Spectroscopic Characteristics of Human Melanin In Vivo*†

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In this paper we present the absorption characteristics of human melanin in the visible range of wavelengths and specifically in the range 620–720 nm. The spectroscopy of melanin is studied by measuring remittance spectra of normal skin and vitiligo-involved skin of volunteers—patients. It is assumed that the spectral differences between adjacent areas of normally pigmented skin, and to some degree amelanotic skin, can only be due to the variations of the melanin filter. The ratio of the remittance spectrum of the vitiligo-involved skin with the spectrum of the normal skin in the range 620–720 nm can be fitted with a straight line for all the volunteers. A very strong correlation is obtained between the intercept and the slope for all the volunteers, which leads us to conclude that it is indeed melanin that we are measuring in all the volunteers and that it is the same substance spectroscopically for all the volunteers.

There has been a large number of studies concerned with the absorption characteristics of extracted as well as synthetic melanins in vitro. It is difficult to characterize these compounds chemically and physically. This is due in part to the fact that the UV-visible absorption spectrum of melanin shows no specific absorption bands [1]. Several laboratories around the world are attempting to do this. In most of the literature it is assumed that epidermal melanin absorbs much like dopa melanin, which is soluble in water, and therefore its absorption spectrum is easily obtained. Extracted hair [2] melanins have been solubilized in very strong basic solutions and their absorption spectrum is similar to that of dopa melanin.

Studies have also been carried out on the photochemistry of these compounds, as melanin is believed to provide photoprotection to more heavily pigmented individuals. This type of study requires a knowledge of the detailed characterization of the absorption spectrum of melanin in vivo, as well as photochemical changes that might occur in the skin and their possible scenario of development.

Some excellent proposals have been made [3–5] for the separation of the absorption by melanin and that by hemoglobin. In these models it is suggested that melanin and hemoglobin act as if they are superimposed optical filters, each altering the absorption spectrum of the skin. The effect of successive optical filters on the remitted intensity can be estimated by multiplying the attenuation caused by the first filter by that caused by the second

\[ I(\lambda) = I_0(\lambda)[T_M(\lambda)] \cdot [T_H(\lambda)] \]

that is to say, the incident intensity, \( I_0(\lambda) \), is multiplied by a factor which is the transmittance of the melanin filter \( T_M(\lambda) \) and this factor is multiplied by a similar one due to the transmittance of the hemoglobin, filter, \( T_H(\lambda) \).

In our study we looked for a natural system where we could control the hemoglobin concentration in order to study the absorption of melanin in vivo. The skin of vitiligo patients provides exactly such a system, where amelanotic areas of skin are surrounded by normal skin. Furthermore there are no structural differences that would affect the remittance spectra between the vitiligo-involved skin and the immediately adjacent normal skin. The only difference should be the absence of melanin in the vitiligo-involved skin areas.

MATERIALS AND METHODS

The Remittance Spectrometer

The objective of this instrument was to quantify "skin color" as we perceive it with our eyes. White light is directed perpendicular to and in contact with the skin area under study and the amount of light coming back out of the skin is analyzed for its spectral components. For light incident at angles other than perpendicular to its surface, the amount of light that is scattered by the surface layer of the stratum corneum would be increased. The desired performance characteristics of such an instrument should include: (a) a very stable light source; (b) the beam of light that strikes the skin should not move with respect to the surface of the skin; (c) the analyzer should be a low-noise instrument, as there would be a large signal to be dispersed; (d) the pressure exerted on the skin area under study should be minimal and the temperature constant; (e) the instrument should be automated so that data acquisition and processing could be accomplished quickly and rapid evaluation performed.

The instrument used is not unlike others described in the literature [3,6,7]. The light source was a quartz halogen, tungsten filament lamp (GE-EJL 200 W) which was powered by a very stable DC power supply (Hewlett-Packard 6034A) whose output was current-regulated better than 0.03%.

The light went into a bifurcated light fiber bundle which delivers the light to the skin. The joint end of the fiber bundle which was in contact with the skin consisted of randomly mixed fibers—half of these deliver light to the skin and the other half conducts the collected light signal from the skin to the analyzer. The analyzer was a double monochromator in order to reduce the stray light (Jobin-Yvon DH10). The output of the monochromator was coupled to a photomultiplier (Oriel 7062), which was connected to a current amplifier (Oriel 7070). The analog output of the detection system was connected to a data acquisition system (Hewlett-Packard 3054DL). Besides the light output, the temperature of the skin at a location 1 cm away from the fiber bundle was measured (Ellab AH2) and recorded through the same acquisition system. Furthermore, the wavelength of the monochromator was recorded and the monochromator scanner was advanced to a new wavelength from 405–720 nm.

Software was developed for the acquisition system to acquire data and to perform enough processing to present on the screen of the computer (Hewlett-Packard 85A) a graph of the remittance spectrum vs wavelength, compared to the spectrum of Al\(\lambda\) as a reference, to correct for the instrument function.

Fig 1 presents a drawing of the probe that was attached to the skin to obtain the remittance spectra. By bringing the optical bundle in contact with the skin we avoided the primary reflection ( specular reflection) by the surface of the stratum corneum. This was found to contribute an unacceptable amount of fluctuation in data collected from run to run on the same individual; while in the geometry used we were able to obtain successive spectra from the same skin site within 2% of each other and within 5% of each other from adjacent skin sites.

The instrument function was obtained each day after warm-up to make sure that we were accounting for variations in the output of the...
as the melanin filter is practically nonexistent in this case. The lower curve is from an area where the pigmentation was normal and the complete procedure was repeated.

**Volunteers**

Forty-three volunteers were selected from the vitiligo patients who were receiving or were about to commence a series of PUVA treatments. No volunteer was under any medication at the time of measurement or had been for the previous 4 days. The volunteers were informed of the details of the experiment and their consent was obtained prior to the experimentation. They were selected to have vitiligenous lesions on their arms and specifically on the inner side of the lower arm or the lower part of the upper arm (people in our region are very sensitive to exposing any other part of the body). The patient was allowed to rest for 10 min before measurements were started.

**Measurements**

The location of the body where the measurements were to be carried out was selected and photographed. The volunteer was seated for a minimum of 10 min in the exact position to be maintained for obtaining the measurements and then the probe was applied to the first location to be recorded. This was usually the vitiligo-involved area; this position was marked on the photograph. The probe was left on the skin for approximately 1 min before the remittance spectrum was recorded, in order to bring to equilibrium the temperature of the skin under investigation and the probe. When a scan was completed the monochromator was returned to 405 nm, the data were recorded on the floppy disc, and a graph of the remittance vs wavelength was displayed on the video screen. The probe was then moved to an area within 2 cm of the original site where the pigmentation was normal and the complete procedure was repeated.

**RESULTS AND DISCUSSION**

The remittance spectra obtained from one of the volunteers are presented in Fig 2. One curve appears higher than the other, indicating a larger remittance; the curve that appears higher was obtained from the vitiligo-involved skin. The absorption bands due to hemoglobin are clearly shown in the upper curve as the melanin filter is practically nonexistent in this case. The lower curve is from an adjacent area of normally pigmented skin and it is for this reason that the hemoglobin absorption bands are severely attenuated.

Pairs of curves as displayed in Fig 2 were obtained from all the volunteers. If we divide the remittance of the vitiligo-involved skin by that of the normally pigmented skin, a curve similar to the absorption of dopa melanin is obtained. Fig 3 shows the ratio of the remittance spectra displayed in Fig 2. Fig 4 shows the ratios obtained for some of the volunteers—all the curves are shown for the sake of clarity. In calculating the ratio of the remitted intensity at 720 nm minus that at 400 nm we find a minimum change of 0.2 and a maximum of 10.8, i.e., a factor of 50.

The problem at this point is how to quantify the ratios observed on the curves in Fig 4. We start by expressing the remitted intensity, \( I_{\text{r}}(\lambda) \), in terms of the transmittance of an absorption filter in the skin. Specifically we write for the remitted intensity from completely amelanotic vitiligo-involved skin, \( I_{\text{r}}(\lambda) \),

\[
I_{\text{r}}(\lambda) = I_0(\lambda) \cdot [T_H(\lambda)] \cdot S(\lambda) \tag{2}
\]

where \( I_0(\lambda) \) is the incident intensity, \( T_H(\lambda) \) the transmittance of hemoglobin, and \( S(\lambda) \) is a scattering term that accounts for the part of the incident intensity that is lost due to scattering.
Similarly we can write for the remitted intensity from the adjacent site (normal skin), \( I_{\text{RN}}(\lambda) \), as

\[
I_{\text{RN}}(\lambda) = I_0(\lambda) \cdot [T_M(\lambda)] \cdot [T_H(\lambda)] \cdot S(\lambda)
\]

where \( T_M(\lambda) \) is the transmittance of melanin. By calculating the ratio of the remitted intensity from vitiligenous skin to that from normal skin we obtain

\[
\frac{I_{\text{RV}}(\lambda)}{I_{\text{RN}}(\lambda)} = \frac{1}{T_M(\lambda)}
\]

If we calculate the natural logarithm of both sides of eq. (4) we have

\[
\ln(I_{\text{RV}}/I_{\text{RN}}) = - \ln T_M(\lambda)
\]

This provides us with a relation between the measured quantities \( I_{\text{RV}} \) and \( I_{\text{RN}} \) and the transmittance of the melanin filter.

The Bouguer-Lambert law states

\[
- \, dl = a \cdot I \cdot dx
\]

which means that a decrease in the incident intensity, \(- dl\), is proportional to the absorbance of the medium, \( a \), that it traverses and also the thickness of the medium, \( dx \). This law holds if \( a \) is constant, \( a \) is the absorbance coefficient times the concentration of the absorbing solute, \( c \), and \( dx \) is the absorbing center thickness.

Furthermore, Beer's law for transparent solvents states that

\[
- \, dl = c \cdot a \cdot I \cdot dx
\]

where \( c \) is the concentration of the solute and the other parameters are defined above.

In the case of skin, especially when we are concerned only with hemoglobin and melanin, there is good experimental evidence that the third condition is satisfied, to a first approximation at least. The first two conditions strictly speaking are not satisfied; however, we get around them by properly designing the experiment. The scattering/structure term, \( S(\lambda) \), allows for the fact that some of the incident light is lost through scattering. And inhomogeneities in the absorbing medium can be overcome by calculating the ratio of the remitted intensity from vitiligo-involved and normal skin. We think that it is reasonable to assume that the same inhomogeneities occur in the normal and in the vitiligious skin areas, if adequately located. In view of the fact that our volunteers, both for religious and psychological reasons, maintain the vitiligo-involved areas under study always covered from sight, we would not expect other than small amounts of solar-induced UV hyperkeratosis. In naked eye comparisons between vitiligious and nonvitiliginous skin no structural differences were observed. Finally, we have measured experimentally the remittance from skin with monochromatic light as well as with polychromatic light (only visible and near-infrared) and we found no significant differences in the range considered here, 405–720 nm.

By comparing eq. (6) and (7) with eq. (5), we recognize

\[
- \ln T_M(\lambda) = - \ln (I_{\text{RV}}(\lambda)/I_{\text{RM}}(\lambda)) = - a(\lambda) \cdot c \cdot 1
\]

where \( a(\lambda) \) is the absorption coefficient of the absorber, \( c \) its concentration, and \( 1 \) the pathlength through the absorber.

Assuming that \( 1 \), the absorption pathlength, is essentially the same among the volunteers and that the absorber were the same for all the volunteers (i.e., \( a(\lambda) \) would be the same) this would leave only the concentration to be determined for different volunteers. Since the mechanisms of absorbance for melanin in the visible–near-infrared are not known beyond doubt, that leaves the additional possibility open that different mechanisms of absorption may be active in skins with different pigmentation levels.

With these points in mind we calculated the natural logarithm of the ratio of the remittance from vitiligo-involved skin to that of normal skin of the same individual. The 5 curves presented in Fig 3 vs wavelength, in nanometers. A straight line is fitted through the points from 620–720 nm range. This was performed for all the volunteers and the results are shown for some volunteers in Fig 6 and are tabulated for all the volunteers in Table I. This means that

\[
a(\lambda) \cdot c = C_1 + C_2 \cdot \lambda
\]

the absorption coefficient times the concentration can be expressed as a function of the wavelength where \( C_1 \) is the intercept and \( C_2 \) is the slope of the straight line fit through the experimental points.

Fig 4. The ratio of remittance spectra from vitiligo-involved skin divided by that of normal skin of the same individual. The 5 curves indicate the variation among different volunteers. In some of these there is no increase in the incident intensity, \( - dl \), so we would expect other than small amounts of solar-induced UV hyperkeratosis. In naked eye comparisons between vitiligious and nonvitiligious skin we would not expect other than small amounts of solar-induced UV hyperkeratosis. In some of these the incident light is monochromatic and collimated (a) the absorbing medium is homogeneous (b) the absorbance coefficient times the concentration can be expressed as a function of the wavelength where \( C_1 \) is the intercept and \( C_2 \) is the slope of the straight line fit through the experimental points.
1. The absorbance of the pigment difference between vitiligo-involved and normal skin for the same volunteer.

2. The mechanism of absorption is the same for all the volunteers.

3. The absorber (melanin) is the same for all the volunteers.

4. The correlation coefficient, $R^2 = 0.979$.

Since in eq. (9) the absorption coefficient is the only term that is dependent on wavelength while $c$ is a different constant for each volunteer, then $C_1$ and $C_2$ should be correlated among all the volunteers if:

(a) the absorber is the same for all the volunteers
(b) the absorption mechanism is the same for all the volunteers in the range $620$–$720$ nm.

Fig 7 is a graph of $C_2$ vs $C_1$ for all the volunteers. It is most satisfying to note the strong correlation among all these volunteers. The relationship between $C_2$ and $C_1$ can be expressed as

$$C_2 = -1.19 \times 10^{-3} \cdot C_1$$

Another way of visualizing the strong correlation between $C_1$ and $C_2$ is to say that one does not really need two constants to describe the absorbance for the different volunteers but simply one. That is, one constant is universal for all the volunteers and the other is different for different volunteers. Rewriting eq. (7) we have

$$a(\lambda) \cdot c = C_1 \cdot 1.19 \times 10^{-3} \cdot C_1 \cdot \lambda$$

$$= C_1 \cdot [1 - 1.19 \times 10^{-3} \cdot \lambda]$$

and therefore

$$c = C_1$$

and

$$a(\lambda) = 1 - 1.19 \times 10^{-3} \cdot \lambda$$

in the wavelength range under investigation.

In conclusion we can say, for the types of skin studied, that

1. The absorber (melanin) is the same for all the volunteers, within experimental error.
2. The mechanism of absorption is the same for all the volunteers in the range $620$–$720$ nm.
3. A coefficient $C_1$ is identified which is correlated with the pigment level in the skin.

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REFERENCES


