Preparation and characterization of rabbit hair keratin hydrogel

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Abstract. Protein-based hydrogel with the characters of environmentally friendly, resource saving and excellent biocompatibility has become a hot topic in the field polymer materials. In this paper, the rabbit hair keratin was extracted from waste rabbit hair and then prepared into hydrogel by means of heating-cooling. The conclusion is that the molecular weight of rabbit hair keratin measured by SDS-PAGE concentrated in 40~60kDa and rabbit hair keratin hydrogel in 11~22 kDa. The forming conditions such as concentration and temperature of preparing hydrogel were experimented by using variable-controlling approach, the formation concentration of keratin should be over 125mg/ml and the temperature over 55°C. Various characterization techniques were used to determine the physical and chemical property of the hydrogel. As proved by scanning electron microscopy (SEM), covalent bonds and three-dimensional porous structure with many interconnected pores had been successfully formed. They were also characterized by thermogravimetric analysis (TG) and swelling test, etc. The consequence showed that the equilibrium moisture content can reach up to 20.21% and the swelling rate as high as 9.55%, which were inversely proportional to the concentration of keratin. Furthermore, the hydrogel had good thermal stability, the initial decomposition temperature is around 204°C. It is indicating that the excellent properties of rabbit hair keratin hydrogel can be further studied.

1. Introduction

Hydrogel is a kind of super-absorbent materials formed by polymer monomers crosslinking by physical or chemical methods. The three-dimensional porous structure, that acts as a skeleton, inside hydrogel can lock in moisture by surface tension. So, hydrogel is a polymer material with strong water absorption and good water retention property. There are numerous hydrophilic groups attached to the network structure to remain hydrophilicity [1]. During absorbing water, the polymer chains form a gel network by crosslinking, which makes hydrogel not to dissolve. That is, hydrogel can absorb water that is hundred times of itself and only volume expands, not dissolves [2]. When the hydrogel full swelling, the softness of it is very similar to the human organic tissues’. Hydrogel also has high permeability to sugar, vitamin, oxygen and other nutrients [3]. Besides, some hydrogels have low hardness and high elasticity, some show good biocompatibility and biodegradability. Based on these characters, hydrogel material can be widely used as a raw material in the area of surgical dressing [4], Human tissue scaffold [5], Agricultural drug release [6], Dye processing [7], Dye processing [7], metal ion absorption [8] and other fields. However, most widely used hydrogel materials cannot meet the requirements of environment-friendly and resource-saving currently, whose degradation products are toxic and
harmful to humans and biocompatibility is poor [9,10]. These limit the application scope of the materials severely. Finding a natural bio-based hydrogel material becomes a hot topic.

Keratin is a kind of structural proteins, which extensively exists in nature. It is usually divided into hard keratin and soft keratin. The hard keratin with a sulphur content among 4%~8% is founded in hair, feathers, hooves, shells claws and horns. The sulphur content of soft keratin is about 2%, which exists in the epidermal skin. Rabbit hair belongs to hard keratin, it has a mass of cystines, forms a disulphide bond by crosslinking, and plays an important role in the physical and chemical properties of keratin. The rabbit hair textile industry in China produces hundreds of tons of rabbit hair waste every year [11]. These scraps are rich in proteins in which the content of keratin is above 90% [12]. If people recycle the rabbit hair, extract the keratin and prepare hydrogel using the materials, it can not only reduce the problem of resource-wasting, but also solve the shortcoming such as poor biocompatibility of hydrogel materials.

This study choose keratin extracted from waste rabbit hair as raw material, method of heat-cooling to prepare the keratin hydrogel materials. Then test the property of swelling, thermal stability and any others, and aim to seek for a way to produce a natural non-polluting protein-based hydrogel.

2. Material and methodology

2.1. Materials

Rabbit hair (Shandong) used to extract keratin. Urea, NaOH, L-cysteine, Ethanol, KBr and Polyethylene glycol 20000 were purchased from Tianjin kermel chemical reagent co. LTD

2.2. Production of keratin hydrogel

Weight the keratin powder for 1.5g, 1.75g and 2.0g respectively, add 10ml distilled water and 0.03g L-cysteine, put the breaker into a thermostatic magnetic mixer, heat for 30 min, then cool down at room temperature. It produces three different concentrations hydrogels as 125mg/ml, 150mg/ml and 175mg/ml.

For another set of experiments, keep the mass fraction consistent at 15%, change the temperature in 55℃, 65℃, 75℃, repeat the above steps and then produce the hydrogels.

2.3. Analysis and characterization

2.3.1. SDS-PAGE. The molecular weight distribution of the rabbit hair keratin was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). First, 15% separation gel and 5% concentrated gel were prepared on the basis of the proportion in Table 1, injected into a mould, placed for a period of time and waited the solution becoming gelatinous. Next, added 10μl of samples into the sample tank, set the constant voltage of 80V until the sample passed the concentrated gel, next adjust the voltage to 120V, till the text finished. Next, took out the adhesive block and dyed in the dye solution for 3hours. Then, decolorized in the decolorizing solution until the bands of the keratin samples can be observed clearly.

2.3.2. SEM. The surface morphology of keratin gel was observed by scanning electron microscope (SEM). Put the samples of keratin powder and hydrogel into the sample chamber, adjusted the contrast ratio, brightness and focal length until the image clear, and then took the pictures.

2.3.3. The swelling ratio. Gravimetric method is used to calculate the swelling ratio of the keratin hydrogel at room temperature. Select distilled water and PBS buffer solution for swelling experiment, respectively. Weigh 8g NaCl, 0.2g KCl, 3.63g NaHPO₄ and 0.24g KH₂PO₄ in 1L distilled water as buffer solution (pH= 7.2). The initial weight of the two pieces of the fully dried hydrogel is both denoted as M₀, one is soaked in 20ml distilled water and another in PBS buffer solution. Weigh once
an hour until the weight stable. The first one is denoted as $M_1$, another as $M_2$. The swelling ratio in distilled water ($R$) of keratin hydrogel was calculated using the following equation (1):

$$R = \frac{M_1 - M_0}{M_0} \times 100\%$$  

(1)

Where $M_0$ and $M_1$ denote the weight of the freeze-dried hydrogel before and after being submerged in the distilled water, respectively.

And the swelling ratio in PBS buffer solution (SR) of keratin hydrogel was calculated using the following equation (2):

$$SR = \frac{M_2 - M_0}{M_0} \times 100\%$$  

(2)

Where $M_2$ denotes the weight of the freeze-dried hydrogel after being submerged in the PBS buffer solution.

Table 1. The proportions of 15% separation gel and 5% concentrated gel

| Solution                       | 15% separation gel | 5% concentrated gel |
|--------------------------------|--------------------|---------------------|
| Distilled water                | 1.1ml              | 2.1ml               |
| 30% Acrylamide                 | 2.5ml              | 0.5ml               |
| 10% SDS                        | 50μl               | 30μl                |
| 1.5mol/L Tris-HCl buffer solution | 1.3ml         | *                   |
| 1mol/L Tris-HCl buffer solution | *                 | 0.38ml              |
| 0.5mol/L Tris-HCl buffer solution | *                 | *                   |
| Tetramethyl ethylenediamine (TEMED) | 2μl               | 3μl                |
| 10% Ammonium persulfate (AP)   | 50μl               | 30μl                |

2.3.4. **TGA (thermogravimetric) analysis of keratin hydrogel.** The thermal property of the samples was measured by TGA in the temperature range of 30 and 600°C at a heating rate of 10K/min in N$_2$ by using a NETZSCH1500 (Shanghai, China).

3. Results and discussion

3.1. **SDS-PAGE**

Figure 1 shows the molecular weight of keratin powder and hydrogel extracted from rabbit hair. Keratin is a kind of natural polymer protein, but the relative molecular weight of keratin hydrogel extracted from rabbit hair is mainly concentrated in 11 ~ 22KDa by SDS-PAGE gel electrophoresis, which indicates that most of the keratin with high relative molecular weight has been decomposed.

Figure 1. SDS-PAGE patterns: (a) molecular weight standard; (b) keratin powder; (c) keratin hydrogel.
3.2. **SEM**

Figure 2 shows the different microstructures between the keratin powder with the keratin hydrogel. (a) is the keratin powder which is full of irregular and lamellar structures, and (b) is the keratin hydrogel. It has an obvious non-uniformed porous structure and a multi-layered cross section. According to the two pictures, it can be seen that in the process of the keratin hydrogel preparation, the chemical bonds in keratin molecules are broken and intermolecular forces are generated, forming a network structure. There is good connectivity of the pores, so it has good adsorption, permeability, water retention, etc.

![Figure 2](image1.png)

**Figure 2.** the microstructures of the keratin powder and hydrogel.

The molecular chain structure of rabbit hair keratin is mainly α-helix. The basic feature of α-helix keratin is that it contains a large number of cysteine residues, some react to form disulphide bonds, and others are free. There are also hydrophobic interactions and hydrogen bonds in it. Figure 3 shows the inter-and intra-molecular bonding in keratin.

When we add cysteine into the solution of rabbit keratin and raise the temperature, the inter-and intra-molecular bonding break, and then some bonds self-assemble. As a reducing agent, the sulfhydryl groups (-SH) can break the disulphide bond (-S-S-), then form a stable β-sheet conformation by physical crosslinking. Figure 4 shows the scheme of proposed self-assembly of rabbit keratin hydrogel.

![Figure 3](image2.png)

**Figure 3.** the inter-and intra-molecular bonding in keratin.

![Figure 4](image3.png)

**Figure 4.** the scheme of proposed self-assembly of rabbit keratin hydrogel.

3.3. **Analysis of the swelling ratio**

The biggest characteristic of hydrogel is swelling. It shows obvious swelling phenomenon in the solvent, and the molecules in the solvent gradually into the network structure of the hydrogel, the volume expand, but won’t be dissolved. In the Figure 5, the volume of the hydrogel expands because
of the molecules of solvent enter the interior of the hydrogel constantly, the chains change and the chains keep moving. When the weight is stable, we call it swelling equilibrium.

Figure 6 shows three stages of the swelling of hydrogels. The first stage is that the molecules of solvent spread to the dried hydrogel, at this point, the hydrogel absorbs few of solvent, filled the internal void constantly, and the volume has not changed much. The second stage is that when the pore structure inside the hydrogel is filled with solvent molecules, the chains inside start to relax and the volume to expand. The third stage is when the molecular chains of the hydrogel start to spread out into the solvent, the structure becomes loose.

Figure 7 shows the swelling property of hydrogels with different keratin concentration. When the keratin concentration is about 150mg/ml, the swelling ratio of hydrogel in distilled water is 20.21% and in PBS buffer solution is 9.55%; when is 175mg/ml, the swelling ratio are 14.89% and 7.02% respectively; when is 200mg/ml, there are 10.54% and 3.48%. The structure of hydrogel with low concentration keratin has many large and loose pores, exposes lots of hydrophilic groups, so that water molecules can enter its interior easily and quickly. But with the increase of keratin concentration, the number of cross-link points increasing and the density of cross-link increasing either, the excessive cross-link causes the number of pores decreasing, hinders the penetration of water molecules into the hydrogel, and results the water content ratio and swelling ratio decreasing.

The swelling ratio in distilled water is significantly higher than the swelling ratio in PBS buffer solution. This can be interpreted in electrostatic repulsion and osmotic pressure. On the one hand, a large number of phosphate anions and chloride ions exist in PBS, which have an electrostatic repulsion to the hydroxyl ions inside the hydrogel. The macromolecular chains are stretched to different levels, and then reduce the swelling equilibrium of the hydrogel. On the other hand, osmotic pressure occurs
between the inside and outside of the hydrogel because of the differences in the concentration of ions. The osmotic pressure provides power to hydrogel swelling. When the hydrogel swelling in PBS, the cations such as sodium (Na\(^+\)) and kalium ion(K\(^+\)) entered into the hydrogel network structure, formed a counterbalance with the carboxylic ions, and shielded the sample to avoid external charges. Based on the above-mentioned conditions, the swelling ratio in PBS is lower than it in distilled water.

3.4. TGA analysis

Figure 8 shows TG and DTG traces of the rabbit keratin hydrogel. The weight lost from 50℃ to 200℃ was attributed to the evaporation of absorbed water. The major weight losses took place in the temperature range from 200℃ to 450℃. It was observed that keratin hydrogel was rapidly decomposed in the temperature range from 200℃ to 450℃ and produced a significant amount of residue. The weight loss specified the degradation of the protein chain molecules, release of Sulphur dioxide and hydrogen sulfide due to breakage of disulfide bond. A peak in the 200–250℃ temperature range, related to denaturation peak of \(\alpha\)-helix crystallites, and the area under the curve is a measure of the \(\alpha\)-helix content. It found that the extracted keratin hydrogel started to decomposed at about 204℃.

When the loss residual weight of keratin hydrogel was about 95%, the decomposition temperature was 85℃; when was 50%, the temperature was 342.6℃; when was 25%, the temperature was 597.5℃.

According to the DTG line, it can be seen that the temperature at the maximum point of weight loss slope is 302℃.

![Figure 7. Swelling ratio: (a)low concentration; (b) medium concentration; (c) high concentration.](image)

![Figure 8. TG and DTG traces.](image)

4. Conclusions

Rabbit hair keratin hydrogel have been prepared by the method of heating-cooling and characterized in terms of SDS-PAGE, SEM, swelling ratio and TG. The effects of keratin addition concentration and temperature on hydrogel formatting ability were studied, under these conditions of experiment, the critical keratin concentration was 125mg/ml, as well as the critical temperature was 55℃, so the conditions of preparing keratin hydrogel are satisfied easily. As revealed by SEM images, the three-dimensional keratin-based hydrogel with a distinctly interconnected porous structure was fabricated successfully. According to the result of swelling assay, the hydrogel material will swell but less dissolve. Such property and special structural characteristic enable the material to retain a large amount of water or biological fluids, so it can be used as raw material in medical dressings and masks. Among the test of TG and DTG, the sample has good thermal stability.
The results demonstrate the correctness and the validity of preparing hydrogel with keratin from waste rabbit hair. Compared with traditional keratin hydrogel materials, rabbit hair has a large production around the world and a lot of short hairs are discarded, businesses can collect and reuse the waste which has low cost and high keratin content. The material is simple in preparative method, controllable in quality and has no need of crosslinking agent. In conclusion, rabbit keratin hydrogel has broad application prospects in the field of medical dressings, body tissue scaffolds, wastewater filters and any other fields, and is worthy of further researching.

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