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A Measurement of Human Hair Oxidation by Fourier Transform Infrared Spectroscopy

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ABSTRACT: Human scalp hair samples were oxidized to determine the sulfonic acid absorption peak. This peak was monitored at 1044 cm^{-1} by Fourier transform infrared spectroscopy (FTIR) in hair samples from 135 whites and found to provide a degree of discrimination in treated and untreated hairs. The effects of moisture, laboratory storage, natural hair color, and variation over time were also studied.

KEYWORDS: forensic science, hair, spectroscopic analysis, Fourier transform infrared spectroscopy, hair treatment

The analysis and comparison of human head hairs is often controversial because of the many subjective parameters used in describing the hair fibers [*1-4*]. Gaudette has suggested that one "approach to the individualization of hair would be to obtain enough additional variable characteristics so that when they are added to present macroscopic and microscopic characteristics a statistical analysis would conclusively demonstrate in all instances that the chances of two people having similar hairs would be negligible" [*1*]. Instrumental methods of analysis will often provide discrete qualitative and quantitative information which can characterize a fiber, removing or supplementing some of the more subjective measurements made by the light microscope.

The treatment of hair beyond normal shampooing, conditioning, and combing is a cultural and environmental phenomenon and may be expected to alter hair in such a way as to be discriminating on the basis of hair treatment. Dyed and bleached hairs are usually distinguishable from untreated hair by light microscopy [*4*]. A hair that has been permanently waved may or may not be as easily distinguished. These changes have been investigated at the molecular level using amino acid analysis, enabling differentiation of treated and untreated hairs [*5*].

The bleaching of hair usually takes place in an alkaline medium with hydrogen peroxide to change black or dark brown hair to blonde. The process proceeds by attack on the cystyl residues in the hair protein to give as a final product a sulfonic acid function. Hair dyes may be oxidative and contain dye precursors, dye couplers (which react with the precursors), and an oxidizing agent (hydrogen peroxide) that reacts at an alkaline pH 8 to 10. By changing the proportions of the dye precursors, coupler, and oxidizing agent, darker or lighter hair color will result. Per-

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manent waving (the cold wave process) produces a sulfonic acid function on reoxidation after reduction of the disulfide crosslinks [6, 7].

Previous studies have shown a significant increase in the amount of cysteic acid and a similar decrease in the amount of cystine in the keratin structure of the hair following oxidation [5, 8–11]. In this oxidation reaction, the sulfur-sulfur bond is cleaved, resulting in two sulfonic acid residues [6, 9]. Those studies using infrared analysis of oxidized wool [12, 13] and oxidized human hair [14] assigned peaks at 1040 cm^{-1} and 1175 to 1180 cm^{-1}, respectively, to these sulfonic acid groups.

An investigation into the oxidative behavior of human head hair was begun using Fourier transform infrared spectroscopy (FTIR) in an attempt to obtain additional variable characteristics. Fourier transform infrared spectroscopy utilizes an interferometer to produce an infrared spectrum. These spectra may be added by means of a computer and weak infrared bands may be observed through a reduced noise level. A diamond cell microsampler was used to analyze 400-ng fragments of single hairs.

Materials and Methods

A Digilab FTS-10 C/D Fourier transform infrared spectrometer with cesium iodide optics, a TGS detector, and a diamond cell with a ×4 beam condenser were used in the scanning of samples. One thousand scans of both the reference cell and the sample were collected at 8-cm^{-1} resolution. The sample chamber was purged with high purity dry nitrogen. Data was processed with a Data General Nova 3 computer. Reagents used included: 30% hydrogen peroxide (Fisher Scientific Co., King of Prussia, PA), 2-mercaptoethanol (Kodak Chemical Co., Rochester, NY), dithiothreitol (Calbiochem, San Diego, CA), and *L*-cysteic acid (Serva Feinbiochemica, Heidelberg, W. Germany).

Scalp hair samples were collected from 135 laboratory staff members, family, and friends and were limited to whites. Donors ranged in age from 6 months to 83 years, with an average age of 33 years. A total of 84 women and 51 men were tested. The following information was collected from each donor: name, age, sex, and type of hair treatment. The treatments of interest were: dyeing or coloring, bleaching, tinting, frosting, permanent waving, or any combination of these. Many types of hair treatment are available, but this study was limited to those of an oxidative nature. Those hairs which had no treatment other than normal shampooing, conditioning, and combing were listed as untreated.

Hairs were not cleaned or washed before mounting in the diamond cell. Two sections of hair, each 0.5 to 0.7 mm in length, were cut from the midpoint of the hair shaft. The oxidation peak assigned to the sulfonic acid group [12–14] was monitored in the range of 1046 to 1042 cm^{-1} and centered at 1044 cm^{-1}. All spectra were plotted from 1300 to 940 cm^{-1}, to enable observation of this oxidation peak.

Results

Untreated hairs were oxidized in 10% hydrogen peroxide at pH 5 for various time intervals and the resulting peak at 1044 cm^{-1} was monitored. An oxidation peak began to appear at 1044 cm^{-1} at approximately 4 h. The pH was increased to pH 10 and the resulting peak was again monitored. The rate of oxidation increased, with an oxidation peak appearing at approximately 1½ h. The infrared absorbance spectra of artificially oxidized hair is shown in Fig. 1. Figure 1*a* represents an unoxidized hair, showing no shoulder or peak at 1044 cm^{-1}. Figure 1*b* shows a hair oxidized for 1½ h. A small peak is distinguishable. Figure 1*c* shows a hair oxidized for 7 h. A large oxidation peak is readily apparent. In the subtraction of an unoxidized hair spectrum from the spectrum of an oxidized hair, a differential spectrum (Fig. 2*b*) shows a sulfur-oxygen peak similar to that of cysteic acid (Fig. 2*a*). Reduction of the oxidation product peak was attempted with solutions of 1% 2-mercaptoethanol at pH 5, 10% 2-mercaptoethanol

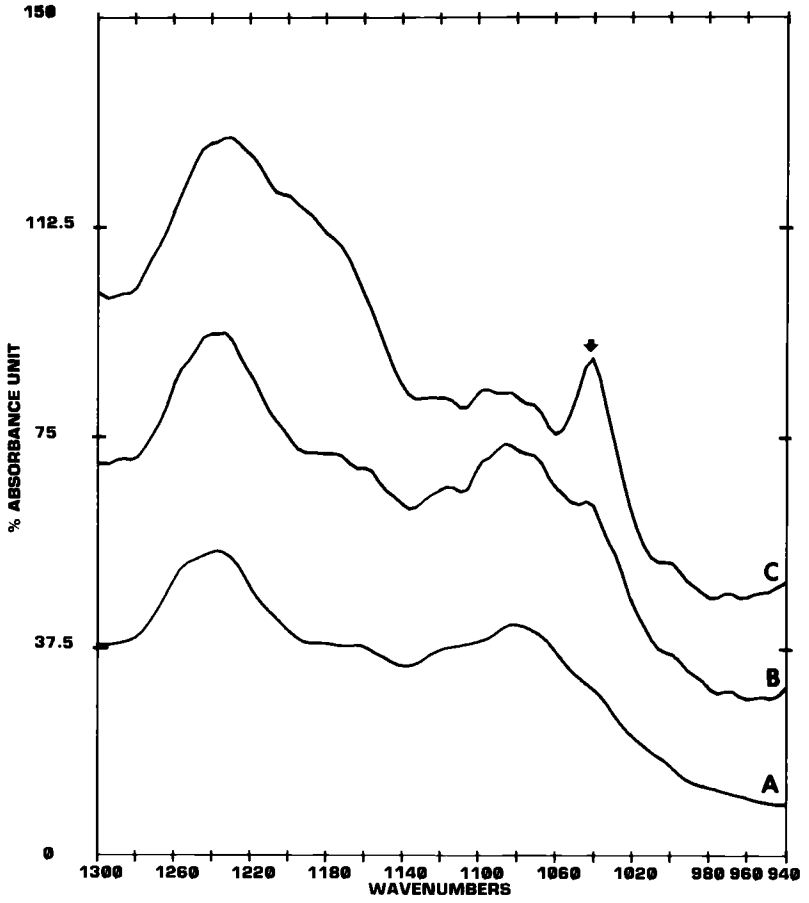

FIG. 1—(a) *Infrared spectrum of unoxidized hair.* (b) *Infrared spectrum of hair oxidized for 1½ h.* (c) *Infrared spectrum of hair oxidized for 7 h. (Arrow indicates oxidation peaks at 1044 cm^{-1}.)*

at pH 5, 10% 2-mercaptoethanol at pH 10, and 1% dithiothreitol at pH 5. All attempts were unsuccessful, although gross changes in the hair were evident.

Variations in the hair of an individual were examined. Five donors were tested, two with treated hair and three with untreated hair, using ten hairs from each person. Each treated hair showed a similar absorbance profile at 1044 cm^{-1}. Likewise, those untreated hairs demonstrated absorbance profiles at 1044 cm^{-1} that were similar in each case.

When treated hairs were examined along the length, changes in the absorbance profile were recorded as shown in Fig. 3. Figure 3*a* shows the root end of a hair that had been permanently waved one month before testing. Very little oxidation product is apparent. Figure 3*b* shows an absorbance profile from a section of hair taken from the midpoint of the same hair. An oxidation peak is present. Figure 3*c* shows an absorbance profile from the tip of the same hair. A slightly larger oxidation peak is present at 1044 cm^{-1}. Near the root no oxidation peak is present since this area had grown out since treatment. There may, of course, be some naturally occurring sulfur-oxygen containing product present in the hair [*10*] which would be seen as a shoulder in the spectrum (as in Fig. 3*a*). An oxidation product peak may or may not be seen in a sample taken from the midshaft region of the hair. The presence of the peak would depend

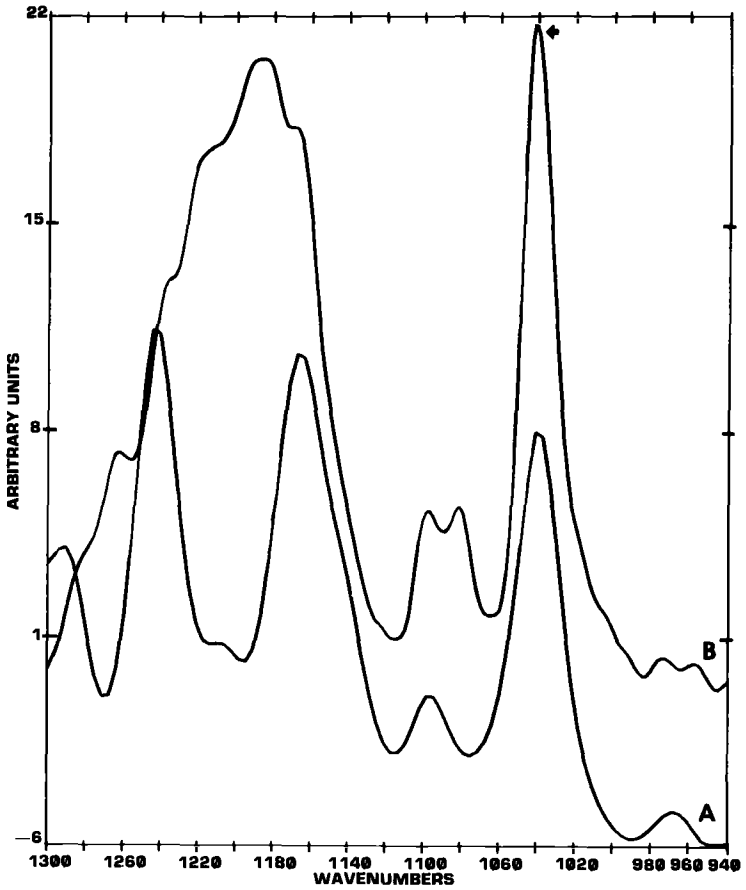

FIG. 2—(a) *Infrared spectrum of cysteic acid.* (b) *Difference spectrum, oxidized minus unoxidized hair. (Arrow indicates oxidation peaks at 1044 cm^{-1}.)*

on such factors as the type and degree of treatment, the time since the treatment, and the amount of naturally occurring sulfur-oxygen containing product. When untreated hairs were examined along the length, no significant changes in the absorbance at 1044 cm^{-1} were observed, although the tip may contain a slightly larger amount of oxidation product as a result of weathering [*6, 10, 13*].

Untreated hair was immersed in distilled water at pH 10 for as long as 72 h, towel dried, and tested immediately. The moisture had no effect on the oxidation peak at 1044 cm^{-1}. Other factors having no effect on this oxidation peak included storage in the evidence storage room for as long as twelve years, and gray versus natural colored hair. No variation over the span of one year was apparent in the hair samples of several untreated individuals.

The results of a population study of 135 whites are shown in Table 1. Hair segments were taken from the upper (distal end) one third of the hair shaft. A total of 51 males was tested. Of these 51 donors, 42 men used no treatment on their hair. No peak or shoulder was detected at 1044 cm^{-1}, in 23 (55%) of the samples. A shoulder was observed in 19 (45%) of the samples; and none of these hair samples showed a well-defined peak. Nine of the fifty-one men treated their hair. A peak was detected at 1044 cm^{-1} in five (56%) of the nine samples; and a shoulder was detected in four (44%) of the samples. None of the hairs showed the absence of a shoulder

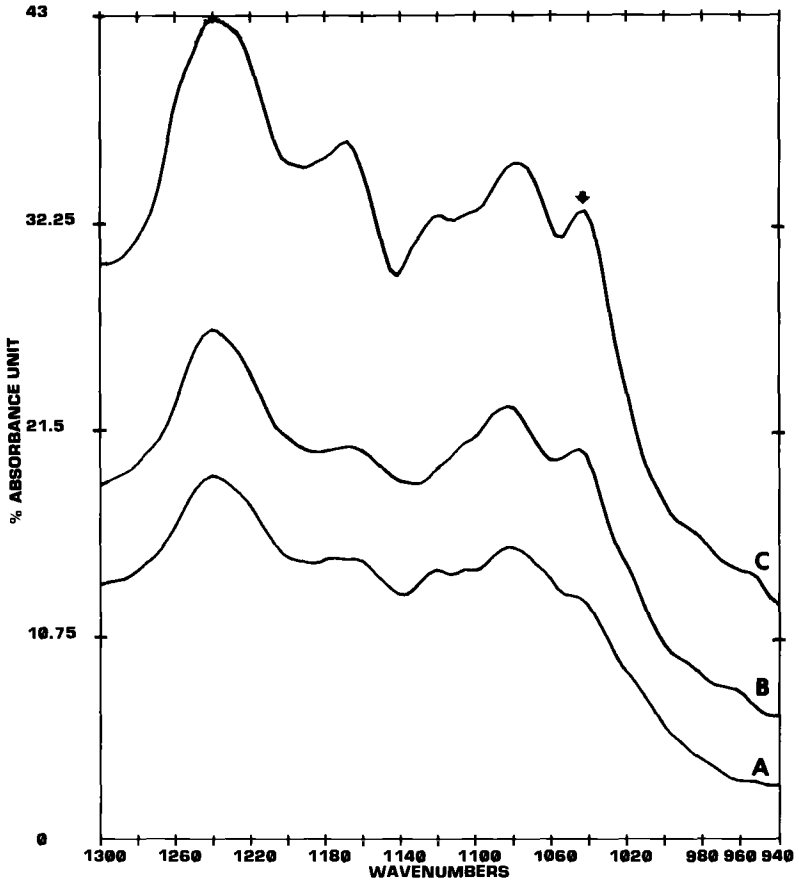

FIG. 3—(a) *Infrared spectrum of the hair root.* (b) *Infrared spectrum of the midpoint of the same hair shaft.* (c) *Infrared spectrum of the tip of the same hair. (Arrow indicates oxidation peaks at 1044 cm^{-1}.)*

TABLE 1—*Population study, showing the presence or absence of an infrared absorbance band at 1044 cm^{-1}.*

	Number of Samples	Neither Peak Nor Shoulder Detected, Number (Percent)	Shoulder Detected, Number (Percent)	Peak Detected, Number (Percent)
Male—not treated	42	23 (55%)	19 (45%)	0 (0%)
Male—treated	9	0 (0%)	4 (44%)	5 (56%)
Total males	51	...	...	...
Female—not treated	28	15 (54%)	9 (32%)	4 (14%)
Female—treated	56	0 (0%)	12 (21%)	44 (79%)
Total females	84	...	...	...
Total population	135	...	...	...

or peak. Lack of apparent large scale oxidation may be due to the milder treatment by the hair products for males, or less vigorous hair treatment by them.

Hairs from a total of 84 women were tested, 28 of whom did not treat their hair. In 15 (54%) of the 28 untreated hairs, no shoulder or peak was detected at 1044 cm^{-1}. Nine (32%) of the hairs showed a shoulder and four (14%) showed a peak. The presence of a peak in the untreated hair is probably due to severe damage to the hair, possibly caused by harsh shampoos, abrasion, or chlorinated pools [*10*]. A total of 56 women donated treated hair samples. A distinct peak was evident in 44 (79%) of the samples; and 12 (21%) gave a shoulder at 1044 cm^{-1}. As with the males, none of the treated samples showed the absence of a peak or shoulder.

Discussion

Observations of the following parameters were made: oxidized versus unoxidized hair, the effects of chemical reduction, gray versus natural colored hair, variations along the hair length, moisture, storage, and variations within an individual over time.

The presence of the 1044-cm^{-1} peak was confirmed when an untreated hair was oxidized in alkaline hydrogen peroxide. This oxidation product gave a sulfur-oxygen spectrum similar to that of cysteic acid. Once the hair was oxidized, simple reducing agents had no effect on this particular oxidation product. These reducing agents apparently do not react sufficiently with the sulfur-oxygen bond of this product to produce a detectable change in the 1044-cm^{-1} region. The resulting oxidation product is not a function of the lack of pigment in gray hair. A gray hair and a natural colored hair from an individual showed similar absorbance profiles whether they were taken from treated or untreated individuals. Variations may occur along the length of a treated hair, but are consistent within an individual. However, an individual with frosted hair may have both frosted and unfrosted hair on their head [*5*]. When comparisons are being made, care should be taken to compare like sections of the hair shaft in all samples. Human head hairs grow at the rate of approximately 12.7 mm (½ in.) per month [*4*], so variations may be due to the length of time since treatment, the type and degree of treatment, the amount of naturally occurring sulfonic acid, and weathering effects at the tip of the hair [*6,10,13*]. Elaborate storage procedures are not necessary as the effects of a usual storage environment are small compared to hair treatment. When the hair of an untreated individual is examined over the span of a year, no noticeable changes in the absorbance profile were detected. The population study shows that if a hair is analyzed using infrared spectroscopy and no peak or shoulder is found at 1044 cm^{-1} in the spectrum, that portion of the hair is untreated.

In conclusion, the presence of the 1044-cm^{-1} sulfonic acid absorption peak has been shown to be a result of the oxidation of sulfur bonds in human hair. This peak may be used to differentiate treated and untreated hair samples. Normal hair color, moisture, and age of hair samples were found to have little or no effect.

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