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Hydrothermal Pretreatment for the Preparation of Wool Powders

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ABSTRACT: Wool powders with low solubility could be easily prepared from wool fibers by combining hydrothermal pretreatment (HTP) and grinding processes. Transforming wool fibers into powders was the first step of exploiting wool as new materials such as bio-thermoplastics. However, wool fibers were difficult to be prepared into powders only by grinding due to their toughness. This article used a chemical-free method to obtain wool powders by grinding HTP-treated wool fibers. Wool fibers after HTP at 130°C for 40 min or above were obviously easier to be broken into snippets with lengths of 25–150 μm after grinding for 1 min and shapeless super-fine powders with diameters of 3–15 μm after ball milling for 10 min. Wool fibers after HTP were brittle mainly owing to decrease of α-helices and S—S bonds. Combination of HTP and grinding could be an eco-friendly and feasible treatment to prepare wool powders on industrial scale. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40173.

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INTRODUCTION

Recently, development of biodegradable and environmentally friendly bio-based materials from renewable natural resources has attracted increasing attention.[1,2] Wool has been conventionally used as a textile material.[3] Wool wastes from textile industry and farm breeding are estimated to be more than 10,000 tons annually in the world.[4,5] Disposing waste wool textiles and wool in landfills brings environmental and economic problems. It is important to transform the waste wool into new materials to increase its value addition.

Many researches have used wool keratin to produce biomedical materials for tissue engineering. Spray-dried wool keratin microspheres were used to improve cell affinity of poly(L-lactic acid) scaffolds.[6] Composites were developed by reinforcing cellulose acetate with cortical cells extracted from wool fibers,[7] blending wool keratin and silk fibroin aqueous solution,[8] and casting solution containing keratin and chitosan in 75% acetic acid.[9] Wool keratin sponge scaffolds fabricated via lyophilization of aqueous wool keratin solution after freezing showed promising support for long-term high-density cell culture.[10] The S-sulfo keratin powders were compression-molded and the resultant transparent S-sulfo keratin film demonstrated good biocompatibility.[11] Highly porous and flexible wool keratin sponges were developed by particulate-leaching and freeze-drying.[12]

Besides biomedical applications, extensive research has been focused on using wool to produce industrial materials. Regenerated protein fibers and bi-component fibers from wool wastes with other polymers could be suitable for use in apparels or technical textile applications.[1] Regenerated wool keratin films were prepared from wool keratin/ionic liquid solution.[13] Superfine wool powders were mixed with glycerol and hot-pressed into films[14] or blended with poly(propylene) and then hot-pressed into films.[15] Wool keratin powders, incorporated with glycerol as plasticizer and sodium sulfite as reductant were hot-pressed into bio-thermoplastics.[2,16] Wool keratin powders also showed higher sorption rates and capacities to heavy metal ions such as Co^{2+}, Cu^{2+}, and Cd^{2+} and dyes in effluent from dyeing industry than wool fibers.[17–19] The sorption rates and capacities to acid dyes by wool powders were comparable with that of activated charcoal.[20]

The prerequisite of transforming wool from fibers to the above-mentioned new materials is obtaining of wool powders. However, wool fibers are difficult to be disintegrated only by mechanical force due to their toughness and flexibility. To obtain fine wool powders, processes were usually tedious and costly extraction using solution of multiple chemicals and

spray-drying, and therefore restricted industrial applications of wool powders.[21,22] For example, ultrafine wool powders were obtained using combined wet media milling for 6 h and air jet milling for 5 h.[22] Oxidation pretreatment was employed to improve the disintegration process of wool fibers.[23] Extracting keratin from wool fibers is also difficult because of high amount of intra- and inter-molecular disulfide (S—S) crosslinkages. Some reductive or oxidative agents that are used to break the S—S crosslinkages, such as sulfites, mercaptoethanol and peroxides are harmful to operators and environment. To overcome pollution of chemicals, protease,[7] keratinolytic bacteria[5] and microscopic fungi[24] were employed to degrade keratin waste biologically. However, biodegradation is expensive and has long production cycle. Therefore, it is particularly important to develop simple and eco-friendly methods to appropriately disintegrate wool fibers.

Researchers have also studied effects of heat treatments on structures and properties of keratin. Tonin et al.,[25] Xu et al.,[26] and Zhao et al.[27] indicated that steam explosion could break S—S crosslinkages in keratin. Zhao and He[28] studied effects of microwave irradiation on structures and properties of wool keratin. Wortmann and Deutz,[29] Huson et al.,[30] and Cao et al.[31–33] investigated thermal properties of wool keratin by Differential Scanning Calorimetry (DSC) and indicated that the bi-modal melting of wool keratin arose from sequential melting of α-form crystallites in the ortho- and para-cortical cells of wool and peak temperatures decreased by increasing moisture content in wool. For example, the melting peak temperature was 205°C for dry wool and decreased to 155°C for wool with moisture content of 23% and the bi-modal melting temperatures was about 140°C for wool in excess water. Senoz et al.[34] pyrolyzed chicken feather fibers in a box furnace with N_2 flow and studied physical and chemical changes in chicken feather fibers during pyrolysis. However, based on our knowledge, there has not been any publication using heat-treatments to prepare wool powders so far.

This article discussed a simple and chemical-free method to prepare wool powders by combining hydrothermal pretreatment (HTP) and mechanical grinding processes. The temperature of HTP was set at 130°C, which was close to the melting temperature of wool keratin. Chemical-free HTP was processed in a high-pressure equipment with excess water for 10–60 min. This paper also discussed the effects of HTP on the molecular structures and properties of wool keratin, especially S—S crosslinkages, α-helical conformation, and solubility.

EXPERIMENTAL

Materials

Australian Merino wool fibers with a mean diameter of 17.9 μm were provided by Jiangsu Sunshine Group (China). The wool fibers were immerged in ethanol (AR, Sinopharm Chemical Reagent, China) for 12 h under ambient conditions and then rinsed with warm deionized water for several times to remove the residual carding oil, which could eliminate the effects of the residual oil on measuring weight loss during the HTP process, solubility, and so on. The rinsed wool fibers were dried at 60°C for 12 h, conditioned at 20 ± 2°C and 65 ± 3% relative humidity for 24 h and was then weighted. Urea, sodium bisulfite and sodium dodecyl sulfate (SDS) were AR grades and produced by Sinopharm Chemical Reagent, China.

HTP Process

HTP was carried out in a high-pressure steam sterilizer with automatic exhaust device (Model: OPS-40IS, Shanghai Ouster Industrial, China). Merino wool fibers were immerged in deionized water at a liquor ratio of 20:1, heated to a proper temperature and then held for varying time periods from 10 to 60 min. According to our previous experiments, it was indicated that treatments at 110 or 120°C with different time periods had no obvious difference among each other. Therefore, the constant temperature of 130°C near the wool melting temperature was selected. The HTP-treated wool fiber samples were dried in air and were conditioned at 20±2°C and 65±3% relative humidity for 24 h.

Grinding Process

A high-speed universal disintegrator (HSUD) FW 100 (Tianjin Taisite Instrument, China) was used to grind wool fibers. The disintegrator was usually used in agriculture, mining and many other industries to prepare powders of grain, soil, medicine, mineral substance, and so on. Wool fibers before and after HTP for different time periods were ground by HSUD for 1 min at the rational speed of 24,000 r/min. The wool fibers after HTP for 40 min also were ground for 10 min using a ball mill TJH-1-4L (Taichi Ring Nano Products, Qinhuangdao City, China) with ceramic balls. The ceramic ball was composed of 95% ZrO_2 and had diameter of 8 mm, density of 6.02 g/cm^3, and Mohs hardness of 9.

Morphology Observation

Morphologies of wool fibers and powders were observed by Hitachi SU 1510 Scanning Electron Microscope (SEM). The wool samples were mounted on conductive adhesive tape, sputter coated with gold palladium, and observed at a voltage of 5 kV.

Measurement of Tensile Properties

Tensile properties of single wool fibers before and after HTP were measured on a single fiber tensile tester YG001N (Nantong Hongda Experiment Instruments, China) under pretension of 0.3 cN, gauge length of 10 mm and extension rate of 20 mm/min. For each sample, 60 fibers were tested and their mean value was calculated.

Measurement of Whiteness

An automatic whiteness tester WSD-3C (Beking Kangguang Instrument, China) was used to measure the whiteness of wool fibers before and after HTP using an illuminant D65 and 10° standard observer. The Hunter whiteness was selected and calculated according to eq. (1). Higher Hunter whiteness means whiter color. Every wool sample was tested for 10 times and the mean value was calculated.

$$W_h = 100 - \left[(100-L)^2 + a^2 + b^2\right]^{1/2} \quad (1)$$

where W_h is the Hunter whiteness, L, a, and b are lightness, redness–greenness, and yellowness–blueness, respectively.

Measurement of Mass Loss Ratio

Percent mass loss of wool fibers was the reduction percent of the dry weights of samples before and after HTP. The wool fiber

samples were dried in an electric thermostatic oven Y802 (Wenzhou Darong Textiles Instrument, China). The percent mass loss after HTP was determined by eq. (2).

$$\%\text{loss} = \frac{W_{d0} - W_{d1}}{W_{d0}} \times 100 \quad (2)$$

where % loss is percent loss of the dry weight of wool keratin, W_{d0} and W_{d1} were the dry weight of wool keratin before and after HTP, respectively. The wool keratin samples were dried at 105°C for 2 h until the weight became constant and then weighed.

Measurement of Molecular Weight

Changes in molecular weights of the wool keratin samples after HTP were studied using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE). About 1 mg of wool powders before and after HTP were mixed with 1000 μL of SDS PAGE 1× running buffer (4.1 m*M* Tris-HCl, 2% SDS, 2% mercaptoethanol, 10% glycerol, double-distilled water) and left standing at room temperature for 2 h. About 20 μL of the clear top layer of each sample was loaded into each slot of gel. After electrophoresis, the protein bands were visualized by staining with Coomassie Brilliant Blue R-250 (Sigma-Aldrich Trading, Shanghai, China). After standing for one night, the gel was flushed with deionized water and put in a destained liquid until a clear background was formed. Standard protein molecular weight marker was used for identification.

Measurement of S—S Crosslinkages and Molecular Conformations

Raman spectroscopy, which is capable of probing content and conformation of S—S linkages has been used to study structural changes of wool or hair keratin after various chemical and physical treatments such as permanent wave,[35] bleaching,[36] photo-oxidation,[37] enzymatic treatment,[38] and stretching.[39] Raman spectra were recorded in a Raman microscope system LABRAM 00 with an optical microscope attachment (Dilor, France). The laser excitation was provided by a He-Ne laser at 6 mW of 632.8 nm output. The laser beam on the fiber samples was focused to a spot diameter of 2 μm using a 100× microscope objective. Spectra were recorded by scanning 400–1750 cm^{-1} region, with a total acquisition time of 200 s. A single wool fiber was scanned to obtain a good signal/noise ratio.[39] Raman spectra of three fibers from one wool sample were nicely overlapped, demonstrating that the spectrum was quite reproducible.

Analysis of Elements and Amino Acid Contents

Wool powders before and after HTP for 40 min were quantitatively analyzed for elements carbon, nitrogen, hydrogen, and sulfur. Analysis was performed with an automatic elemental analyzer Vario EL III (Element Analysis System, Germany). About 5 mg of the wool powder was weighed to the nearest 0.001 mg using an ultra-microanalysis scale (model Auto balance AD-2, Perkin Elmer). In the absence of air, the sample was introduced into burning chamber of the elemental analyzer. After injection of a defined amount of oxygen, the sample was burned at a temperature of 1150°C. The generated different gases were separated according to the purge-and-trap principle and the amount of each gas was determined by thermoconductivity.[40]

Amino acid composition of wool was determined after acid hydrolysis with 6*M* HCl at 110 ± 1°C for 24 h under vacuum. Free amino acids were analyzed by HPLC 1100 (Agilent, USA). The eluate was detected at 338 nm. The cystine content was determined by external standard calibration and expressed as mass percent of total amino acids.

Analysis of Crystallinity

Crystallinity of wool keratin was analyzed by X-ray diffraction (XRD) and DSC. An X-ray diffractometer D8 Advance (Bruker AXS, Germany, wavelength = 1.54 Å, Cu Kα radiation) was used. The intensity and current of the generator were 40 kV and 40 mA, receptively. The powder wool samples were scanned from $2\theta = 3$–40° at a rate of 4°/min and a step of 0.02°. Area of the crystalline peak was calculated after subtracting the background and air scatter using the XRD pattern processing software MDI Jade 5.0. TA Q200 DSC (TA Instrument, USA) was used. About 5–7 mg of wool samples before and after HTP were placed in sealed aluminum pans and heated from 0 to 250°C at a heating rate of 10°C/min. The aluminum cell was swept with N_2 during the analysis process. Melting enthalpy ΔH (J/g) was calculated from the DSC curves by TA Instrument Universal Analysis.

Measurement of Solubility

Solution of urea (480 g/L), sodium hydrogen sulfite (50 g/L), and SDS (30 g/L) was prepared to dissolve wool keratin. About 5 g of wool samples before and after HTP at 130°C for varying time respectively were added into 100 mL of the above solution and were stirred using magnetic stirrer at 70°C for 12 h. The liquor was then filtered with filter paper (Medium rate, Hangzhou Wuhua Filter Paper, China) and cooled to room temperature. The undissolved residues were washed with 200 mL pure water, dried at room temperature and weighed. Each wool sample was independently repeated for three times. The degree of solubility of the wool keratin was determined by eq. (3).

$$\text{wt }\%_{\text{sol}} = \left(1 - \frac{W_{\text{insol}}}{W_i}\right) \times 100 \quad (3)$$

where wt $\%_{\text{sol}}$ is the weight percent of dissolved components and expressed the degree of solubility, W_i is the weight of initial wool keratin, W_{insol} is the weight of the final undissolved components.

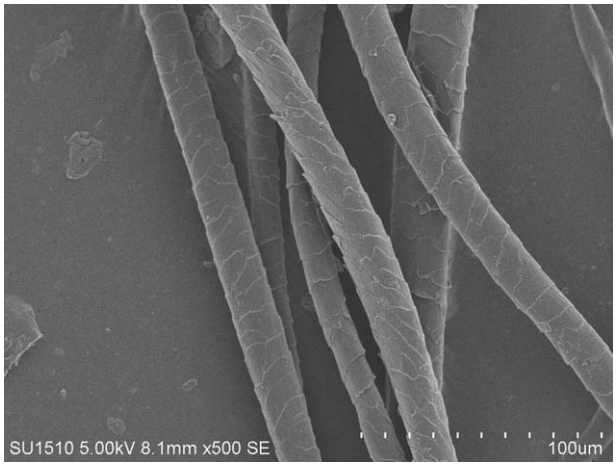

Figure 1. SEM image of longitudinal views of wool fibers before HTP.

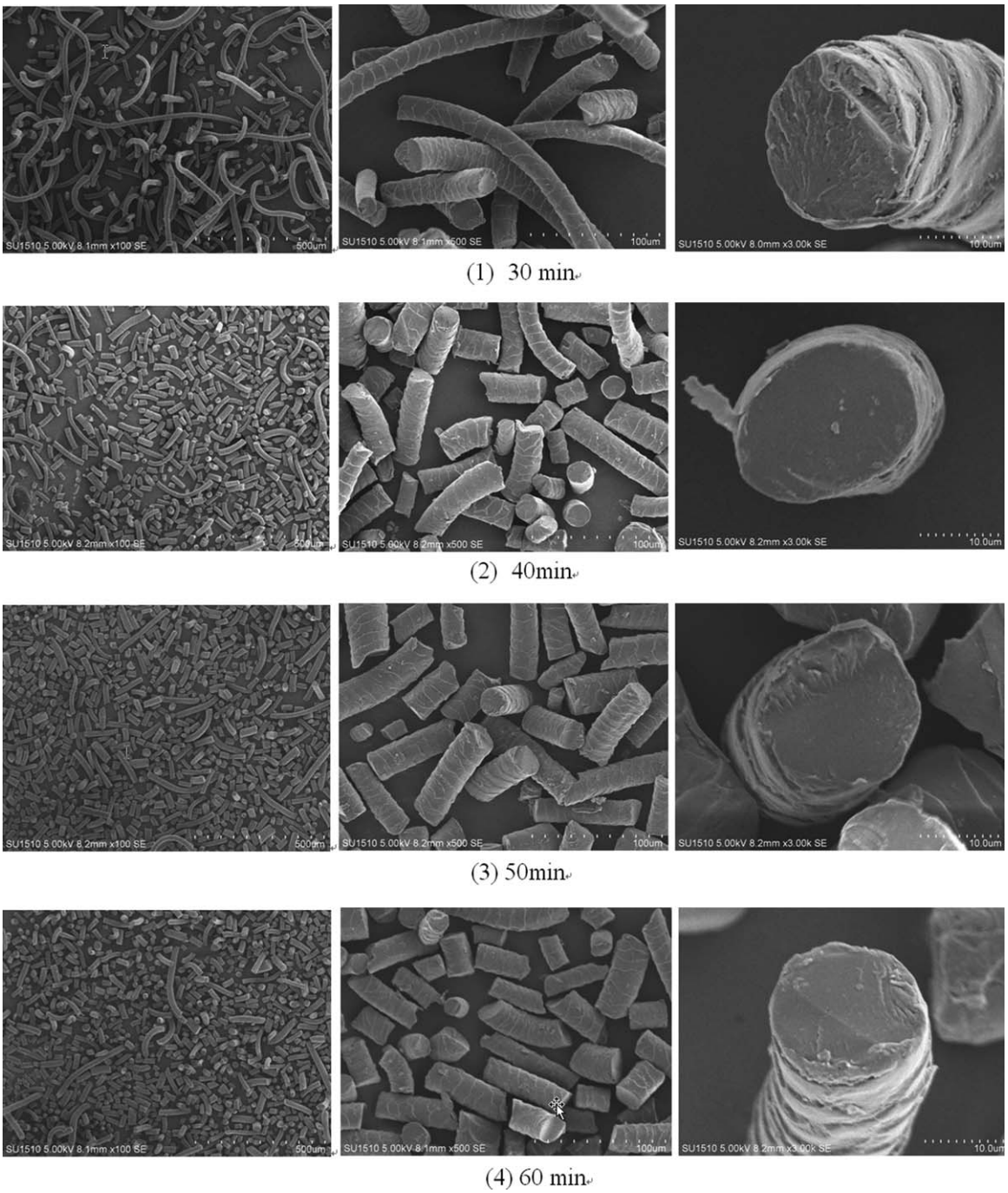

Figure 2. SEM images of the wool powders prepared by combined HTP at 130°C for different time and disintegrating by HSUD for (1) 30 min, (2) 40 min, (3) 50 min, and (4) 60 min.

RESULTS AND DISCUSSION

Effect of HTP on the Preparation of Wool Powders

Wool fibers before and after HTP for 10 and 20 min could hardly be ground into powders by HSUD. However, wool fibers after HTP for longer than 30 min were easily disintegrated into powders. Figure 1 shows SEM images of longitudinal views of wool fibers before HTP. Figure 2 shows SEM images of wool powders prepared by combining HTP at 130°C for 30, 40, 50, and 60 min, respectively, and disintegrating by HSUD for 1 min. As can be seen from Figure 2, for the wool powders from the fibers with 30 min of HTP, there were some remaining long wool fibers which have not been sufficiently broken to small snippets in disintegrating process. However, after 40 min of HTP and 1 min grinding, wool fibers were broken into much shorter fiber snippets. After HTP for 40 min or longer time, wool fibers could be easily disintegrated into snippets with lengths of 25–150 μm. The lengths of disintegrated wool snippets showed no obvious differences among HTP for 40, 50, and

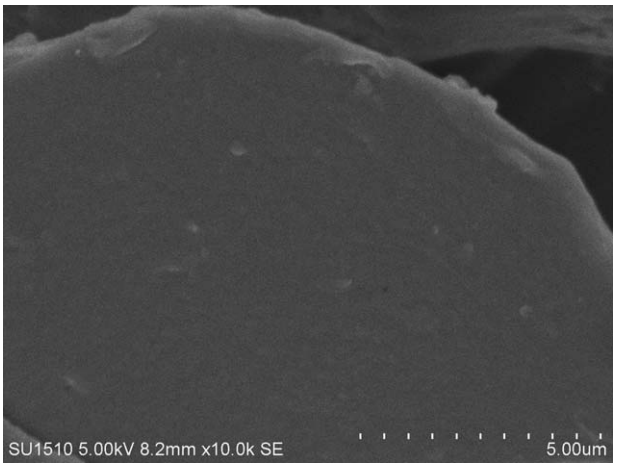

Figure 3. SEM image of the typical broken surface of wool snippet in Figure 2 (2) (×10,000).

60 min. It was also showed that there was a small quantity of fiber fragments with lengths above 200 μm, which is a critical size for sieve separation. Typical scaled surface structures of cuticle cells in Figure 1 were still recognizable for all the disintegrated wool snippets by HSUD. As shown in Figure 3, a typical cross-section of wool snippets after HTP for 40 min was plane and smooth, which indicated a brittle breakage.

In order to further decrease sizes of wool powders, after HTP for 40 min, wool fibers were ground by ball mill for 10 min. Figure 4 is a SEM image of ground wool powders. The wool fibers were converted into irregular shaped particles with sizes ranging from 3 to 15 μm. The scales on the surface of wool fibers had been completely destroyed by the huge impact energy from violently moving ceramic balls. It could be indicated that wool snippets could be smashed not only along the axial direction but also along the longitudinal direction. HTP at 130°C for 40 min was an effective pretreatment process to develop fine wool powders.

Effect of HTP on the Tensile Properties of Single Wool Fibers

Figure 5 shows the tensile properties of single wool fibers before and after HTP at 130°C for 10, 20, and 30 min, respectively.

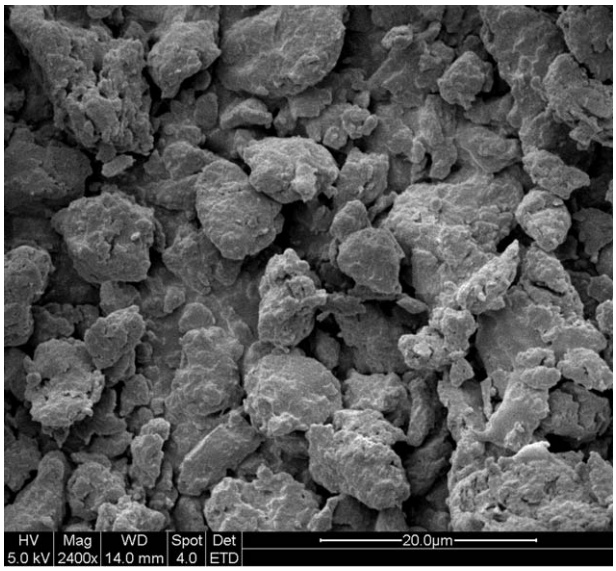

Figure 4. SEM image of wool powders after HTP at 130°C for 40 min and grinding with ball mill for 10 min.

Single wool fibers after HTP at 130°C for 40, 50, and 60 min were too weak to be measured for their tensile properties. Compared with untreated wool fibers, the HTP-treated wool fibers had lower breaking strength and breaking elongation. For the wool fibers after HTP for 30 min, their breaking strength decreased from 7.45 to 2.12 cN and only 28.5% of strength was retained, their breaking elongation decreased from 48.77% to 4.73% and the decreasing percentage was around 90%. The results indicated that wool fibers lost their flexibility and became brittle and thereby facilitated disintegration of wool fibers to powders by mechanical grinding process.

Degradation of Wool Keratin During HTP

Yellowing of Wool Keratin. Color of wool keratin after HTP for varying time periods changed gradually from natural white to creamy yellow. The Hunter whiteness values of wool keratin before and after HTP at 130°C for varying time from 10 to 60 min are shown in Figure 6. Hunter whiteness values of the wool keratin after HTP were significantly lower than that of the

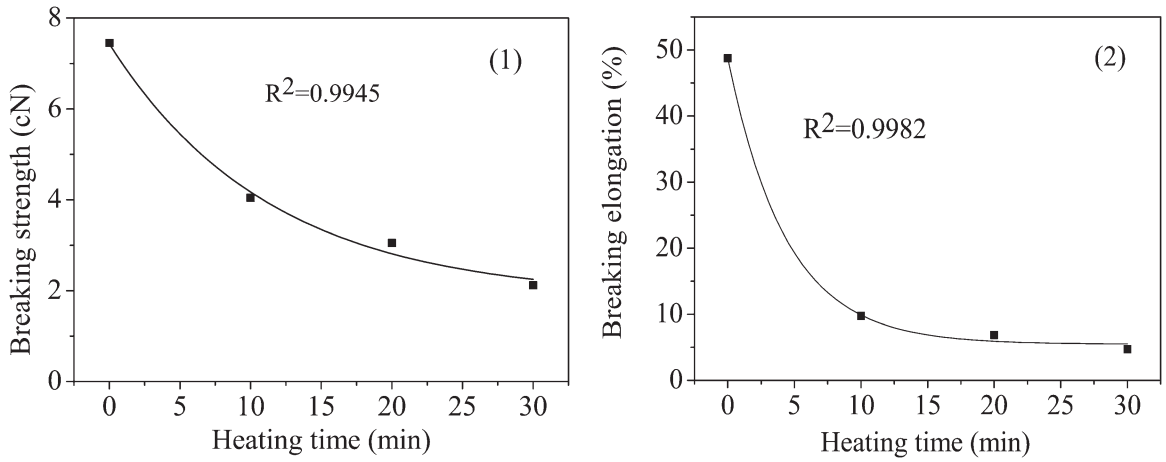

Figure 5. Tensile breaking strength (1) and elongation (2) of single wool fibers before and after HTP at 130°C for varying time periods (10–30 min).

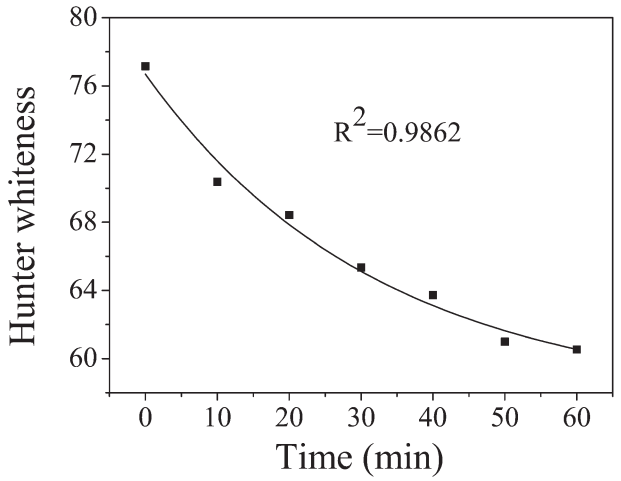

Figure 6. Hunter whiteness values before and after HTP at 130°C for varying time periods of 10–60 min.

untreated wool and decreased as time prolonged. Lower Hunter whiteness value means yellower color. Yellowing of wool keratin indicated that some unstable amino acid residues may be formed.[41]

Mass Loss of Wool. The mass loss indicated that degradation occurred during the HTP process. Percent mass loss of wool after HTP at 130°C for varying time periods from 10 to 60 min are shown in Figure 7. The % mass loss increased as HTP time increased. When wool was HTP treated for 60 min, its % mass loss was as low as 4.05%.

Decrease in Molecular Weights of Wool. SDS–PAGE images in Figure 8 show that HTP at 130°C decreased molecular weights of wool. Wool can be considered as two-phase, filament/matrix composites, in which partly crystalline, α-helical intermediate filaments (IFs) are embedded in an amorphous matrix of IF-associated proteins.[42] IFs are the low-sulfur proteins, molecular weights of which fall in the range of 43–67 kDa and the matrix is composed of high-sulfur proteins, molecular weights of which fall in the range 11–28 kDa.[25] As shown in Figure 8, the untreated wool (Lane 8) showed prominent IF bands in the

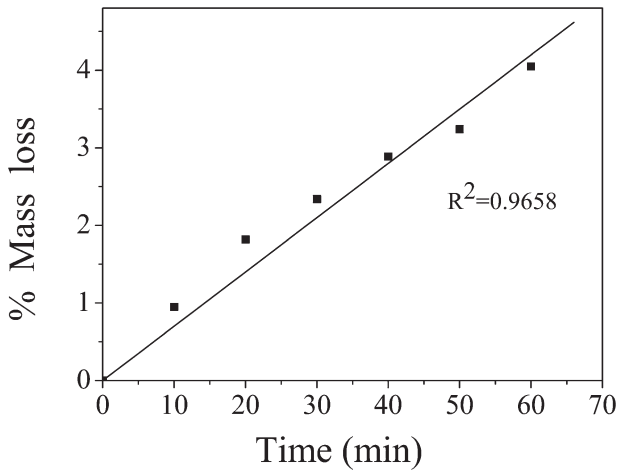

Figure 7. Effect of HTP at 130°C on % mass loss of wool.

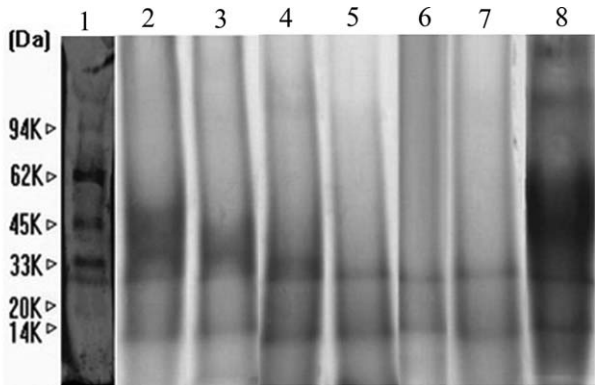

Figure 8. SDS–PAGE images of wool. Lane 1, molecular weight standard; Lanes 2–7, wool keratin after HTP at 130°C for 10, 20, 30, 40, 50, and 60 min, respectively; Lane 8, wool keratin before HTP.

range of 43–67 kDa and matrix bands in the range of 11–28 kDa. Compared with untreated wool, wool after HTP at 130°C for 10, 20, and 30 min (Lanes 2–4) had fewer high molecular weight proteins in the region of 43–67 kDa. Increasing HTP time further reduced molecular weights of wool. The wool after HTP at 130°C for 40, 50, and 60 min (Lanes 5–7) hardly showed high molecular weight proteins in the range of 43–67 kDa. These results indicated that HTP degraded the crystalline IF of wool and scission of the protein backbones occurred. It was in accordance with the reports that steam treatment at or above 130°C could decrease molecular weights of wool keratin[25] and soy proteins.[43]

Effect of HTP on the S—S Crosslinkages and Molecular Conformation of Wool Keratin

Figure 9 shows the normalized Raman spectra of wool keratin before and after HTP at 130°C for 40 min. Normalization was carried out based on the band at 1450 cm^{-1} assigned to CH_2 bending mode, the peak area of which was large and not influenced by HTP.[35,36] Table I summarizes the assignments of some Raman vibration bands for wool keratin. Peaks at 510 ± 5, 525 ± 5, and 540 ± 5 cm^{-1} were assigned to S—S stretching vibrations of gauche-gauche-gauche (g-g-g), gauche-gauche-trans (g-g-t), and trans-gauche-trans (t-g-t) conformers, respectively.[44,45] Among the three conformers, g-g-g conformer was energetically most stable.[45] As shown in Figure 9, the S—S region of 485–570 cm^{-1} for wool keratin before HTP was dominated by the most stable g-g-g conformer. Compared to wool keratin before HTP, Raman spectrum for wool keratin after HTP demonstrated obviously decreased intensity in the range $485-570$ cm^{-1} and increased contents from the energetically less stable g-g-t and t-g-t conformers. According to the studies by Kuzuhara,[35,36] ratio of peak area of S—S bands (calculated from the peak to a baseline that was drawn between 485 and 570 cm^{-1}) to the peak area of the CH_2 band (calculated from the peak to a baseline that was drawn between 1428 and 1500 cm^{-1}) of Raman spectrum expressed the content of S—S crosslinkages in wool keratin. The peak area was expressed by weighting the cut peak area from an even paper. The calculated ratio of peak area of S—S bands was 0.75 for untreated wool and was 0.32 for HTP-treated wool, indicating that the content

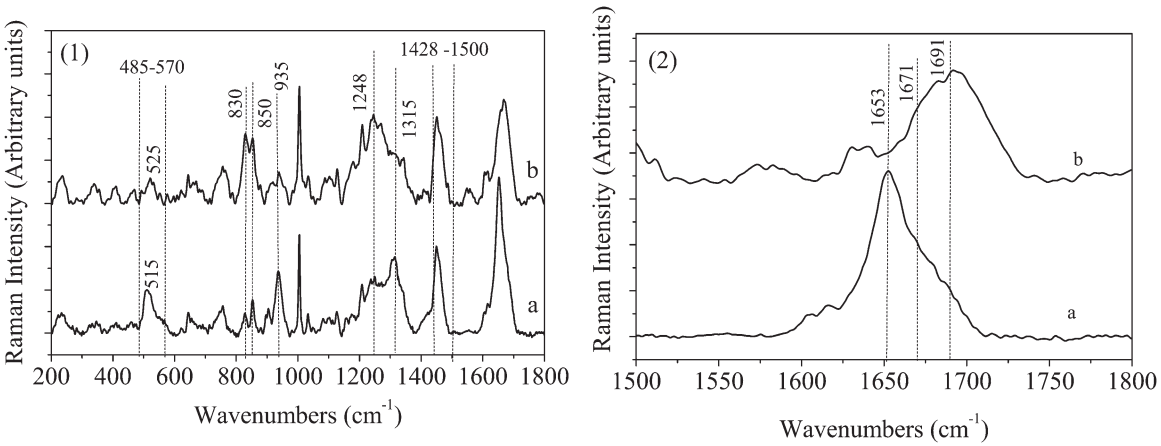

Figure 9. Normalized Raman spectra of wool keratin before (a) and after HTP at 130°C for 40 min (b) (1) 200–1800 cm^{-1}; (2) 1500–1800 cm^{-1}. Note: normalization was based on the CH_2 bending vibration at 1450 cm^{-1}.

of S—S crosslinkages for wool keratin after HTP significantly decreased and HTP at 130°C for 40 min broke about 60% of the S—S crosslinkages in the untreated wool keratin. Analysis of amino acid compositions of wool keratin showed that the cystine content decreased from 6.29 wt % to 2.54 wt % after HTP for 40 min. The above results proved effective cleavage of S—S crosslinkages via physical approaches, and ensured feasibility of industrial application of wool keratin. Table II shows the percentages of organic elements in wool keratin before and after HTP for 40 min. Difference in sulfur contents between the two samples was most significant. Sulfur content of wool keratin decreased from 3.1% to 2.5% after HTP, indicating that some sulfur compounds were volatilized during HTP.

From Figure 9, compared with untreated wool, Raman spectrum of wool keratin after HTP at 130°C for 40 min had lower intensities at 935, 1315 in amide III and 1653 cm^{-1} in amide I, and higher intensities at 1248, 1665, and 1691 cm^{-1}. According to Table I, Raman bands at these wavelengths are sensitive to conformation of molecular chains of proteins. For example, band at 935 cm^{-1} assigned to C—C skeletal stretching of peptide backbones is a marker for α-helix conformation.[35–37] Qualitative analysis of the broad amide I peaks in Figure 9 indicated that α-helical forms (1653 cm^{-1}) dominated. Random coil (1671 cm^{-1}) forms increased and α-helical forms decreased after wool keratin was processed with HTP at 130°C for 40 min. These changes demonstrated that the wool keratin after HTP had lower amount of α-helix and higher amount of random coils, which indicated transformation of the secondary structures of keratin from α-helix to random coil during HTP at 130°C for 40 min. The decreases of α-helix, S—S crosslinkages and molecular weights all contributed to brittleness of wool fibers after HTP.

Tyrosine in wool keratin gives Raman spectra with a doublet at 830 and 850 cm^{-1}. Intensity ratio of the two peaks, I_{850}/I_{830}, is sensitive to hydrogen bonding of phenolic hydroxyl groups. As shown in Figure 9, ratio of 10:7 for untreated wool keratin was higher than 1, indicating that the phenolic hydroxyl groups were exposed.[46] Ratio of 5:6 for wool keratin after HTP at 130°C for 40 min was lower than 1, indicating that the phenolic hydroxyl groups were buried. The change could be resulted from change in conformation of the protein molecules.[46]

Table I. Assignments of Some Raman Bands for Wool Keratin

Wavenumber (cm^{-1})	Vibrational assignment	References
510 ± 5	S—S stretching (g-g-g)	43, 44
525 ± 5	S—S stretching (g-g-t)	
540 ± 5	S—S stretching (t-g-t)	
Doublet at 850 and 830 cm^{-1}	Tyrosine	46
935	C—C skeletal stretching (α-helix)	35-37, 47, 48
1248	Amide III (random coil)	
1315	Amide III (α-helix)	
1428-1500	CH_2 bending mode	
1450	CH_2 bending mode	
1653	Amide I (α-helix)	
1671	Amide I (random coil)	

Effect of HTP on Crystallinity of Wool Keratin

Figure 10 shows XRD intensity patterns of wool keratin before and after HTP at 130°C for 40 min and indicates that crystallinity of wool keratin decreased significantly after HTP. The diffraction intensity patterns for wool keratin before and after

Table II. Percentages of Organic Elements of Wool Keratin Before and After HTP at 130°C for 40 min

Sample	Nitrogen (%)	Carbon (%)	Sulfur (%)	Hydrogen (%)
Wool keratin before HTP	15.56	47.58	3.105	6.757
Wool keratin after HTP at 130°C for 40 min	15.44	46.86	2.471	6.680

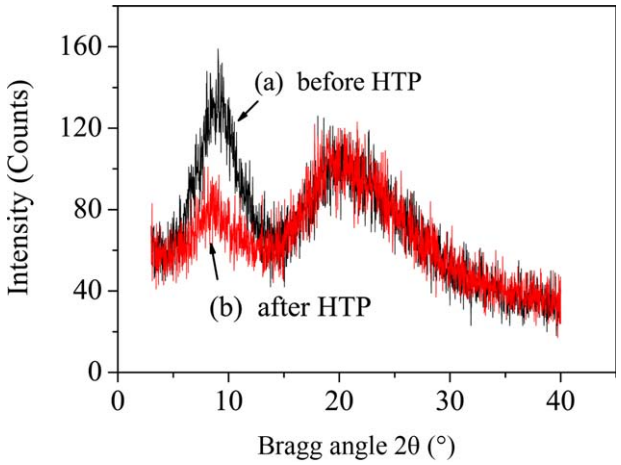

Figure 10. X-ray diffraction intensity patterns of wool keratin before (a) and after HTP at 130°C for 40 min (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

HTP both showed two broad peaks around $2\theta = 9°$ and $2\theta = 20°$, respectively. According to the investigation,[32] the peak around $2\theta = 9°$ was the main diffraction peak from crystalline phase in wool keratin. Diffraction intensity of the peak around $2\theta = 9°$ for wool keratin after HTP was significantly lower than that of wool keratin before HTP and the lower diffraction intensity indicated lower crystallinity. Area of the crystalline peak around $2\theta = 9°$ of wool keratin after HTP at 130°C for 40 min was 1773 counts and that of wool keratin before HTP was 6324 counts, which demonstrated that crystallinity of HTP-treated wool decreased by 71.9% when compared with that of untreated wool.

Figure 11 shows DSC curves of wool keratin before and after HTP at 130°C for 40 min. As shown in Figure 11, DSC curve of wool keratin before HTP appeared an endothermal bi-peak from 224°C to 242°C. The corresponding temperatures of the bi-peak were respectively 231 and 234°C. Bi-peak was the same as bi-melting peak of α-helical crystals from wool keratin, as

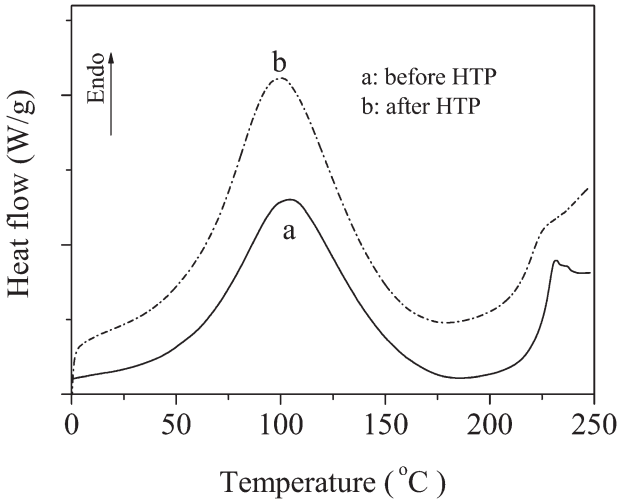

Figure 11. DSC curves of wool keratin before (a) and after HTP at 130°C for 40 min (b).

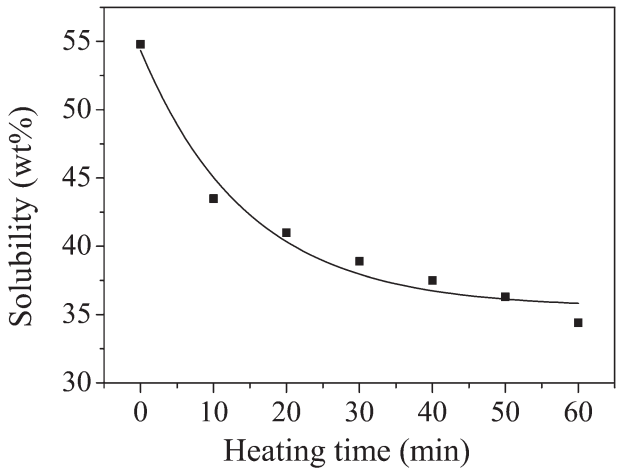

Figure 12. Solubility of wool keratin before and after HTP at 130°C for varying time periods (10–60 min).

reported by Huson et al.,[29] Wortmann and Deutz.[30] Therefore, the bi-peak in Figure 11 was the bi-melting peak of α-helical crystals in wool keratin. However, the DSC curve of wool keratin after HTP appeared a very smaller single melting peak around 225°C. Melting enthalpy ΔH (J/g) wool keratin before HTP was 12.65 J/g, which was obviously higher than 3.68 J/g for wool keratin after HTP. Decrease in ΔH for HTP-treated wool indicated that HTP at 130°C for 40 min had decreased crystallinity of wool keratin and the decrease percent of 70.9% was similar with that of 71.9% calculated from XRD. Figures 10 and 11 both indicated that HTP at 130°C for 40 min had destroyed most of α-helical crystalline IF of wool keratin.

Effect of HTP on Solubility of Wool Keratin

Figure 12 shows solubility of wool keratin before and after HTP at 130°C for varying time periods from 10 to 60 min. For untreated wool, 54.8 wt % of wool keratin was soluble. Solubility obviously decreased as heating time increased, and solubility of wool keratin after HTP for 60 min was 34.4 wt %. Wool keratin after HTP at 130°C with lower molecular weights and higher specific surface should be more prone to be dissolved in urea-bisulfite solution. However, studies by Rombouts et al.,[49] Jones et al.,[50] and Senoze et al.[34] indicated that some cystines in wool keratin were possibly converted to covalent lanthionine (LAN) cross-linkages during HTP. LAN thio-ether linkages do not tend to react with reductant and are probably more stable in the urea-bisulfite solution. These extra nondisulfide linkages could protect wool keratin against solubilization and resulted in decrease in solubility.[34,50] Increasing heating time from 10 to 60 min allowed sufficient conversion from disulfide to LAN bonds. The lower solubility of wool keratin after HTP was beneficial for using wool powders to develop new materials such as biocomposites, bio-thermoplastics, and bio-adsorbents.

CONCLUSIONS

Powdered wool with low solubility could be easily obtained by combination of HTP and mechanical grinding processes. Compared with untreated wool, wool fibers after HTP at 130°C for 40 min or longer time were obviously easier to be disintegrated

into small snippets with lengths of 25–150 μm by HSUD for 1 min and irregular shaped powders with sizes of 3–15 μm by ball mill for 10 min. Convenience of disintegrating wool fibers into powders resulted from brittleness of HTP-treated wool fibers. Fibers only retained 28.5% and 9.7% of their breaking strength and elongation after HTP at 130°C for 30 min. Brittleness of HTP-treated wool fibers resulted from changes in structures of wool fibers, especially decreases in contents of S—S crosslinkages, α-helix, and molecular weights. For wool keratin after HTP at 130°C for 40 min, contents of S—S crosslinkages and cystine both decreased by about 60%, the conformation of residual S—S crosslinkages was more unstable. Sulfur content decreased by 20.4%, and crystallinity decreased by about 70%. Raman spectroscopy showed that content of α-helical forms decreased and that of random coil forms increased, while SDS–PAGE showed that α-helical IF were degraded during HTP. Disintegrated powders after HTP showed lower solubility in mixture solution of urea, sodium hydrogen sulfite, and SDS, resulted from conversion of S—S crosslinkages to LAN bonds, which were not susceptible to reductive solution. The lower solubility was beneficial for utilizing wool powders to develop new materials such as bio-composites, bio-thermoplastics, and bio-adsorbers. Moreover, wool fibers retained most of their weight during HTP and the highest mass loss was as low as 4.05% after HTP for 60 min. HTP at 130°C for 40 min could be an eco-friendly and practically feasible physicochemical pretreatment method for large-scale preparation of wool powders, and could ensure industrial development of wool keratin as new materials.

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