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Near-infrared spectroscopy: Applications in hair research

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Synopsis

We have evaluated the potential of NIR (near-infrared) spectroscopy as a tool in hair research. We find that this technique is ideally suited for measuring the relative moisture content of hair *in situ* under practical hair-grooming conditions. It also allows measurement of melanin absorption in the NIR region, where there is no interference from synthetic hair dyes. Thus, rapid evaluation of "lift," the bleaching produced by oxidation dye products, can be performed on hair swatches as well as on live heads.

INTRODUCTION

Human hair fibers are roughly 50–80 μm in diameter and are primarily composed of keratin proteins. Nearly 95% of the dry hair mass is proteinaceous, and 10% of this derives from the disulfide-containing amino acid, cystine. The remaining is made up of lipids, pigments, and some bound ions (1). Under ambient conditions of 25°C and 50% RH (relative humidity), hair fibers bind as much as 10% water by weight (2). Hair fibers have a complex morphology, with three distinct regions: the outermost cuticle layers, the inner cortical cells, and occasionally an innermost and porous medulla. The cortical cells contain α-helical protein, assembled in a fibrillar arrangement, embedded in an amorphous protein matrix (3).

Water acts as a plasticizer for hair and plays a critical role in determining its tactile, mechanical, and other cosmetic properties. The amount of water bound to hair depends on the ambient humidity, with more water bound at higher humidities. The affinity for water mainly arises from the polar amino acid side chains of keratin, with negligible contribution from the peptide bonds (4,5). As the humidity increases, one would expect the binding to become less specific, with water binding to low affinity sites as well as existing as free water. The moisture content of hair at any given RH, as measured by the regain from the dry state, is different and less than that obtained by way of dehydrating hair from 100% RH. Such hysteresis is also seen in other synthetic polymers and biopolymers and has been explained to arise from differences in the ratio of the "bound" to the "free" water (6). Watt (2) provides an excellent review of the water-binding properties of keratin, with ample references. Cosmetic hair treatments, either with heating appliances such as a blow dryer and curling iron or with grooming products such as hair fixatives, modify the water-binding ability of hair and, therefore, its physical

properties such as hold and feel. It is, therefore, very important to be able to measure the effects of hair care products on their ability to effect changes in the kinetic and equilibrium water-binding properties of hair.

Human hair fibers derive their natural color from melanin pigment. Unlike synthetic hair colors that are mixtures of small molecules dispersed throughout the fiber, including the surface, the natural pigment is polymeric and exists as discreet granules only in the hair cortex. This structural difference between these two types of coloring matter has a profound influence on their optical properties. While the synthetic hair dyes do not absorb above 750 nm (red light), the natural pigment shows semiconductor-like optical properties and absorbs (scatters) light from the UV region all the way into the near-infrared, at least up to 1400 nm.

NIR energy is relatively weak in causing electronic transitions to excited states in all but the highly delocalized electronic systems. The hair pigment melanin is unusual in this regard, and it has been suggested that its absorption does not fit the classical definition of chromophoric absorption and is more semiconductor-like (7). Typically, NIR absorption by molecules results from overtones and combinations of the characteristic bond vibrations. For biological tissue, this region is dominated by bands due to O–H, mainly from water, and N–H from the protein backbone. NIR spectroscopy has been used extensively in skin research (8–11). Use of NIR second-derivative methodology to monitor the water content of hair has also been reported (12).

Here we report the results of our study aimed at evaluating the potential of NIR spectroscopy as a tool in hair research. We find that this technique allows measurement of the water content of hair under hair-grooming conditions. Although these measurements provide relative water concentrations, they can be converted to absolute values by appropriate calibration. We have also exploited the difference in the light absorption characteristics of the natural hair pigment and the synthetic dyes to measure "lift," or the bleaching of the natural pigment due to the oxidative hair coloring process in the presence of the deposited dye. This latter aspect is particularly important when testing competitive products for which information on the ingredient concentrations is often lacking.

EXPERIMENTAL

Spectra were collected with a Magna 760 FT-IR system with NIR capability (Nicolet Instrument Co.), using the SAB-IR accessory. The latter is a bifurcated fiber-optic probe consisting of a *ca.* 3-mm fiber-optic bundle in which the fibers that bring in the incident light and those that take the reflected light to the detector are randomly arranged. The assembly tip is encased in a screw-in cover with a Sapphire angled window to cut down the specularly reflected light. The system uses a PbS detector and runs under the OMNIC® operating system. Background correction was performed using Spectralon®. Typically 32 scans (38 sec) were collected for each sample at a resolution of 8 cm^{-1}. The data were collected in the reflectance mode. Absorption curves were generated as log (1/R). Baseline correction was performed on the spectra using the packaged routine.

In reflectance measurements, both absorption and diffuse scattering of the incident light contribute to the observed signal. The depth of penetration by light depends on the wavelength and the nature of the sample. In biological materials, for example, the longer wavelength NIR radiation will penetrate more deeply than the ultraviolet light. Mea-

surements on skin have revealed that NIR radiation can easily penetrate the thickness of hair, which is approximately 50 μm in diameter (10,11). It should be noted, however, that since natural hair pigment absorbs NIR radiation (see below), it will attenuate the incident beam, thereby affecting the apparent absorption due to water, compared to the unpigmented hair. Thus comparative measurements should be made only on hair of similar color.

Measurements were either made on human heads or on hair tresses. The tresses were typically 6" in length and were made from Piedmont hair, dark brown hair, or from blended gray hair, all obtained commercially (DeMeo Brothers, New York).

Hair drying at precise temperatures was carried out in a convection-air oven. Moisture uptake was studied by allowing hair tresses that had been partially dehydrated in an oven to pick up moisture in a humidity- and temperature-controlled room.

Deuterium oxide/water exchange in hair was studied by incubating hair in a 99.9% D_2O solution at various temperatures and for various times in an oven.

The experiment, involving evaluation of the effect of a leave-in treatment on the water-binding properties of hair, was performed on a Piedmont hair tress. This test product contained the following ingredients: amodimethicone, cyclomethicone, panthenol, tocopherol, hydrolyzed protein, polyquaternium 37, polyquaternium 11, dicaprylate/dicaprate, tallowtrimonium chloride, nonoxynol-10, phenoxyethanol, propylene glycol, and water. A tress was shampooed and dried with a towel. The lower half of the tress was dipped in the product, and the excess was squeezed out. This tress was then blow dried, combed, and heated in an oven at 93°C for 30 min. Subsequently this was transferred to a humidity-controlled room and measurements were made on the treated and untreated sites. Two measurements were made on each site, and the data collection was done in a manner that scrambled any sampling preference. Care was taken to insure that the measurement sites on the treated and untreated portions of the tress were not separated by more than 1.5", to minimize intrinsic differences in hair properties due to weathering. An average value for each site was obtained and the data were collected periodically for 4 h. An "infinite time" measurement was made the following day, after which the sample was heated at 120°C for 30 min to provide a baseline ("no water") reading.

Hair dyeing was performed on tresses using the dark brown shades of two commercial permanent hair-coloring products according to package instructions.

RESULTS AND DISCUSSION

The NIR spectrum of human hair under ambient conditions of 22°C and 50% RH is shown in Figure 1. The shoulder at 1450 nm (6896 cm^{-1}) in the spectrum most likely is the first overtone of the O–H stretching vibration of water observed in the mid-IR spectra at about 3450 cm^{-1}. The doublet at about 1740 nm is the overtone of the methylene C–H stretch of protein side chains and lipids. In the 2000-nm region, the hair spectrum shows a strong band at 1935 nm, a shoulder at about 1984 nm, and an overlapping band at 2051 nm. The 1935-nm band has been assigned to a combination of the O–H stretch and H–O–H bending vibrations of water (12). The main changes in the hair spectrum upon heating occur at about 1450 nm and 1935 nm, due to loss of

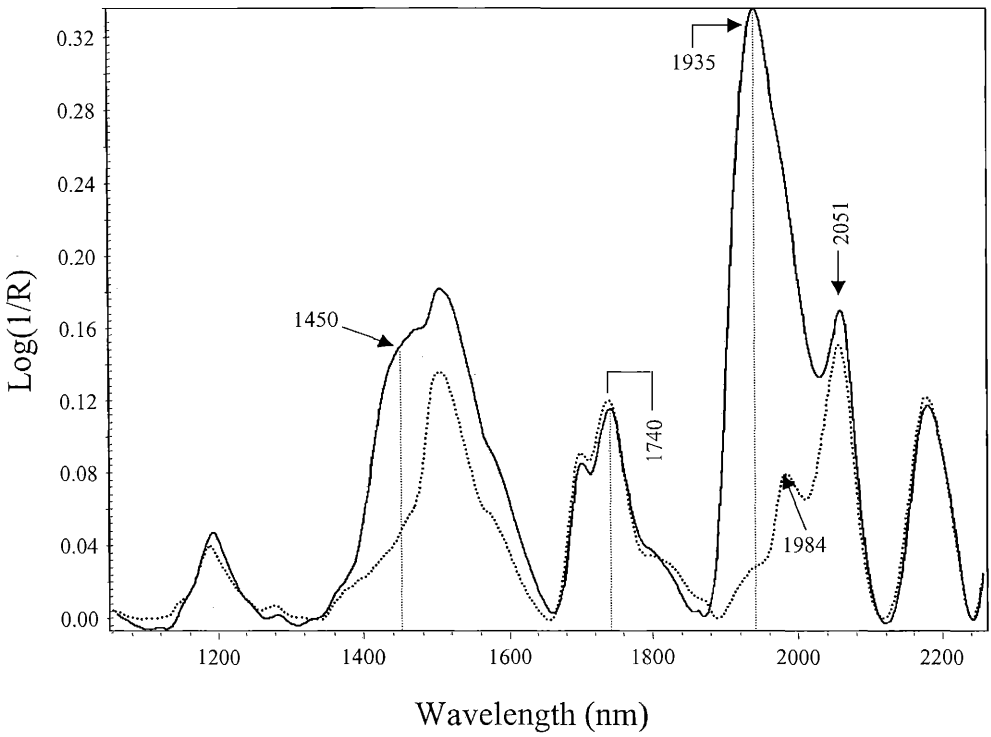

Figure 1. NIR absorption spectrum of human (Piedmont) hair and the effect of water. The solid lines represent the spectrum at 22°C and 50% RH. This hair sample was then heated at 110°C for 90 min and the dotted-line spectrum results. Notice the significant changes in the 1440-nm and 1935-nm regions, which are associated, respectively, with the harmonic of the O–H stretch and a combination of O–H stretch and H–O–H bend. The other bands are likely due to protein.

water. The small residual "bump" at 1935 nm after heating may either be due to small residual water that is very tightly bound, water uptake during measurement, some underlying protein band, or some combination of the above. Clearly the 2051-nm band is due to protein, since it is unaffected by heating. The band at 1984 nm is also likely due to protein. The data clearly show that of the two bands assigned to water, the 1935-nm band will be clearly more sensitive for moisture content studies since the interference from protein will be minimal at this wavelength and the absorption cross section is higher. It should be noted that the bands associated with water in hair are very similar to the corresponding bands observed in the NIR spectrum of the epidermis component of skin with respect to their positions and relative intensities (11).

The above assignment of bands at ca. 1450 and 1935 nm to water is further substantiated by the results of the experiment in which the water in hair was replaced with D_2O, the isotopic analogue. Since the mass of deuterium is twice that of hydrogen, the vibrational frequencies of O–D are different and lower in energy than the corresponding O–H vibrations. Thus, when hair treated with water is soaked in D_2O, due to mass action the bound water will be replaced by D_2O. This, in turn, will be reflected in the NIR spectrum as a frequency shift of the water-related bands. The effect was most pronounced in the 1400–1500-nm and in the 1900–2000-nm regions. For example, the

1935-nm water band decreased in intensity and a new band appeared at 2023 nm, as seen in Figure 2. This latter band has been assigned to the stretch/bend vibrations associated with deuterium oxide. The ratio of the intensities of the bands at 2023 nm to 1935 nm increased with increasing temperature and the time of incubation of the tress in D_2O (data not shown). This suggests that this ratio could be used as an index of D_2O/H_2O exchange in hair. This line of experimentation can be used to distinguish between the various types of bound water and the bulk water. The D_2O for water exchange depends on the diffusion of D_2O into the hair followed by the replacement of water already present in the hair. Since hair damage, either due to weathering or chemical processing, affects the hair integrity and damages the constituent protein, both

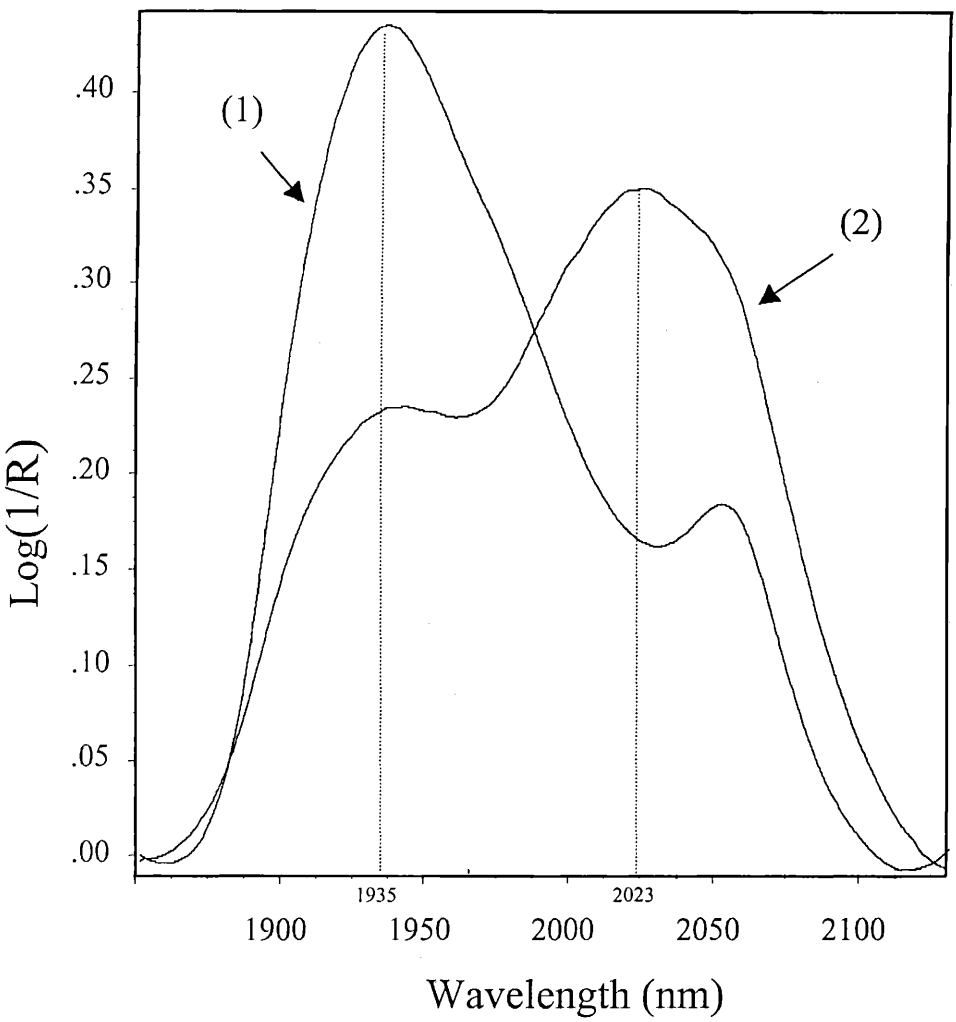

Figure 2. Deuterium oxide (D_2O)/water exchange in human hair. Spectrum 1 is due to hair soaked in water and towel dried. This was then soaked in D_2O for 10 min at 80°C, towel dried, and remeasured (spectrum 2). These data clearly suggest that the 1935-nm band in the hair spectrum is due to water. The ratio of the bands at 1935 nm and 2023 nm is an index of H_2O/D_2O exchange and may be related to the structural integrity of the fibers.

these processes are likely to be affected. It is, therefore, interesting to speculate that the kinetics and equilibrium parameters of this exchange may provide information on the structural integrity of the fibers.

Figure 3 shows the effect of moisture on the NIR spectrum of hair in the 1900-nm region. This figure clearly shows that the intensity of the band at 1935 nm can be used to measure both the dehydrating effects of heat, as in blow-drying or upon using a curling iron, as well as in water uptake by dried hair. It should be noted that heating the hair to 110°C for 90 min did not cause any apparent irreversible change in the water-binding properties of hair and that the process was totally reversible. Also, the bands due to protein that are used to normalize the data were also unaffected. This suggests that no apparent damage to protein, as judged by these markers, occurs under these conditions. A previous study on the use of NIR to measure water content of hair used second-derivative methods (12). We have chosen to use baseline subtraction of the raw spectra as the method of choice because it is simple, straightforward, and as the data show, not influenced significantly by the protein bands. The derivative method, we feel, is complicated when deconvolving bands of differing bandwidths.

Hair treatments, particularly the "leave-in" kind, may affect the water-binding properties of hair. Mechanistically, this may result from one or more of a variety of effects, such as the interaction of the product with the hydrophilic sites in hair, or be due to the hydrophobicity of the product, etc. The result of such an experiment is seen in Figure

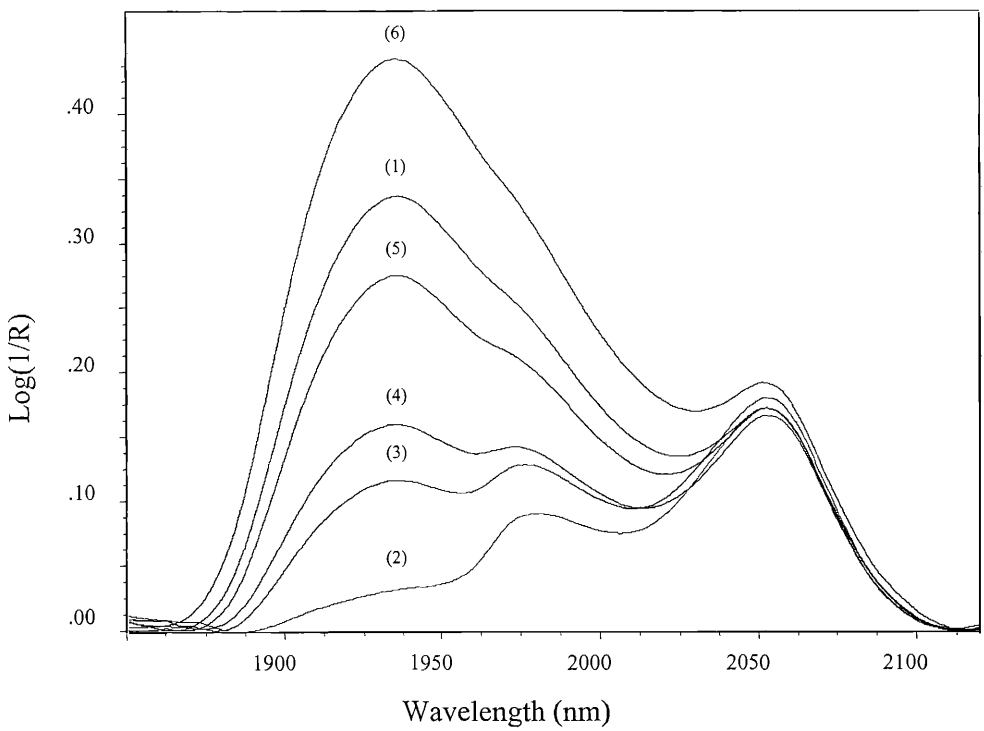

Figure 3. Changes in the ambient hair spectrum (1) upon heating at 110°C for 90 min (2), followed by water regain in a room, maintained at 20°C and 50% RH, after 5 min (3), 10 min (4), and 75 min (5). The hair tress was then soaked in water and dabbed with a paper towel, and the spectrum was measured (6).

4. It compares the results obtained with the untreated (control) half of a hair tress with the other half of the tress treated with a leave-in conditioning product. After the treatment the tress was heated at about 90°C for 30 min, following which the measurements were made (see Experimental section for details). As is clearly seen, there appears to be more water in the treated portion of the tress right after heating and for short water-take times, after which the two seem to converge. The data obtained after 24 h (infinite time) were indistinguishable from those obtained after 4 h, and have not been shown in the figure for clarity. The data obtained at 4 h at this humidity may, therefore, represent the equilibrium amount of water under these conditions. Thus, these data show that this product allowed more moisture to be retained in hair under the drying conditions of high temperature, similar to those encountered during blow-drying

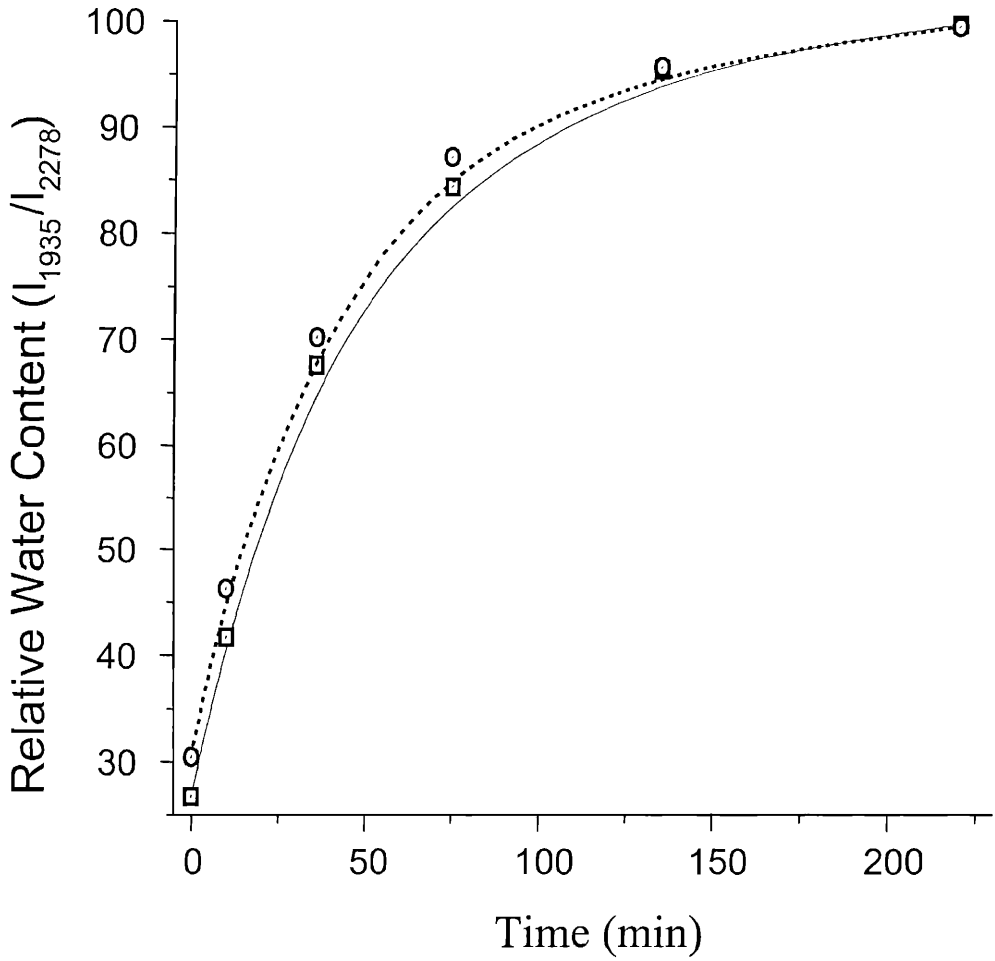

Figure 4. The water regain by hair as a function of time. The hair samples were heated to 93°C for 30 min, following which they were measured in a room maintained at 20°C and 50% RH (see text for details). The 1935-nm band was normalized against a water-insensitive protein band. The open circles represent the treated site, while the open squares represent the untreated control. Notice that at early times the treated sample contains more water than the untreated control, but that the two converge as the system approaches equilibrium.

and heat setting. At this time we do not have a mechanistic interpretation for these findings.

It should be noted that water uptake measurements are typically performed gravimetrically under isothermal conditions with RH as the only variable. The gravimetric method, by its very nature, is susceptible to interference by the convection currents set up during changes in temperature. Volumetric methods, which are less frequently used, are also performed under isothermal conditions. Furthermore, these methods cannot be performed on live heads. Our measurements, on the other hand, are not "real" equilibrium uptake measurements. The heated sample is allowed to relax to a new final RH and temperature. Two variables, temperature and RH, are changed simultaneously, so in that sense it is a composite of a temperature-jump and an RH-jump experiment, both of which affect the water content of hair. Superimposed on this complexity is the hysteresis associated with the heating of hair, which is well documented in the literature (1,2). Nevertheless, this protocol models the real-life situation better than the isothermal measurements. Also, the "infinite time" measurements represent true equilibrium.

The data presented above show that this method has the sensitivity to allow identification of materials that would alter the water-binding property of hair. In the later stages of product development, such experiments can be performed either on hair tresses or directly on live heads in a controlled-humidity environment, for product optimization and for claims substantiation.

We have also found this technique to be extremely useful in the characterization of oxidative hair-coloring products. The measurement of "lift" (melanin bleaching) during oxidative coloring, particularly with dark brown and black shades, is not possible with a typical colorimeter due to interference from the dyes themselves. A way around this problem is to use a dyeless base. This strategy does not work when evaluating competitive products. As noted in the Introduction section above, the synthetic hair dyes do not affect the reflectance properties of hair beyond 750 nm, while the natural hair pigment also absorbs the NIR radiation. This difference in the light absorption characteristics of the natural hair pigment and the synthetic dyes can be exploited to measure bleaching or "lift" produced during oxidative hair coloring, without interference from the deposited hair color, using NIR spectroscopy.

Figure 5 shows the tail end of the absorption of human hair. Spectrum 1 is from black hair from one of the authors (C.P., Asian Indian) while spectra 2 and 3 are from commercial dark brown and blended gray hair, respectively. The effect of pigmentation in this region of NIR wavelengths is clear when one compares the above spectra with spectrum 6, which is due to Piedmont hair that lacks melanin pigment.* These data reveal that the darker the natural hair color, the higher the absorption in this region. It would follow that this region of the spectrum could be used to follow changes in the natural pigment concentration in hair. Indeed, spectra 4 and 5 represent blended gray hair (spectrum 3) dyed with dark brown shades of two oxidative hair-coloring products. It is clear from the spectra that oxidative coloring reduced the intrinsic melanin concentration in the blended gray hair sample. Furthermore, the product corresponding to

* The band at *ca.* 1285 nm, seen clearly in the Piedmont hair spectrum and only as a shoulder in the other spectra, is due to protein backbone.

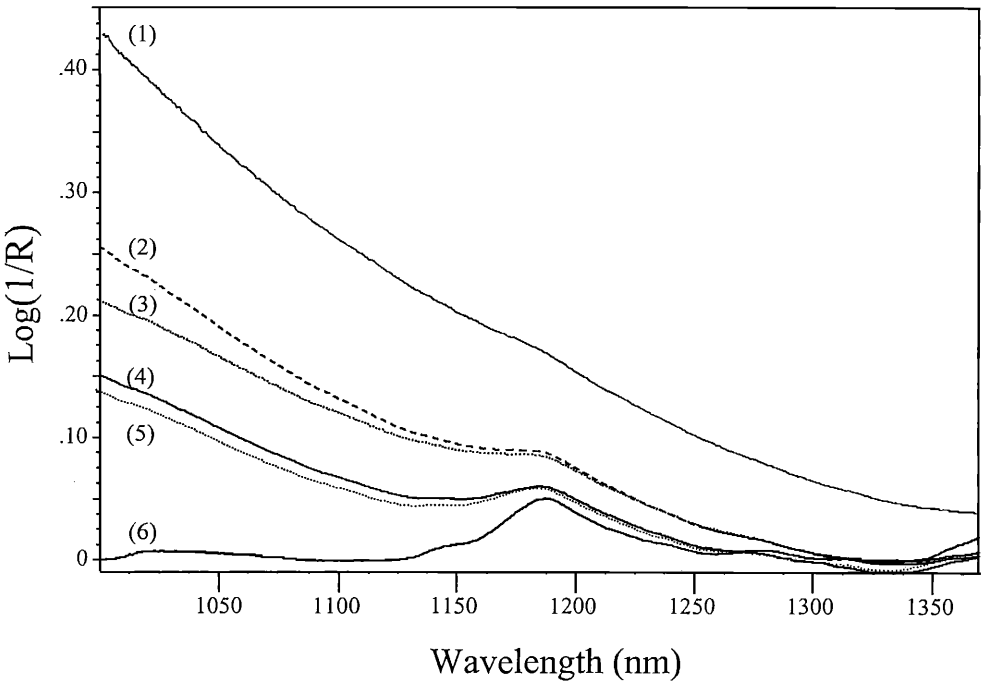

Figure 5. Absorption spectra of human hair. The spectra, from the top, represent: (1) hair from one of the authors (C.P.); (2) commercially blended medium brown hair; (3) commercially blended gray hair; (4) commercially blended gray hair dyed with product A; (5) commercially blended gray hair dyed with product B; and (6) Piedmont hair. The spectra represent the tail end of melanin absorption. Comparison of spectra 1 and 6 reveals the absorption due to melanin pigment, while comparison of spectra 3, 4, and 5 reveals the "lift" produced by permanent hair dyes. Notice that product B provides slightly more "lift" compared to A, even though they are both dark brown shades. Such subtle differences between products translate into differences in the appearance of hair color and wearing properties.

spectrum 5 produced more bleaching (lift) than the one responsible for spectrum 4. Thus, this methodology can be used during the product development process to optimize lift to the desired level, based on the market positioning of the product.

It should be recognized that conventional UV/visible reflectance measurements would be more sensitive than the NIR methodology described above for monitoring chemical or photochemical bleaching, due to higher extinction in this region of the spectrum. Such instrumentation, however, cannot distinguish between natural pigment color and synthetic colors.

In summary, we have evaluated NIR spectroscopy for use in hair research. We show that it will prove to be a valuable tool in hair care and hair color product research, development, and claims substantiation. This fiber-optic-based instrumentation is capable of measurements on live heads, which also makes it suitable for salon use.

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