (~25 %) determined by the ratio between the weight after lyophilization and the original weight of the wool relative to the other procedures; It is possibly due to extensive protein hydrolysis that would lead to a large amount of protein lost during dialysis [1, 11].

### 2.6. Ionic Liquid

The extraction of keratin using BMIM was performed to use a process described earlier. In short, wool fiber snippets were gradually added to BMIM IL at 130 °C at a ratio of 1:10 (w / w) wool to BMIM after 120min full dissolution of fiber was observed [12]. The resultant hot viscous solution was decanted to precipitate the keratin protein into distilled water at room temperature [22].

It has also been found, in general, that the degree of the success of the reaction depends strictly on the nature of the extract solution, the method variables, and the form of starting keratin biomass wool (Table 1).

## 3. KNP Synthesis

### 3.1. Method 1

In deionized water, keratin powder isolated from wool and doxorubicin hydrochloride (DOX) was dissolved separately at a final concentration of 1 mg / mL and 2 mg / mL respectively. Then, using a 450 nm filter cut-off, the keratin aqueous solution was filtered and held under

stirring 730 rpm during the dropwise application of varying quantities of doxorubicin. The sum of keratin-related doxorubicin added varied from 5 to 45 wt%. The mixture

was held for 60 min under stirring and deposited overnight at 4 °C. Afterwards, the mixture was dialyzed against deionized water with a cellulose membrane (cut off 12–14 kDa) for 24 h to extract free doxorubicin and then centrifuged for 10 min at 4000 rpm to eliminate particle aggregate (Fig.3). Finally, a specified supernatant volume was frozen, and the resulting powder was weighted to determine the yield of keratin nanoparticles [31].

### 3.2. Method 2

The keratin sponge was dissolved in formic acid (0.3% w/v) at 70 °C for 24 h under magnetic stirrer to prepare the electrospaying solutions (Fig.4). Keratin sponge will only be dissolved up to 5 percent in formic acid. Considering that no prior knowledge on electrospaying keratin solution was available, a spectrum was considered for each of the essential parameters of electrospaying; In specific, test and error concentration (levels of 0.5, 1.5 and 2% w/v), voltage (15, 20 and 25 kV), nozzle tip to collector distance (10, 15 and 25 cm) and feed velocity (0.02–0.04 ml / h) and the mixture of conditions were chosen for the processing of small-size electrospayed keratin nanoparticles. For the concentration of keratin polymer in the electrospaying solution, And the distance between the nozzle-collector and the total particle size of electrospayed nanoparticles. Tests revealed that for the electrospaying keratin nanoparticle, the correct concentration of keratin in formic acid was around 0.5% (w / v) [18].

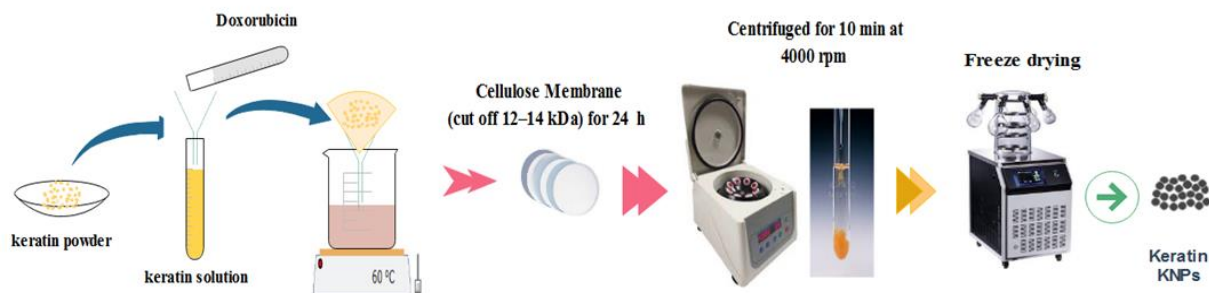


Fig. 3. Preparing nanoparticles for Keratin by doxorubicin.

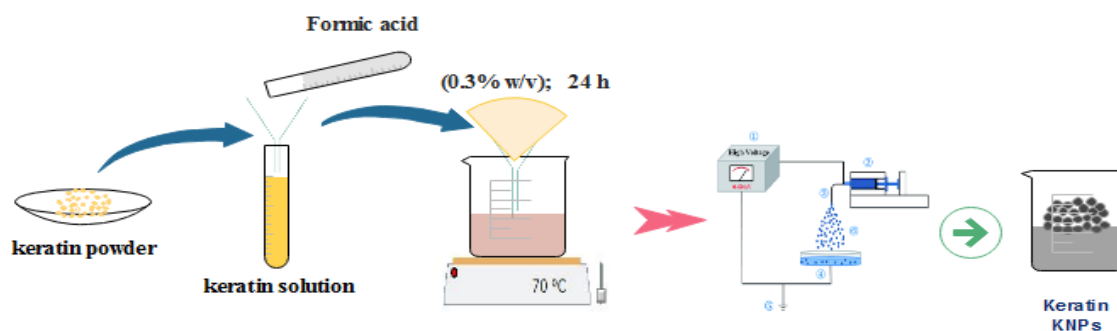


Fig. 4. Synthesis of nanoparticles Keratin by electrospaying.

### 3.3. Method 3

Synthesis of magnetic keratin nanoparticles (MKNPs), 0.540 g of ferric chloride, and 0.199 g of ferrous chloride (2:1 molar ratio) are dissolved in aqueous KNP dispersion (0.5 g / L, 100 mL). A three-necked synthesis flask was used. The solution was mixed and degassed for about 30 min with the use of argon as a neutral gas. The chemical co-precipitation of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  happened after immediately applying 7 mL of ammonium hydroxide at an average temperature of 22 °C and for 30 min under intense stirring at 1000 rpm. The MKNPs were isolated using a magnet after the reaction, and washed with deionized water three times [14].

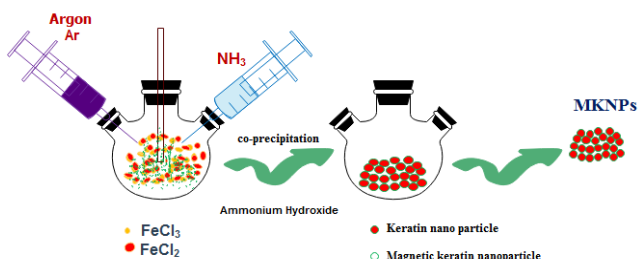


Fig. 5. Synthesis nanoparticles for Keratin by  $\text{Fe}_3\text{O}_4$ .

## 4. Conclusion

Keratin is one of the easiest natural materials to fill in as an effective protective surface layer, even though it is made simply of proteins. It shows incredible potential for new bioinspired models in which there is an intense knowledge of the natural chemistry, structure, and mechanical properties.

Developing techniques for effective poultry biomass production of keratin should prove to be very useful for the sustainable management of huge waste. Researchers are working independently and in combination, mode to establish different chemical, biological, and physical methods for keratin extraction. Denaturing techniques, such as methods based on reduction, oxidation, and sulphitolysis, are the benchmark for obtaining good yields and undamaged keratins.

Keratin-based materials are commonly used in the form of powders, micro/nanoparticles, keratin nanoparticles, and samples of an average particle size of 36–72 nm were processed using electrospay techniques. To sum up, the anticancer drug doxorubicin was successfully encapsulated into keratin nanoparticles using ionic gelation and aggregation processes, magnetic keratin was synthesized as

a new nanocomposite to overcome the problem of handling and isolation coexisting with keratin nanoparticles (KNPs). The nanocomposite was prepared in a dilute aqueous solution of KNPs by the in-situ synthesis of Fe<sub>3</sub>O<sub>4</sub>.

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