Studies of Wool Keratin by EPR Spectroscopy

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ABSTRACT: Electron paramagnetic resonance (EPR) tests were conducted on four kinds of interrelated samples—natural undyed wool fibers (SW3, SW5), fibers dyed with model direct dyes (1W, 5W), fibers treated destructively with formic acid for descaling and dyed with the same dyes (K1W, K5W), and the dyes themselves (1%). For all samples, a radical signal of $g = 2.007$ was detected. The presence of Mn^{2+} and Fe^{3+} ions in the wool fiber structure was postulated. Modifications of disulfide regions of fiber matrix were also analyzed. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 1459–1465, 2006

Key words: EPR; dyes/pigments; keratin fibers; microstructure

INTRODUCTION

There have been relatively few analyses of natural polymers, especially wool fiber keratin, using electron paramagnetic resonance (EPR) spectroscopy. Many have investigated these processes using electron spin resonance (ESR) or EPR. These include Gasimov et al.[1,2] and L'vov et al.[3] (fibrous proteins), Cope et al.[4] (hoof horn material), Alonso et al.[5] (stratum corneum from rats), Chandra and Symons[6] (1987) and Symons et al.[7] (1995) (human fingernail), Shavachko et al.[8] (human hair), and Chipara et al.[9] and Gasimov et al.[10] (collagen irradiated with protons). EPR spectroscopy has been used to study aspects of other natural materials such as wool keratin (Mamedov,[11] Smith,[12] Shatkay,[13] and Gogginger[14]) and silk fibroin (Gasimov[15] and Kweon[16]). The signals were analyzed in terms of characteristic features associated with specific sulfur-centered radicals.

EXPERIMENTAL

For examination, 10 model azo dyes were selected and synthesized at the North Carolina State University at Raleigh, NC (Bae[17]). The fleece was degreased for 8 h through Sokshlet apparatus extraction in methylene chloride (temperature 45°C). A sample of wool fiber (0.5 g) was wet out with hot water and then, with 10 drops of 3% acetic acid and 0.005 g of dye, added to a dyebath (40 mL) at 60°C. The bath temperature was subsequently raised to 95°C and kept at this level for 30 min. After adding Na_2SO_4 (0.05 g), dyeing was continued for another 30 min. The dyed fiber was then rinsed with cold water and air-dried. Descaling was carried out in 98–100% formic acid at 80°C for 2 h.

Electron paramagnetic resonance (EPR) spectra were recorded at room temperature (293 K) and liquid nitrogen temperature (77 K), using an EPR spectrometer ELEXSYS E-500–10/12 (Bruker) with a Unix workstation. As a spin standard, $VOSO_4$ diluted with K_2SO_4 was used with the number of spins standing at 4.935×10^{17}. The description of the experimental technique is shown in particular in Figures 1, 3–5.

RESULTS AND DISCUSSION

EPR spectra were recorded for raw, i.e., undyed, wool fibers, samples dyed with direct dyes, and dyed samples earlier treated destructively with formic acid. All EPR spectra reveal a radical central signal of $g = 2.007$ (Figs. 1–5).[11] Figure 1 compares the EPR spectra of two undyed samples of wool of the same breed but from different batches. Differences in spectrum profiles were found for spectra recorded at both temperatures (room and liquid nitrogen). These prove structural diversification of fibers, which was observed earlier by Włochowicz[18] in wool keratin.

FTIR and FT-Raman spectroscopies show subtle changes in the structure of the fiber. FTR spectroscopy is much more affected by structural changes within the fiber than FTIR. For example, as a reflection technique (using samples of whole fiber fragments), FTR spectroscopy is much more affected by structural changes within the fiber than FTIR (for which KBr

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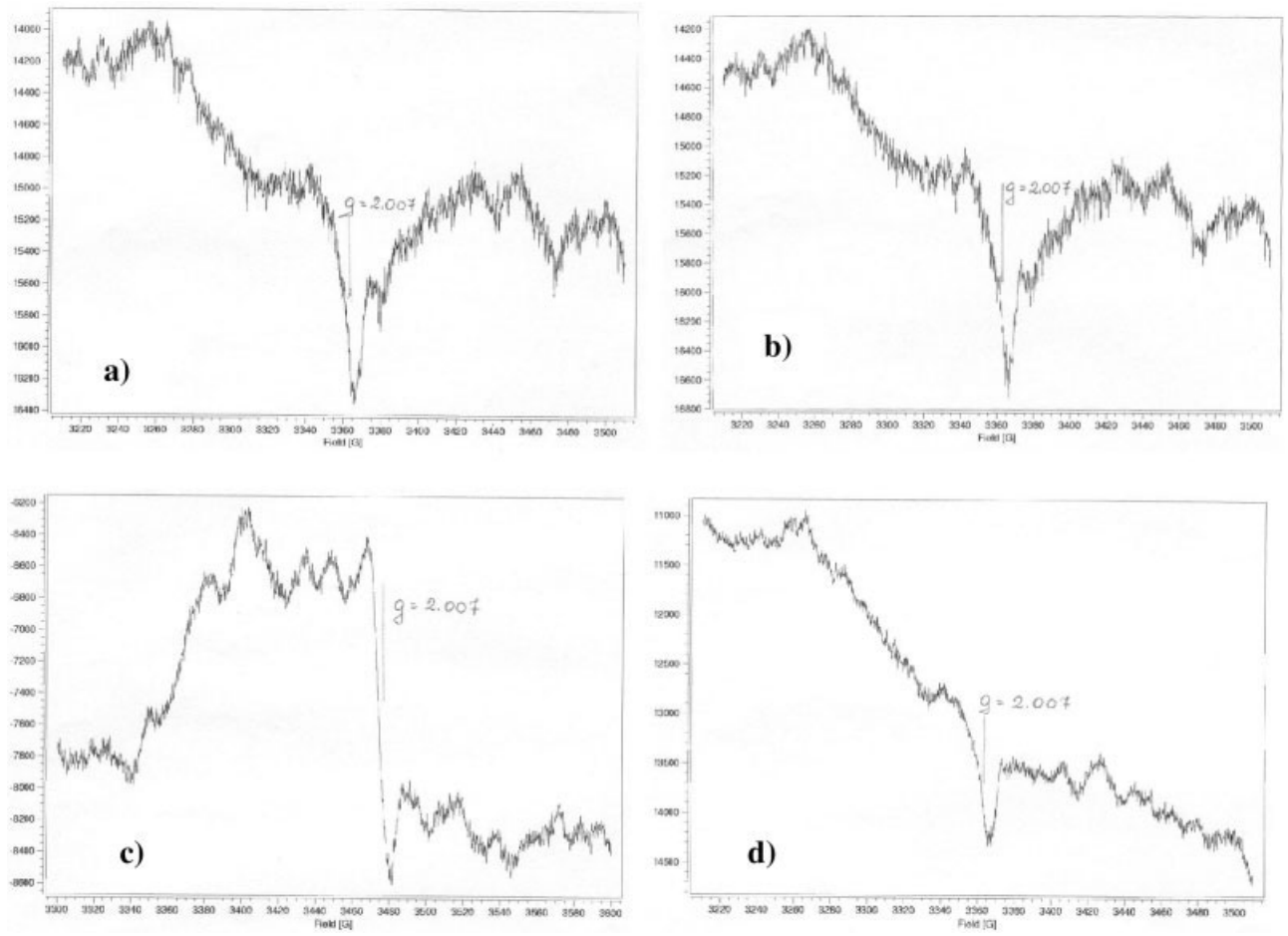
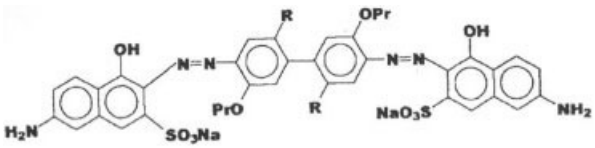


Figure 1 EPR spectra of wool fiber. (a) Undyed wool fiber (SW3) recorded in 293 K; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (b) Undyed wool fiber (SW3) recorded in 77 K; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (c) Undyed wool fiber (SW5) recorded in 293 K; instrument settings: center field 3450 G; microwave frequency 9.763 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (d) Undyed wool fiber (SW5) recorded in 77 K; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s.

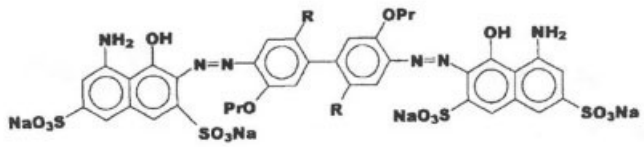
pellets of powdered fibers are prepared). Differences in percentage content of disordered forms are caused by this structural heterogeneity of wool fiber.[19]

The fibers analyzed were dyed with model direct dyes with the following structural formulas (Structure 1).

Dye spectra reveal a hyperfine structure, which may prove the presence of isolated Mn^{2+} ions. The structure is particularly visible for spectra recorded in liquid nitrogen [Figs. 2(b)–5(b)]. The number of paramagnetic centers per 1 g of sample is similar in all cases, except for pure dyes, where it is much bigger (notably for Dye 5) (Table I). Most likely, the clearly visible hyperfine structure of pure dyes is due to their structural symmetry, when compared with the complex (crystalline and amorphous) structure of natural polymers.



a) dye 1 (R = CH_3)



b) dye 5 (R = CH_3)

Structure 1 Structures of model dyes.

TABLE I
EPR Investigation Results for Samples recorded at 293 and 77 K

	293 K			77 K		
Samples	ΔB (B)	I (j.u.)	Spin no (10^{16} N/g)	ΔB (B)	I (j.u.)	$N_{77\ K}/N_{293\ K}$
SW 3	13.9	2132	1.36	22.0	1517	1.78
SW 5	14.0	2979	1.89	18.7	1420	0.85
1W	13.4	3275	1.76	11.9	963	0.23
5W	14.5	3168	1.76	9.4	855	0.11
K1W	13.1	3378	2.52	9.8	2115	0.35
K5W	13.0	3945	1.82	12.3	1214	0.28
1	10.4	16230	6.03	12.6	5475	0.50
5	13.0	64019	5.64	14.3	34480	0.65

Table I reveals a decreased number of EPR-detectable paramagnetic centers at low temperatures, when comparing the intensity of signals at room temperature and in liquid nitrogen (except for sample SW3). This may imply increased antiferromagnetic interactions caused by the presence of transition metal ions in

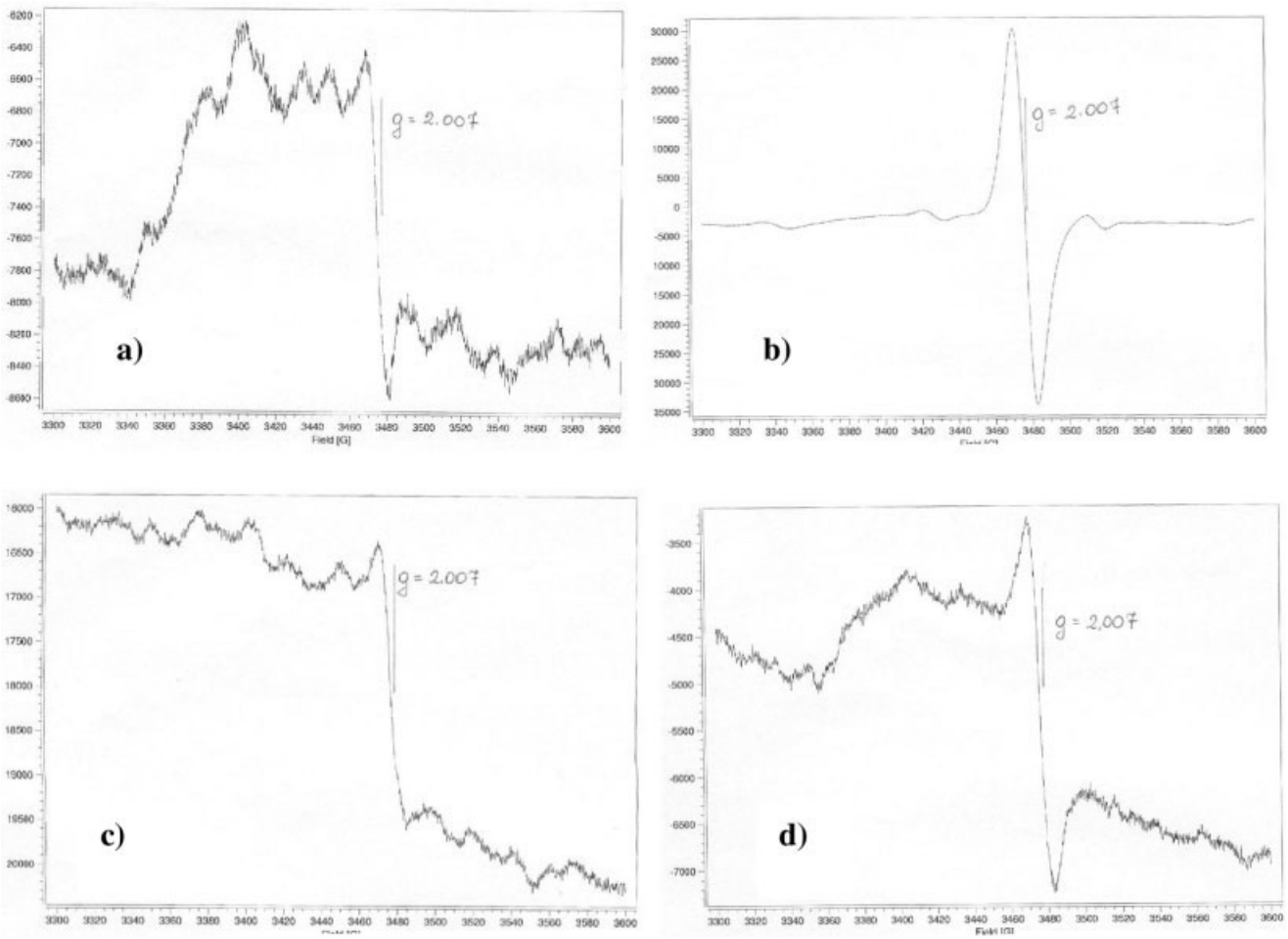


Figure 2 EPR spectra of wool fiber (recorded in 293 K). (a) Undyed wool fiber; instrument settings: center field 3450 G; microwave frequency 9.763 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (b) Dye 5; instrument settings: center field 3450 G; microwave frequency 9.769 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (c) Wool fiber dyed with Dye 5; instrument settings: center field 3450 G; microwave frequency 9.766 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (d) Wool fiber descaled in formic acid and then dyed with Dye 5; instrument settings: center field 3450 G; microwave frequency 9.767 GHz; microwave power 0.0102 W; receiver time constant 0.164 s.

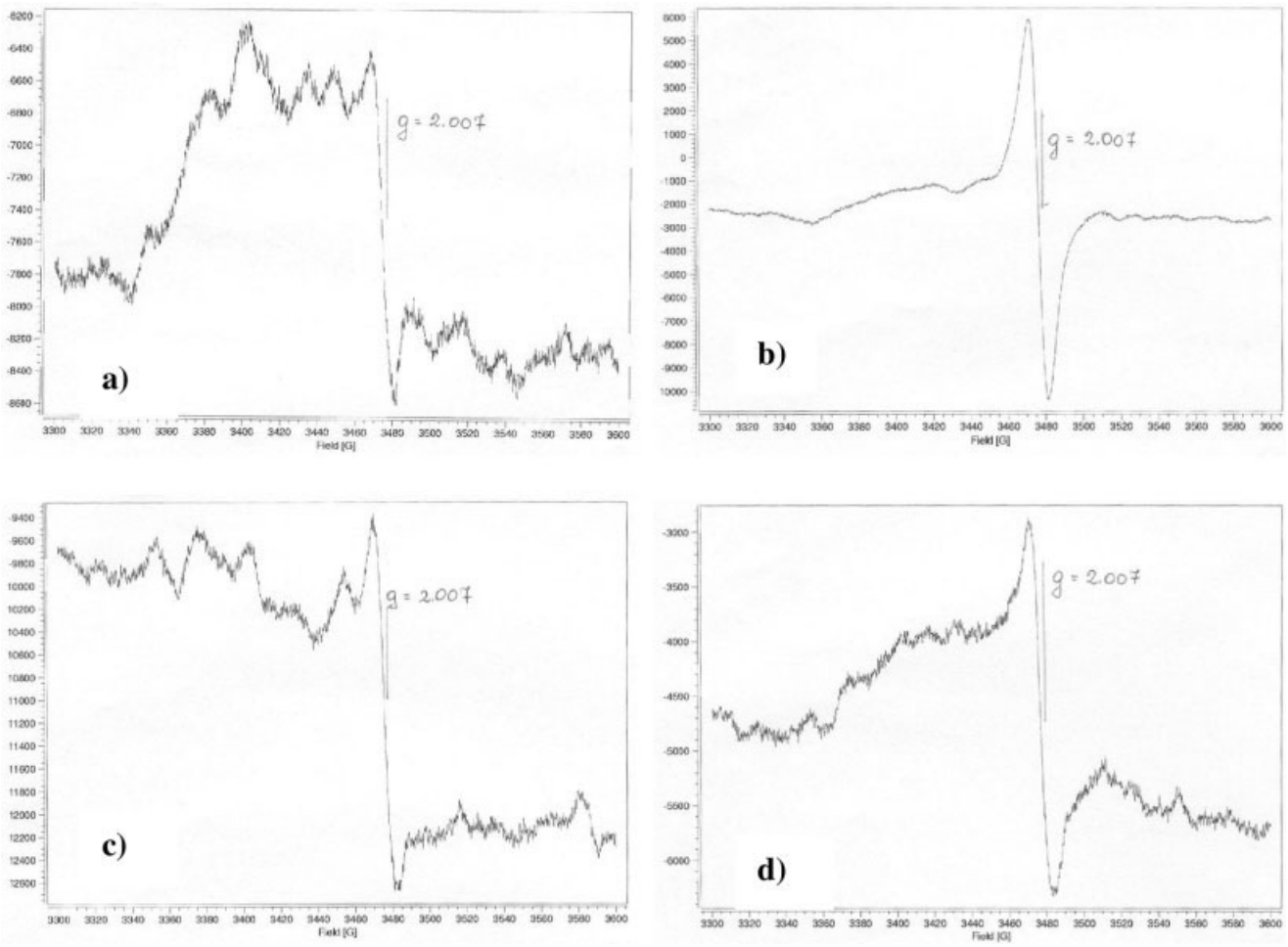


Figure 3 EPR spectra of wool fiber (recorded in 293 K). (a) Undyed wool fiber; instrument settings: center field 3450 G; microwave frequency 9.763 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (b) Dye 1; instrument settings: center field 3450 G; microwave frequency 9.766 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (c) Wool fiber dyed with Dye 1; instrument settings: center field 3450 G; microwave frequency 9.767 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (d) Wool fiber descaled in formic acid and then dyed with Dye 1; instrument settings: center field 3450 G; microwave frequency 9.769 GHz; microwave power 0.0102 W; receiver time constant 0.164 s.

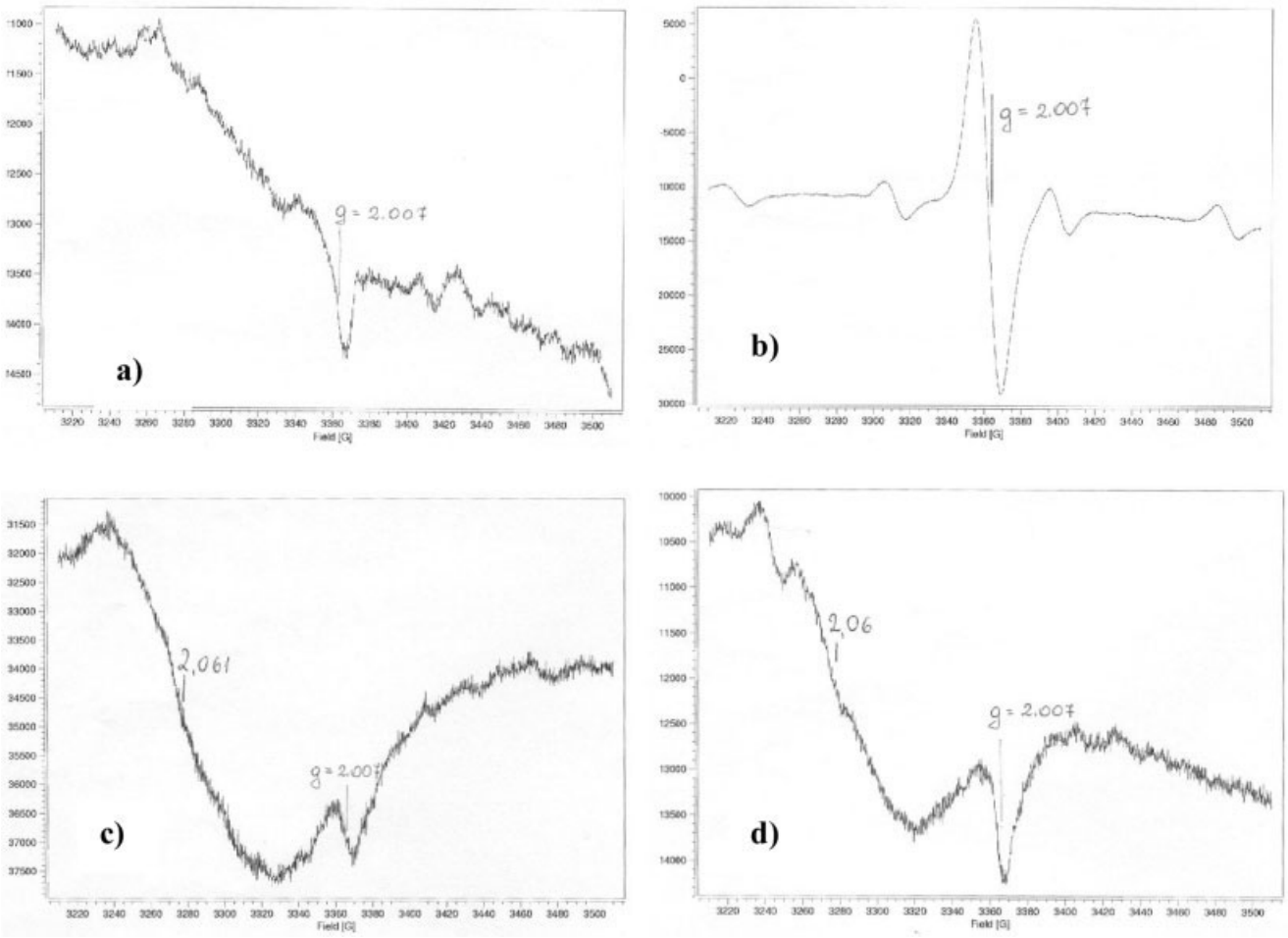


Figure 4 EPR spectra of wool fiber (recorded in 77 K). (a) undyed wool fiber; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (b) Dye 5; instrument settings: center field 3360 G; microwave frequency 9.445 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (c) Wool fiber dyed with Dye 5; instrument settings: center field 3360 G; microwave frequency 9.451 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (d) Wool fiber descaled in formic acid and then dyed with Dye 5; instrument settings: center field 3360 G; microwave frequency 9.448 GHz; microwave power 0.0102 W; receiver time constant 0.164 s.

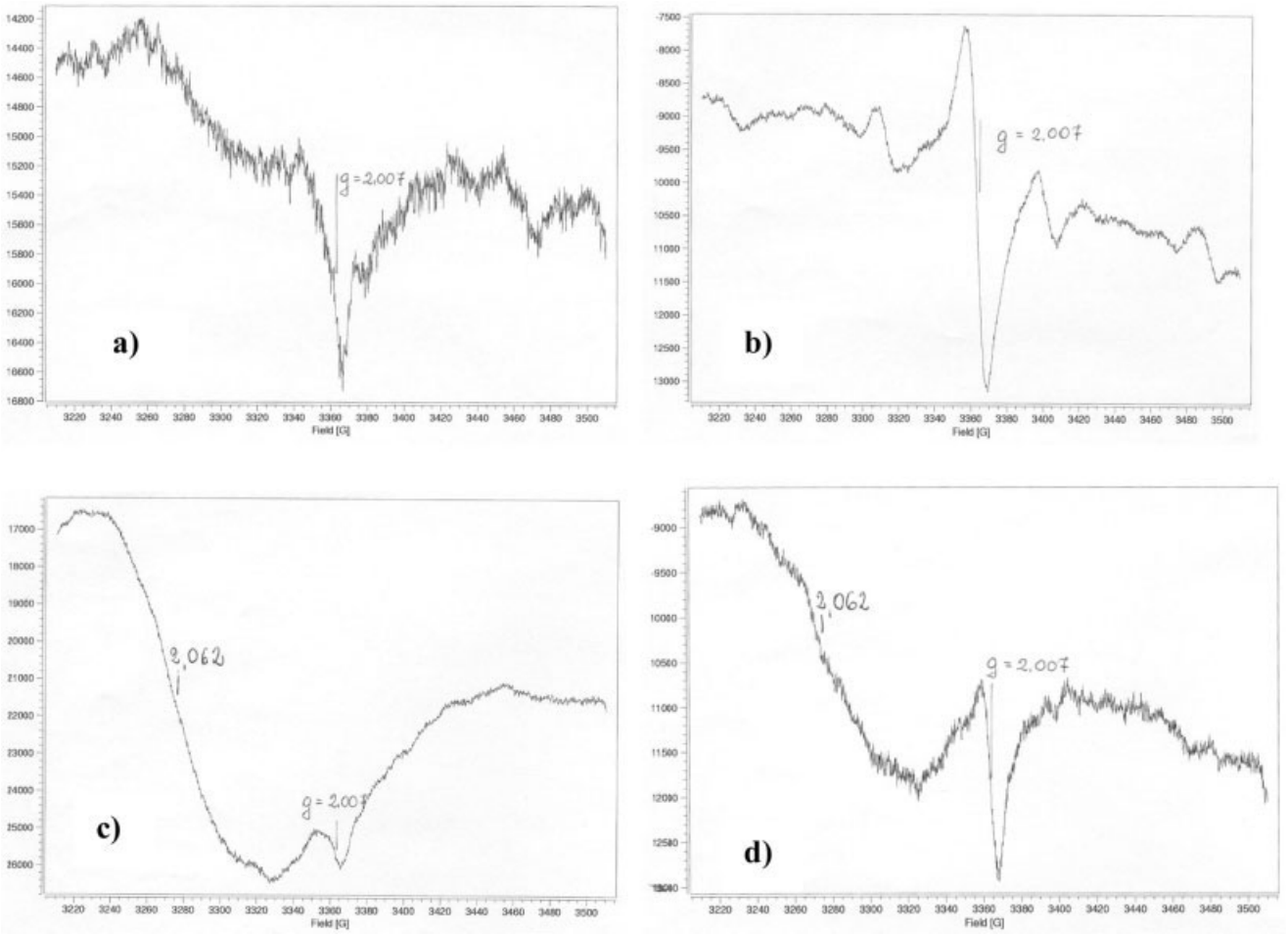


Figure 5 EPR spectra of wool fiber (recorded in 77 K). (a) Undyed wool fiber; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (b) Dye 1; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. (c) Wool fiber dyed with Dye 1; instrument settings: center field 3360 G; microwave frequency 9.448 GHz; microwave power 0.0102 W; receiver time constant 0.164 s. Wool fiber descaled in formic acid and then dyed with Dye 1; instrument settings: center field 3360 G; microwave frequency 9.447 GHz; microwave power 0.0102 W; receiver time constant 0.164 s.

the samples examined. In the samples of undyed fibers and pure dyes, the width of EPR signals was found to increase as the temperature dropped (Table I). Typically, lowering the temperature in paramagnetic preparates results in narrower but more intense EPR signals.

Samples 1W, 5W, K1W, and K5W recorded at liquid nitrogen temperature, have an intense, wide signal of $g = 2.06$ [Figs. 4(c) and 4(d) and Figs. 5(c) and 5(d)], which may be attributed to a number of possible sources. One of them is the presence of Fe^{3+} ions in wool fibers, as earlier confirmed (Pielesz[20]). However, it is known that the supermolecular structure of fiber, particularly its matrix, is densely crosslinked with disulfide bonds (Pielesz,[19,20] Alix,[21]). It is also known that even nonirradiated wool fibers contain free radicals[14]. However, it was found out (Gogginger[14]) that both treating fibers at high microwave power and recording spectra at low temperatures resulted in a higher number of signals detected. In particular, cysteinyl R-S· radicals were observed, resulting from homolytic rupture of the disulfide bonds of cystine residues, as reported earlier in the literature (Shatkay[13,22]). Hence, the presence of the $g = 2.06$ signal should be probably attributed to the R-S· radicals, the amount of which may change when the amorphous structure of a fiber is modified with dye solutions and formic acid. The attribution of the $g = 2.061$ signal to R-S· radicals from the matrix of the hoof horn material has been confirmed by Cope et al.[3] In this study, the intense $g = 2.06$ signal occurs for samples 1W, 5W, K1W, and K5W, i.e., for the chemically treated samples.

Meanwhile, the higher values of signal intensity obtained at 293 K (Table I) for samples K1W and K5W, when compared with samples 1W and 5W (treated with the same dyes), imply an ongoing process of modification of wool fiber caused by formic acid. Conformation changes in the structure of wool fiber caused by its

dyeing and, particularly, destructive treatment with formic acid were analyzed earlier (Pielesz,[19,23]).

Dyeing a wool fabric with the model dyes does not affect the secondary fiber structure too strongly.[19] Samples of undyed wool fibers, dyed samples treated with formic acid and samples not treated with acid were chosen to see the destructive results of fiber descaling in fiber structure and the dye introduced.[19]

CONCLUSIONS

To conclude, it should be said that the EPR spectra obtained do not differ substantially. Nevertheless, the results confirm the observations made earlier,[19,23] using different analytic techniques (FTIR, FTR, electrophoresis, electron, TEM, and fluorescence microscopies). It seems that the discussed structural changes in wool keratin occur mainly in the fiber matrix. This region, particularly densely crosslinked with disulfide bonds, is also the most susceptible to any outside interference.

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