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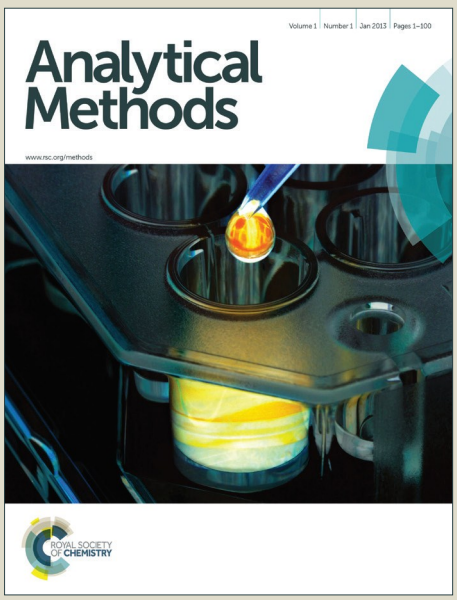

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Application of Electron Paramagnetic Resonance Spectroscopy, Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance and Scanning Electron Microscope to the study of the photo-oxidation of wool fiber

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Abstracts: An investigation into the influence of ultraviolet-irradiation (UV) on wool fibers was studied using Electron Paramagnetic Resonance (EPR) Spectroscopy, Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR) and Scanning Electron Microscope-Energy Dispersive Spectrometer (SEM-EDS). By the means of SEM-EDS and FTIR-ATR spectra, it found out that there are other observed components precipitated out of the fibers and the precipitated elements was related to S element， which indicated that cysteine residue was involved during the degradation procedure. The EPR spectra of the exposed wool fibers to UV radiation and untreated wool fibers showed a characteristic absorption peak centered at the highly isotropic g= 2.0029±0.0005 with the peak-to–trough width (~1mT) and without hyperfine structure, which was turned out to be carbon radicals.

Key words: UV-irradiation; Wool fiber Degradation; EPR spectra; FTIR-ATR; Free Radical

1. Introduction

Ancient wool textiles were welcomed with their beautiful texture, design, excellent hydrophobicity and tensile properties. Due to its structures, wool textile is damageable, as a result of being susceptible to different external factors e.g. heat, light, moisture and microorganisms [1-9] during the long burial period. Wool fiber turned to be fragile when under high temperature and its intensity would obviously decrease. To the worse, wool fiber became yellowing and degrading when exposed to continuing heating. What's more, it was expanding when exposed to high humidity environment. Water, as inter media of chemical reaction, facilitated the harmful chemical reaction and microorganism multiplying simultaneously. However, when decrease the ambient humidity, wool fibers became carbonized and being fragile because of losing water. The alkaline environment, which could lead to the leakage of molecular chain and exfoliation of the cuticle layer, could destroy 3D-net structure of wool fibers. The photochemical degradation mechanism of wool fibers has been studied extensively in recent years. According to literatures, the main obvious change of photochemical degradation is chemically bleached photo-yellows rapidly, or a yellow discoloration depending on the wavelength of the incident radiation [3, 4, 7]. From these literatures, scientists have used Fourier Transform Infrared Spectroscopy (FTIR), Fourier Transform Raman, Diluted Solution Viscometry, Scanning Electron Microscope (SEM), Amino Acid Analysis (AAA) and X-ray Diffraction (XRD) in the characterization of wool fiber keratin degradation at holistic level [10-18]. By using these characterizations mentioned above, it was known that the degradation of wool fiber was related to the changes with the amino acid residues and breaking of inter or intra chains bonds. However, it was still unknown how the changes happened.

In the light of above challenges, scientists now suggested that mechanical damage might give rise to detectable free radicals. From the literatures[19,20], scientists have hypothesized the degradation and fracture mechanism of polymers and biopolymers with two main mechanism: (1) the degradation process in which cracks due to the breaking of inter- and intra-chains bonds as the energy accumulates on some parts of polymer chains with thermal fluctuation; (2) the chain reactions of free radicals. Therefore, research on free radicals is a very critical way to exploit the degradation mechanism of wool fibers on a micro scale. Free radicals, which are commonly produced by ultraviolet, x-ray, γ-ray, or other irradiation and also by pyrolysis [21-22] are very important to proteins and enzymes due to their functions of catalyzing in organism. And it is very important to detect the lifetime and formation mechanism of the free radicals caused by external factors including light, heat, pH, mechanic force and molecular structure, since they revolved in many reactions such as in the oxidation reactions of hydro-carbon and many organic chain reactions.

Electron Paramagnetic Resonance (EPR), a powerful

technique to trace the free radical of artificially aged protein caused by gamma-ray and ultraviolet irradiation, can be employed to investigate the aging kinetics of wool keratin at the electronic and atomic level and probe the radical chemistry. J.J. Windle [21] et al studied the effect of mechanical action on EPR of wool and silk, which found that a –CHS · radical resulting from the rupture of the disulfide bond in the cysteine residue was observed in wool fiber.

In this paper, the deterioration of wool fiber by photo-oxidating was studied mainly via EPR, SEM-EDS and FTIR. The results demonstrated that carbonyl radicals took part in the deterioration process under UV-irradiation aging process. The data will provide an analysis method about the molecular chain and the secondary structure to study the aging mechanism of wool fiber. The findings also contribute to the explanation on deterioration mechanism of ancient wool textiles and pave the way for better preservation of these textiles heritages.

2. Materials and method

2.1. Sample Preparation

Under laboratory conditions, modern wool fiber from Xinjiang Province were immersed in 98% ethyl alcohol for 15 mins to remove out visible impurities. Then, the fibers were washed in deionized water for five times and dried at ambient temperature. The cleaned wool fibers were immersed into prepared detergent (prepared with deionized water and detergent with a proportion of 800:5) in order to remove grease more efficiently, and then put into ultrasonic cleaners for 15 mins. With this process, the wool grease can be removed to a great extent. Then, the de-greased fibers were washed in deionized water five times and dried at ambient temperature. Hence, the cleaned wool fiber was already obtained.

2.2. UV-irradiation exposure

Three samples of cleaned wool fibers were exposed to UV light of 356nm, from a Philips 15W UV lamp at ambient environment. And the height between lamp and samples is 30cm. The fibers were exposed for 6, 13 and 24 days respectively. Untreated wool fiber was used as control sample.

2.3. Characterization techniques

The samples were observed and images recorded using SEM-EDS (JSM-5610LV, Japan). FTIR-ATR was performed at ambient temperature using Nicolet 5700 (Perkin Elmer, USA) with the resolution ratio of 0.09cm^{-1}. The spectra were recorded in the wavelength range of 400cm^{-1}-4000cm^{-1}. Using samples of 10 mg in the standard quartz EPR tube, a German model EB200-SRC EPR spectroscope was used for EPR measurement, at room temperature. The parameters of EPR were microwave frequency of 9059.471±2MHz, microwave power of 0.99800mW, modulation frequency of 100.00 KHz, modulation amplitude of 2.000 * 1mT, time constant of 0.03s and sweep time of 1min.

3. Results and Discussion

3.1. The comparison of wool fiber morphology

The morphological differences between four samples mentioned above were studied by SEM. The different wool fiber surface images are shown in Fig.1 (a-d), which was enlarged 2500 times.

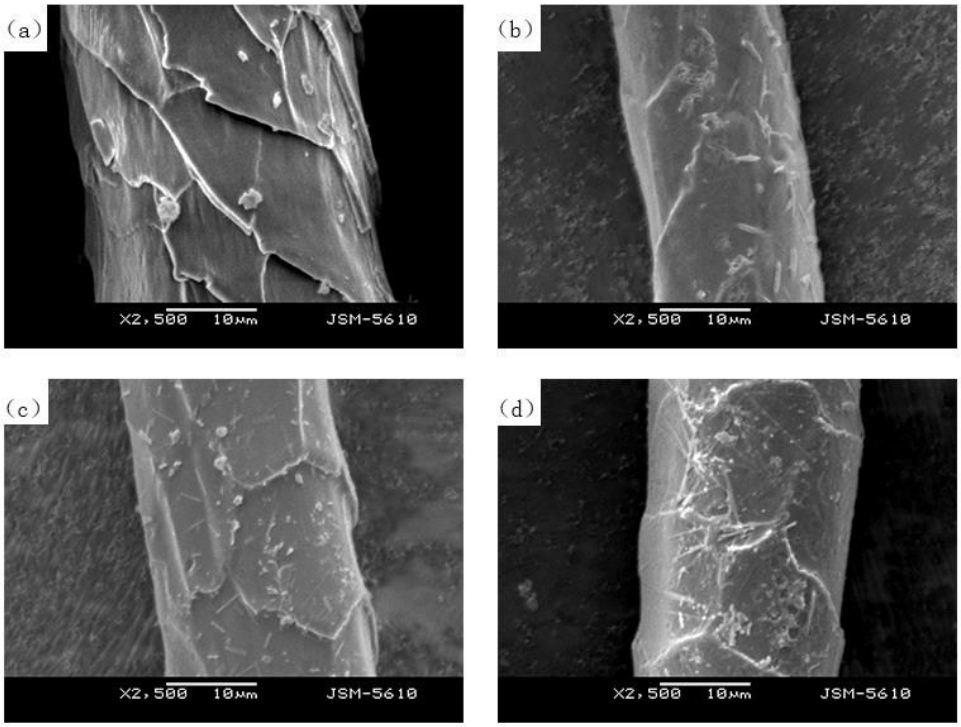

Fig 1. Morphology of (a) untreated; (b) exposed for 6 days; (c) exposed for 13 days; (d) exposed for 24 days samples

Note-worthy, the color is one of the degradation characteristics of the undying wool(which is not showed).The color of untreated wool fiber was white, while it changed to light yellow (after 6 days), medium yellow(after 13 days), dark yellow(after 24 days) with increased exposure time. Further observation shows that the cuticle layers were gradually destroyed, which were obviously observed on untreated wool fiber, as shown in Fig.1a, b, c and d. Also, the cuticle layer isn't much smoother than untreated wool fiber after UV exposure, which leads to loss of smoothness. Besides, there are other observed components precipitated out of the fibers and the precipitated elements were sharply increased with constant exposure times.

In order to further investigate the composition of the fibers, crystalloid substances and other contaminants, EDS, which can provide elemental information at a specific location was employed. As shown in Fig.2, the pink square areas analyzed by EDS are marked.

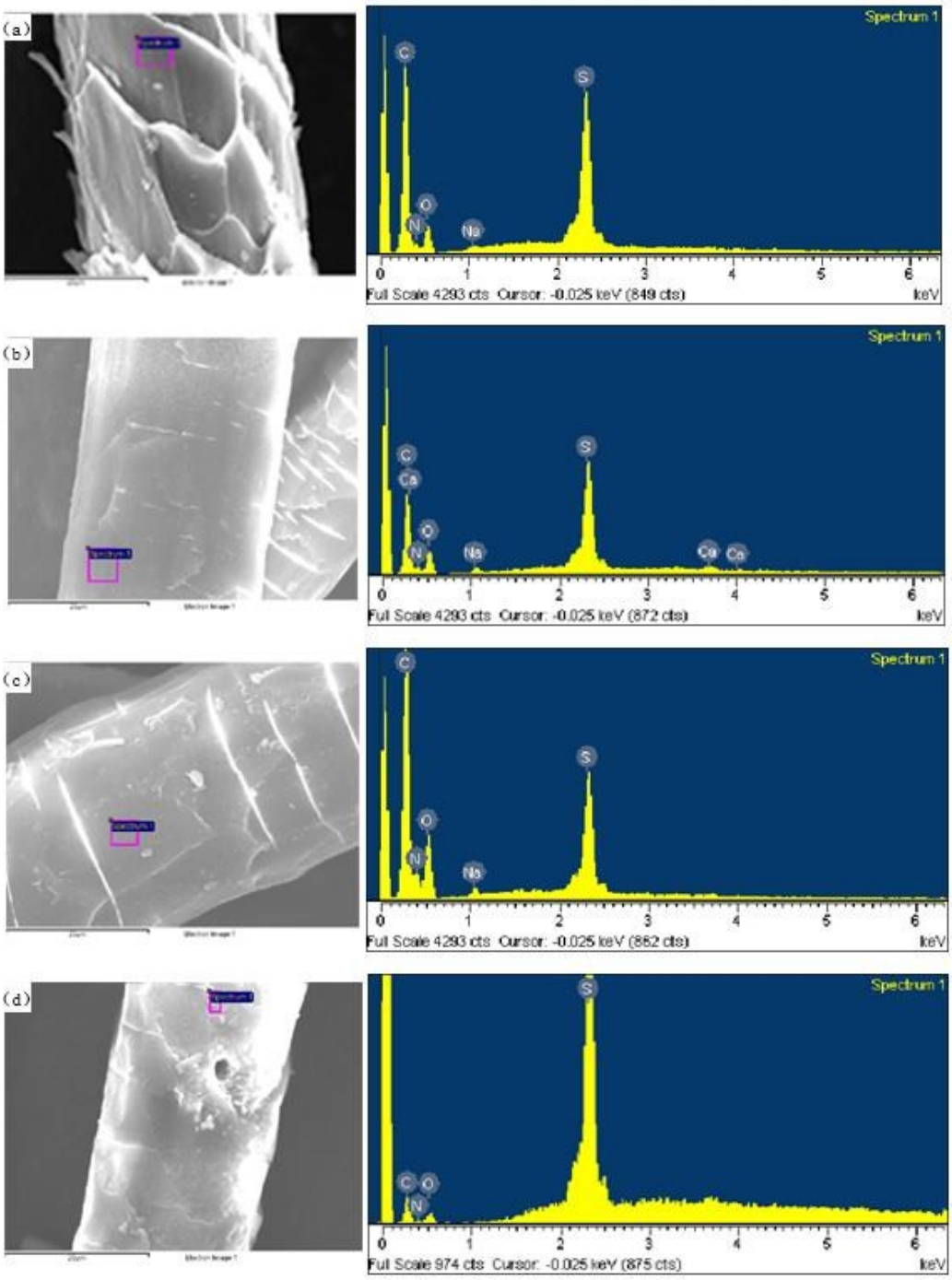

Fig 2. The EDS of wool fiber: (a) untreated; (b) exposed for 6 days; (c) exposed for 13 days; (d)exposed for 24 days

The result of the analysis applied on untreated wool fiber (Fig.2a) shows that the content of element S accounted for 3.21%. With increasing exposure time, the amount of element S precipitated out increased dramatically. The percentage of element S was found to be 3.80% in wool fibers exposed for 6 days (Fig.2b), 3.81% in fibers exposed for 13 days (Fig.2c), and 12.90% in fibers exposed for 24 days (Fig.2d) respectively. It is worth noting that during the deterioration processing, a pungent smell produced. It is supposed that these crystalloid substances may result from leakage of the –S-S- bond between protein polymer and cysteine residue in wool fiber.

3.2. FTIR spectroscopy analysis

It is known that the 1650 cm^{-1} absorption peak, a α-helix amide Ⅰ characteristic peak, is induced by the stretching vibration of carboxyl bond [11]. The characteristic peak of amide Ⅱ, induced by bending vibration of N-H and stretching vibration of C-N, is at 1540cm^{-1}; at 1230cm^{-1},there is the characteristic absorption peak of amide Ⅲ, which is induced by C-C stretching vibration and C=O bending vibration[4,11-15,23].

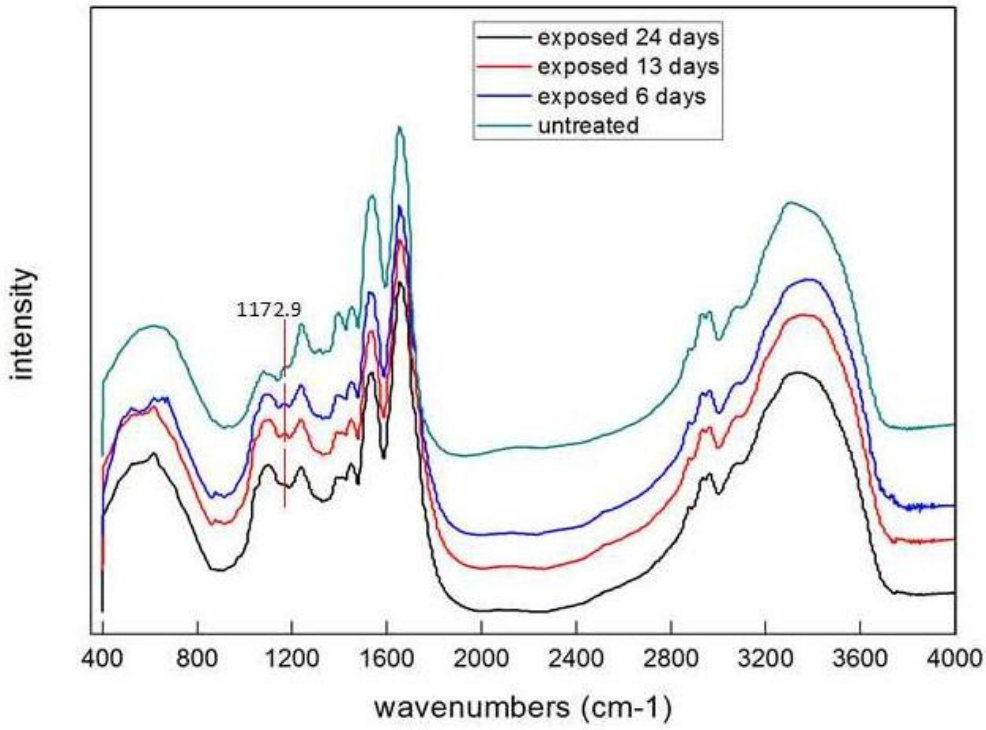

Fig 3. FTIR spectroscope of untreated wool fiber and different exposed times wool fiber

As to the literatures, the wavelength from 1000 to 1170$cm^{-1}$is the obviously changing of the aging wool fiber. The character peak at 1021cm^{-1} indicates bunte salt group, and the character peak at 1041cm^{-1} represents cysteic acid (-SO_3-)[13]. As shown in the Fig.3, although the wool chemical chain is broken and degraded during photo-oxidation deterioration, the main characteristic peak of wool protein did not apparently change. Besides, a small absorption peak disappears, and an ester bond was formed by amino acid residue, at 1172.9cm^{-1}. Furthermore, the intensity of this peak was increased with increased exposure time, which indicates that inter and intra molecular chains were seriously broken. As presented in the literature, cysteine is oxidized from sulfur monoxide to sulfuric acid, and the amount of bult salt increased with the increased exposure time [4, 11, 16]. The FTIR-ATR data implied that cysteine in wool fiber was seriously destroyed during exposure process. But FTIR-ATR spectroscopy is unable to study the cysteine aging behavior quantitatively at a micro degree.

3.3. EPR spectroscopy analysis

The obvious morphological changes imply that wool fiber was damaged, EDS and FTIR-ATR spectroscopy indicated that the deterioration of the exposed wool fiber is related to cysteine residue. However, further in-depth information of the cysteine aging behavior process is not clear; in other words, the kinetics of the cysteine residue is unclear. Therefore, EPR spectroscopy was used to detect free radicals during the process of wool fiber deterioration and the result is shown in Fig.4.

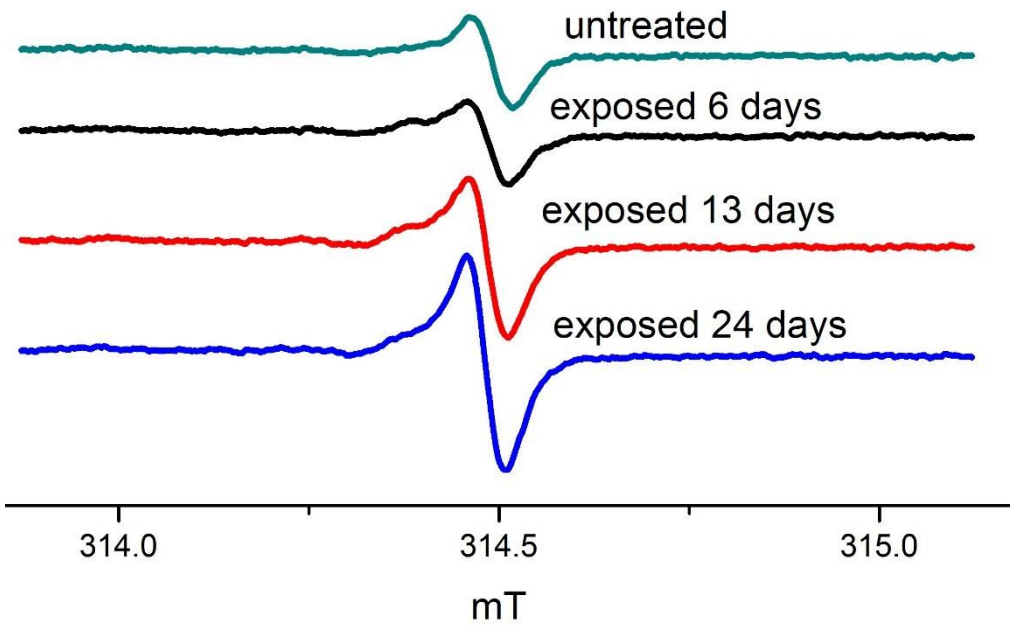

Fig 4. EPR spectra of untreated wool fiber and different exposed times wool fibers

All the EPR spectra had the same resolved characteristic absorption peak centered at the highly isotropic g-factor, whose value is 2.0029 ± 0.0005 indicating that the signals originate from the cubic symmetry of the radical center. After a study using Raman spectroscopy, as Gong [24] et al pointed out that the deterioration of ancient textiles is related to the carbonyl formation; there are carbonyl radicals formed during the aging process. What's more, in their study, it pointed out that these single caves without hyperfine structure indicate that spin density is localized at the atom(s) possessing the zero nuclear spin, and due to the peak-to–trough width (~1mT) of the curves, the common stable paramagnetic metal species, like Fe^{3+}, Mn^{2+} and Cu^{2+}, are excluded. As from the literatures [24–29], the value of g-factor ranged from 2.0028 to 2.0037 means that EPR absorption peaks are assigned to be carbon radical and the g-factor value of sulfur radical is 2.0218. Furthermore, thanks to the narrow peak-to –trough width, and the light asymmetry of the spectra, it turns out that the unpaired electron localized in the carbon radicals in the wool fiber matrix. According to Fig.4, with an identical weight for each sample, the EPR absorption peak intensity of untreated wool fiber is the weakest, while absorption peak intensity of the sample exposed of 24 days is the strongest. Considering the morphological changes (shown in Fig.1) and the degree of wool fiber chain structure changes (shown in Fig.3), the variation in concentration of the formed radical coincides with these two changes. Conclusively, the exposure ageing of the wool fiber was concomitant with the intensity of carbon radical.

For the sake of explaining the formation kinetics of free radical, the scheme of the chemical structures of the formation of radicals is shown in Fig.5. As it is shown in Fig.5, the main cleavage of the peptide bond is on the cysteine. Combining with the EPR spectra, the hemolytic cleavage may occur at one or both of the two sites marked ①、and ②(Fig.5).

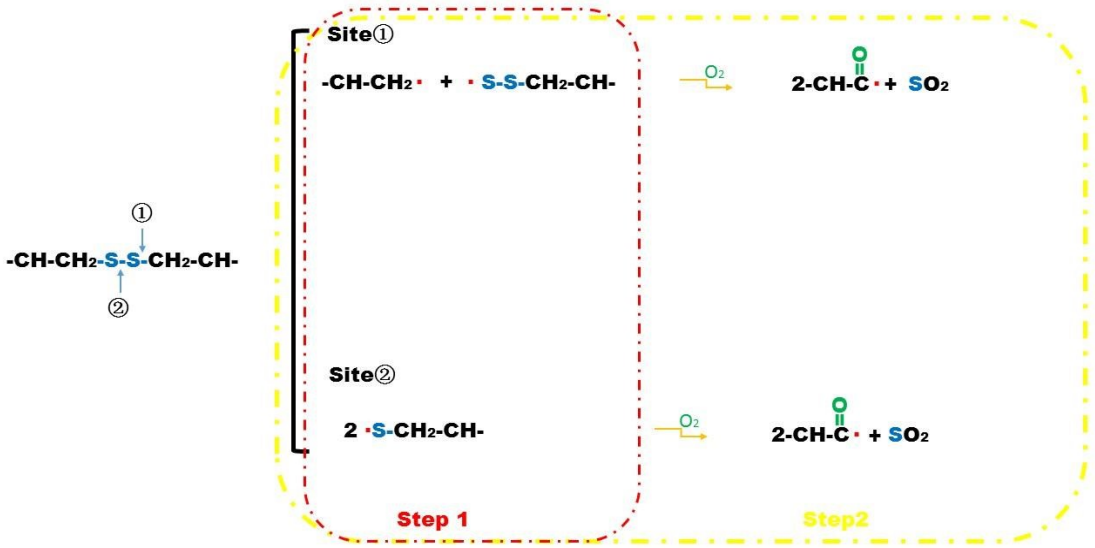

Fig 5. Scheme of chemical structure of possibly formed radicals in aging wool fiber

If the hemolytic cleavage occurs only on step 1, the residual radicals in the aging wool fiber are –C-C•, -S-S• and –C-S•, and the hyperfine structure will be observed due to the magnetic interaction between the spin of electron and nuclear. On the radical of –C-C•, the proton (1H=1/2) ligated to the carbon radical center in the –C-C• will complicate the hyperfine structure in the EPR spectra. As to the -S-S• and –C-S•, as it was mentioned above that the g-factor of -S-S• is approximately 2.0218, while in the EPR spectra, the g-factor is 2.0029. More remarkable, the sulfur-centered radicals and the carbon-centered radicals derived from saturated hydrocarbons, while the nitrogen radicals and the oxygen radicals are prone to be oxidative. The ambient aerobic environment can catalyze the oxidation reaction undergone by exposed wool fibers that arise to the formation of carbonyl groups [9, 10]. Vilaplana et al [10] had found out that there may be multi-band hyperfine structures if there are sulfur-centered radicals in the vacuum condition. Therefore, the step 2 occurs in an aerobic environment. As shown in Fig.5, –C-C•, -S-S• and –C-S• were all oxidized, and the carbonyl groups were the final product with SO_2 simultaneously. The carbonyl radical will give rise to the EPR spectra without hyperfine structure, and only carbonyl radicals can last and accumulate during the aging process [24]. In fact, wool fiber degradation is a complex process with several chemical bonds fractured simultaneously. However, it is not clear whether the hemolytic cleavage occurs at site ① or at site ② or the both during the degradation process. This requires a deeper explanation.

4. Conclusion

In summary, the different UV-irradiation exposure times of exposed wool and untreated wool fibers were studied and the photo-oxidation mechanism was investigated by means of SEM-EDS, FTIR-ATR and EPR spectroscopy. The morphological differences indicated that the degree of degradation of wool fibers was concomitant with the exposure times. From the EDS, the crystalloid substances on wool fiber implied that chemical substances which contained the element of S separated out and there may be some bonds broken which are related to element of S. The FTIR-ATR data imply that cysteine in wool fiber was seriously destroyed during the exposure process. Furthermore, the carbonyl radicals were found using the EPR spectroscopy. Without the hyperfine structure, the peak-to –trough width (~1mT) and little asymmetry of the EPR curves, it was certified that the degradation of wool fiber was a process with free radical chemistry. This paper provides a basic scientific knowledge of the aging process of wool fiber, and the study on conservation of ancient wool textiles.

Acknowledgements

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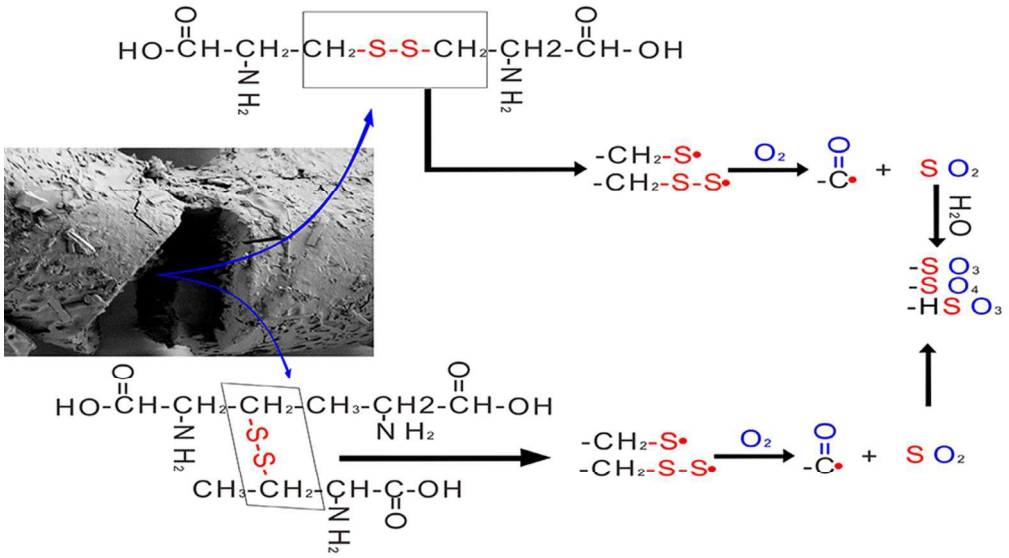

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