Supporting Information

From Feather to Adsorbent: Keratin Extraction, Chemical Modification, and Fe(III) Removal from Aqueous Solution

Sujuan Pan [1,2,*], Changqing Wang [2], Yibo Wang [2] and Rongmin Wang [1]

[1] College of Chemistry & Chemical Engineering, Northwest Normal University, Lanzhou 730070, China; wangrm@nwnu.edu.cn
[2] Key Laboratory of Resource Utilization of Agricultural Solid Waste in Gansu Province, Tianshui Normal University, South Xihe Road, Qinzhou District, Tianshui 741001, China; wcqing2046@163.com (C.W.); wangyibo2021008@163.com (Y.W.)
* Correspondence: pansujuan_002@163.com; Tel.: +86-13830866425

1. Extraction of feather keratin (FK)

In this experiment, FK was extracted by reduction method as Yin *et al.* reported [1] and but slightly modified. The operation includes four steps, such as degreasing, pretreatment with hydrochloric acid, reduction by 2-mercaptoethanol, and precipitation. The extraction process is described as follows:

(1) Degreasing process

Firstly, the colored chicken feather (Figure S1a) was washed by tap water to remove sediment, then washed with detergent powder for three times, and rinse well with plenty of tap water then drying. Secondly, 6 g clean feather was placed in soxhlet extracter, adding 150 mL industrial alcohol and 100 mL acetone, and reflux for 4 h to obtain degreased feather. Finally, the degreased feather was pulverized into feather powder by a small multifunctional pulverizer.

(2) Pretreatment of HCl process

Put 6 g into 500 mL flask. Add 300 mL distillation water and 2 mL concentrated hydrochloric acid in it, stir and heat at 37~40 °C for 2 h, then washed to neutral and dry to obtain the hydrochloric acid pretreated feather powder (Figure S1b).

(3) Reduction process

Placed 6 g pretreated feather powder into a three-neck flask, added 0.6 g Tris, 4.4 g SDS, 6 g urea, 250 mL distilled water, and 2 mL 2-Mercaptoethanol, adjusted pH=8.0 by NaOH dilute solution, under N_2 atmosphere, at 70 °C reacted for 2 h. Then filtration to remove undissolved residue, collection the filtrate obtained the brown FK extracting solution (Figure S1c).

(4) Precipitation process

Add the diluted hydrochloric acid (~10%) to above FK extracting solution until the FK was precipitated completely. After filtration, the filter cake was dispersed in 100 mL ethanol to

remove the residue 2-Mercaptoethanol, still standing for 2 h, pumped and filtered, washed with water until neutral, frozen overnight, and then lyophilized with a lyophilizer at -50 °C. The clay like FK powder was obtained with a yield was 85.3% (Figure S1f).

2. Purification of FK

In the characterization, to avoid results affected by residual reagents and small molecular proteins, the following methods were employed to purify FK:

（1） The filtrated keratin extraction solution was dialyzed with a dialysis bag (MwCO: 8000-14000 Da) for 48 h in deionized water, and it was exchanged at intervals of 2, 4, 6, 8 and 12 h, respectively. To avoid FK aggregation, according to Hill *et al*. [2] reported method, dialysis medium was adjusted pH>7.0 with 0.1M NaOH solution.

（2） The dialyzed keratin solution was concentrated to ~2% (W/V) via ultrafiltration cup (MwCO: 5000 Da), then poured it into a 24 well culture plate about 2 mL in each well. It was frozen at -20 °C for 48 h and then lyophilized with a lyophilizer at -50 °C. Finally, the pure FK was obtained. It appears lustrous and sheetlike (see Figure S1g).

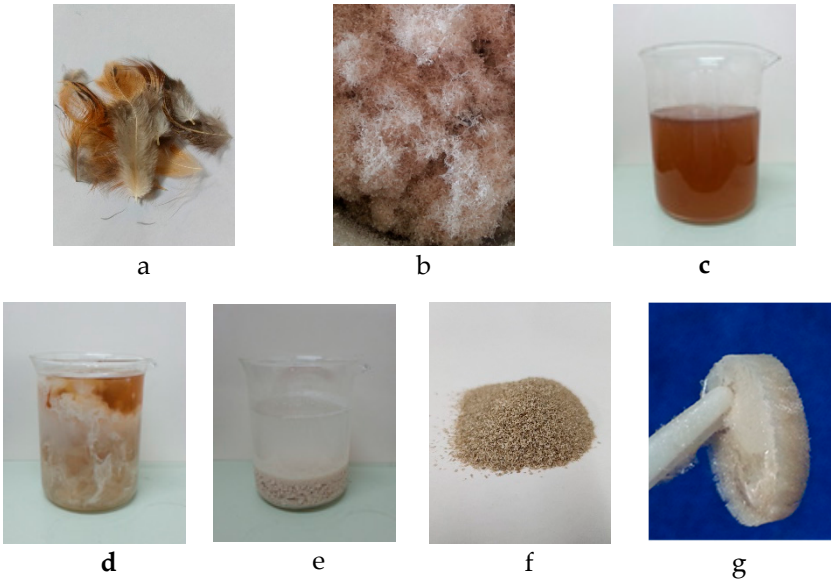
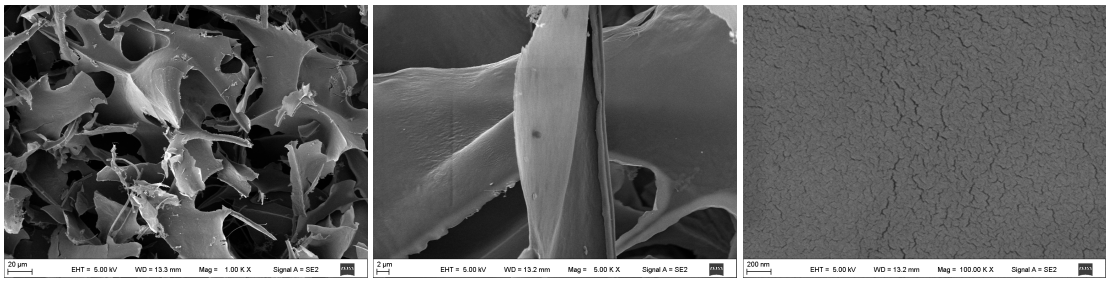


Figure S1. Photos of Chicken feather and FK at extraction process. (**a**) Chicken feather; (**b**) Chicken feather podwer; (**c**) Extraction solution; (**d**) Precipitating; (**e**) After precipitation; (**f**) FK podwer; (**g**) Purefied FK.

The microscopic morphology of purified FK is shown in Figure S2. As can be seen from the Figure S1a, the lyophilized FK presents irregular sheets, its surface looks flat and smooth (see Figure S1b). When magnified to 100K times, the surface is flat but cracked (see Figure S1c). These results indicate that the β -sheets of FK molecular affects the aggregation of a large number of molecules. This β - sheets makes FK molecules packed tightly, and the strong hydrogen bonds between FK chains leading to the aggregation of a large number of molecules

into the lamellar structure. Due to the hydrophobic effect of molecular chains and the strong hydrogen bond between molecular chains, feather keratin has strong contraction stress, which leads to interlayer separation and surface micro-cracks during lyophilization.



a b c

Figure S2. Different magnified SEM images of purefied FK. (**a**) 1K magnification; (**b**) 5K magnification; (**c**) 100K magnification.

3. Preparation of GA-FK gel

FK was chemically modified with GA, at same time via crosslinking the amino and carboxyl groups on FK chains to prepare GA-FK gel.

In this process, to assess the influence of crosslinking on the morphology structure and strength of the GA-FK gel, the dosage of activation reagent (EDC-NHS) was investigated, firstly. The experiments were conducted as follows:

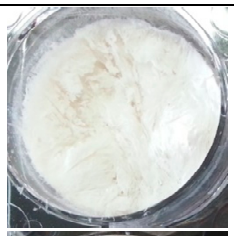
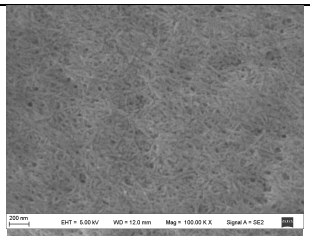
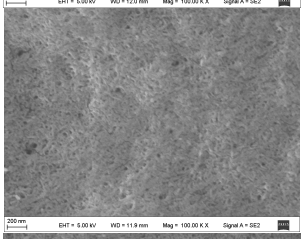
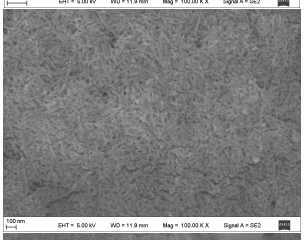
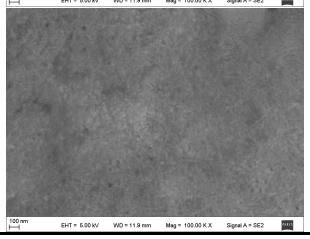
(1) Pretreatment of FK: 1 g FK powder was put into a 250 mL flask, added 100 mL urea aqueous solution (8 mol·L^{-1}), 0.1g Tris and 0.1 g DTT, adjusting pH=8 with 1M NaOH solution, at 70 °C stirred for 1h under a N_2 atmosphere. The obtained FK dispersive solution was dialyzed in deionized water for 24 hours (MwCO: 8000~14000 Da). The water was exchanged at intervals of 2, 4, 6, 8, and 12h, respectively. Then, the dialyzed FK solution was concentrated to 1% (W:V) by ultrafiltration cup (MwCO : 5000 Da), obtained the concentrated FK solution.

(2) Crosslinking: 12 mmol of EDC and 6 mmol NHS were dissolved in 20 mL 0.05 mol·L^{-1} MES buffer to form mixture, then certain amount of mixture was added into the 10mL concentrated FK solution and blending. After reacted 37 °C for 24 h, washed with deionized water, and lyophilized the EDC-NHS crosslinked FK gel was obtained. The dosages of crosslinking agent and morphology of obtained FK gels are shown in Table S1.

It is known from Table S1, when the amount of EDC-NHS liquid was 0.1 mL (EDC was 0.06 mmol) FK gel-1 was the layered structure still, and SEM photo shows it has a loose and

porous network of structures. This indicates that only mild cross-linking occurred in FK. As the dose of EDC-NHS increased, the macro morphologies of FK gels gradually transformed from layered to sponge like structure. SEM results show that the pores of the three-dimensional network structure changed from large to small, and from sparse to dense. When the amount of EDC-NHS liquid was 2.0 mL (EDC was 1.2 mmol), the FK gel-4 presented large cracks in macro morphology, and dense almost has no pore in microscope. This indicates that FK gel-4 was highly cross-linked due to the excessed EDC-NHS, and it fragmented into more pieces under the contraction stress during lyophilization.

Table S1. The dosages of crosslinking agent and morphology of obtained FK gels.

Product	Dos. of mixture (mL)	EDC (mmol)	Macro morphology	Micro morphology
FK gel-1	0.1	0.06		
FK gel-2	0.5	0.3		
FK gel-3	1.0	0.6		
FK gel-4	2.0	1.2		

Note: In 10 mL concentrated FK solution contains 0.1 g FK, pH=5.5, reacted at 37 °C for 24 h.

In addition, as the dosage of EDC-NHS increased, the strength of the FK gel improved, it was brittle at low crosslinking and tenacity at appropriate crosslinking. On the other hand, when the FK gels were immersed in water, they would be swelled in different degrees. FK gel-

1 becomes soft and loses its shape after being exposed to water, FK gel-4 disintegrates into fragments, only FK gel-2 and FK gel-3 could maintain their shapes like sponge.

According to Yin *et. al.* reported [1], it is estimated that 0.1 g FK contains about 0.05 mmol of free amino group available for reaction, and the EDC-NHS dosage for preparing FK gel-2 is far too much. Therefore, to ensure that the prepared FK gel will not disorganize and maintain its properties and certain strength when it is used in aqueous environment, the 0.3 mmol was selected as the optimum dosage of EDC (0.5 mL mixture).

Secondly, GA was employed to chemically modified the FK, and it was activated by EDC-NHS, then grafted on -COOH of FK. In this procession, the FK was crosslinked by EDC-NHS also. Because the number of $-NH_2$ in FK is extremely limited (0.1 g FK contains about 0.05 mmol $-NH_2$), therefor it is very necessary to investigate the dosage of GA. The experiments were conducted as follows:

(1) Activation of GA: 12 mmol EDC and 6 mmol NHS were dissolved in 20 mL 0.05 $mol \cdot L^{-1}$ MES buffer, then 1~4 mmol GA was added in it, reacted 15 min at room temperature to obtain car-boxy-activated GA solution (GA-NHS).

(2) Modification and cross-linkage of FK: 0.5 mL activated GA solution was added into 10mL concentrated FK solution (containing FK ~0.1 g) and mixed, reacted at 37 °C for 24 h. The product was immersed in 200 mL deionized water to remove the free GA and other small molecule compounds, and it was exchanged at intervals of 2, 4, 6, and 8 h, respectively. Then lyophilized, the GA modified, and EDC-NHS crosslinked FK gel (GA-FK gel) was obtained. The eluent of 5 times was collected for quantitatively analysis the unreacted GA by UV-Vis spectrophotometer. The dosages of GA and morphology of obtained GA-FK gels are shown in Table S2.

It is known from Table S2., the color of GA-FK gel deepened with the increase of GA dosage, which might be caused by the oxidation of GA in the reaction process. When the dosage of GA was 0.025 (do not overdose) and 0.05 mmol (equivalent dose), the residual of GA was about 9% and 15%. This indicates that GA could not be 100% grafted onto FK. When the amount of GA is 0.10 mmol (overdose), the residual of GA was 41%, and a large amount of GA was retained in GA-FK gel by adsorption, resulting in color of GA-FK gel deepening due to oxidation.

In theory, the maximum grafting amount of GA was 0.05 mmol. However, after the bonding reaction with 0.05 mmol GA, the eluted GA (the amount of GA was eluted) was about 15%. This indicates that excessive amount of GA was useless, and only part of the amino groups were bonded to GA and the other part were crosslinked. Therefore, 0.05 mmol is selected as the optimum dosage of GA.

Table S2. The dosages of GA and morphology of obtained GA-FK gels.

Product	Dos. of GA (mmol)	GA in Elution (C/C_0)	Macro morphology
GA-FK gel-A	0.025	0.09	
GA-FK gel-B	0.05	0.15	
GA-FK gel-C	0.10	0.41	

Cond: EDC: 12 mmol, NHS: 6 mmol, MES buffer: 20 mL (0.05 M, pH=5.5); concentrated FK solution (W/V:1%), reacted at 37 °C for 24 h.

References

1. Yin,X.-C.; Li,F.-Y.; He,Y.-F.; Wang,Y.; Wang,R.-M. Study on effective extraction of chicken feather keratins and their films for controlling drug release. *Biomater. Sci.* **2013**, *1*, 528–536.
2. Hill, P.; Brantley, H.; Van Dyke, M. Some properties of keratin biomaterials: kerateines. *Biomater.* **2010**, *31*, 585–593.