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Fluorescence-free UV/VIS reflection spectra of human skin

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Synopsis

A fluorimeter operated in synchronous mode was used to record fluorescence-free UV/VIS reflection spectra of human skin. $BaSO_4$ or Teflon served as reflection standards. The variation coefficient of the spectral data showed a maximum around 350 nm. The relative remittance (normalized spectra) increased dramatically after cyanoacrylate stripping around 305 nm equivalent with loss of UV-absorption. Increase of moisture level by occlusion or washing of skin caused a similar but reversible change, washing with surfactants was provocing changes lasting for more than 24 hours. Exaggerated use of soap and surfactant bars drastically enlarged remittance around 300 nm.

INTRODUCTION

UV/VIS radiation impinging on the skin surface will be only partially directly reflected. About 5% of the total radiation energy is reflected from the surface (1). The remaining radiation entering the stratum corneum will be reflected, diffracted, and refracted, processes that are described in total as scattering (2). Therefore, the light beam will lose its original direction and tend to gain a more diffuse distribution within the skin. During its chaotic way through tissue, light will be diminished by endogenous and exogenous chromophores absorbing at different wavelengths: the longer the optical pathway, the more the loss by absorption. With increasing wavelengths, irradiation reaches deeper layers of the skin (3). A fraction of the incident radiation of different wavelengths, scattered from different penetration depths, will reach the skin surface again (= diffuse reflection).

Depending upon the quality and quantity of the chromopheres that rays encounter on their penetration path, the recorded spectra will show more or less intense absorption peaks. The whole level and general shape of the spectra will be determined by the scattering properties of the skin getting increasingly turbid or less permeable with shorter wavelengths (1).

To standardize spectra, the particular influences of lamps, optical geometry, and skin probes have to be eliminated. This is optimally achieved by producing relative spectra that show the diffuse remittance of skin in percentages of the reflectivity of a reflection standard like $BaSO_4$ or Teflon (reflection close to 100%, irrespective of wavelength).

Traditionally the reflection of monochromatic light is recorded using an integrating sphere connected to a photometer (4–6). Such an instrumental array does not allow discrimination of reflection from fluorescence: when using a UV-photometer the reflected light after interaction with skin is no longer limited to a controlled wavelength.

Fluorescence contributes considerably to the recorded signal when skin is irradiated with UV (1) because the epidermis contains a series of UV-absorbing fluorescent molecules: aromatic aminoacids, NADH, flavines, porphyrines, possibly Maillard products, and lipofuscin (7). To record only reflection and eliminate fluorescence of different wavelengths, a commercial fluorimeter with monochromators in the excitation as well as in the emission pathway has been used and operated in the so-called synchronous mode, i.e., both monochromators are moved synchronously through the whole spectrum with a wavelength difference of zero.

To measure optical data at arbitrarily chosen skin sites, light guides transporting light from the instrument to the skin, and after interaction with the skin back to the instrument for further analysis, are preferentially chosen.

The moisture content of the excised horny layer is steadily related to the relative humidity of the environment (8). A frequently used technique uses electrical capacitance to measure relative water content of the skin (9). We found that application of dry silica gel under occlusive dressing decreases electrical capacitance measured with an appropriate instrument (Corneometer, Courage-Khasake, Cologne, Germany) and therefore probably skin moisture content (10). This technique to reduce skin moisture content was used to get optical data on dry skin.

Contact of skin with water elutes amino acids, urocanic acid, lactic acid, etc., from the horny layer (11). UV-spectrometric measurements of such aqueous extracts show absorption maxima around 260–280nm. Quantification and identification of the main components as urocanic acid and tyrosine can be achieved by HPLC (10).

EXPERIMENTAL DETAILS

INSTRUMENTS

To measure reflection spectra a fluorimeter (LS 50, Perkin-Elmer) was operated in synchronous mode with a wavelength (λ) difference between excitation and emission monochromator ($\Delta\lambda = 0$ nm). To avoid overloading of the photomultiplier, filters were introduced into the excitation pathway: attenuator 1%. Both arms of a bifurcated quartz guide (UV-type, Schott, Mainz, Germany) were installed into the fiber-optic holder (Figure 1a). The end, C, of the fiber-optic should contain the individual fibers coming from branches A and B in a statistical or optimally evenly distributed manner to avoid as far as possible specular reflection (Figure 1b). C is surrounded by a transparent and stiff disc of plastic (radius: 3 cm). Transparency is necessary to precisely select skin areas; a disc should minimize changes of dermal blood volume caused by pressure.

REFLECTION STANDARDS

Commercially available standards of $BaSO_4$ and Teflon were used.

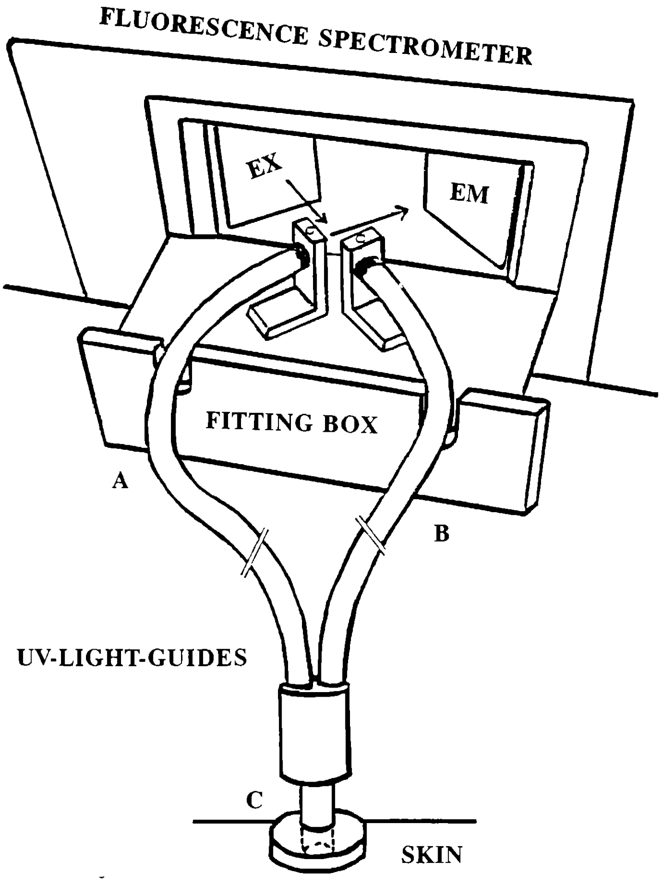

Figure 1a. Adaptation of bifurcated quartz guide to fluorimeter LS 50.

STANDARDIZATION OF SPECTRA

The spectra of skin sites were defined as relative reflection in relation to the reflectance of $BaSO_4$, the reflectance of which is assumed to be 100% irrespective of wavelength: reflectivity of sample (1/nm)/reflectivity of standard (1/nm) = reflectance (% of standard over the spectral range 250–800 nm).

NORMALIZED SPECTRA

To visualize spectral changes caused by treatment and physiological effects, relative spectra were calculated: standardized reflectivity of treated skin (1/nm)/standardized reflectivity of untreated skin (1/nm) = normalized reflectance (% of untreated standardized skin).

MODE OF STATISTICAL DISTRIBUTION

The mode of distribution of individual spectral intensities at 310, 340, and 480 nm selected from spectra of the forearms of 20 individuals was determined by the Kolmog-

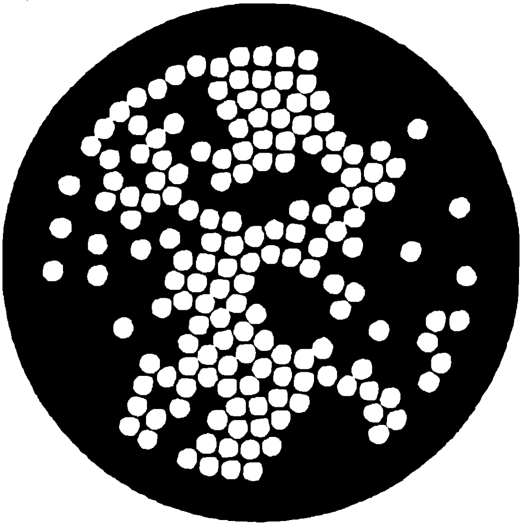

Figure 1b. Distribution of individual fibers within common end C (dark circles = fibers from branch A; light circles = fibers from branch B).

oroff-Smirnow test. Special software to plot mean value + standard deviation of spectra was prepared, allowing statistical calculations.

SPECTRAL DIFFERENCES BETWEEN BODY SITES

With three groups of volunteers (ten male, five female, ages 10–20a; eleven female, one male, ages 31–55a; nine male, seven female, ages 56–72a) the following skin sites were investigated: lower volar forearm, forehead, back of hand, calf, nose, palm, and post auricular area.

The spectral range was 250–750 nm, excitation and emission slit 3 nm, and scanspeed 500 nm/min. These conditions were also selected for the following investigations. The time of recording was summer and fall of 1991.

OCCLUSION OF SKIN

The center of the volar forearms of ten volunteers was occluded for one hour. Before and immediately after stopping occlusion, and several minutes later, reflection spectra were recorded. The mean spectra were normalized, referring to the mean spectrum before treatment.

TAPE STRIPPING OF SKIN

The center of the volar forearms of ten volunteers was stripped with tape 20 times. Before and after removal of strips, spectra were recorded. On neighboring sites, spectra were recorded after removal of up to four cyanoacrylate strips. The spectra were normalized, referring to the corresponding mean spectrum before treatment.

DRYING OF SKIN

The middle volar forearm of five volunteers (three female, two male; mean age = 25 + 4.2a) was dried by means of dry silica gel under occluding polyethylene film for 60 minutes. Before application and after removal of the film, spectra were recorded and normalized.

CHANGING BLOODFLOW

On the volar forearm of five volunteers, erythema was induced by pressure (dermographism), nicotinic acid benzylester (1% in ethanol), and infrared radiation. Reflectivity was measured before and after applying pressure on the erythematous skin. Venous occlusion was induced by interrupting circulation: two and five minutes later reflection spectra were recorded.

INFLUENCE OF A SINGLE TREATMENT WITH SURFACTANTS

Tap water (3 ml), soap slurry (10% aqueous dispersion, 3 ml) and SDS (10% aqueous dispersion, 3 ml) were applied for ten minutes on the volar forearms of ten volunteers under a funnel pressed against the skin surface. Before and 1, 10, 20, and 40 minutes after treatment, reflection spectra were recorded.

INFLUENCE OF FREQUENT WASHING WITH DETERGENTS

Thirty volunteers washed their hands and lower forearms four times per day for two minutes each and for two weeks with a classic soap bar free of perfume and refattening agents. Two, seven, nine, and 14 days after starting treatment, reflection spectra were recorded.

RESULTS

STANDARDIZATION OF SPECTRA AND IDENTIFICATION OF ENDOGENOUS ABSORBERS

The original reflection spectra of $BaSO_4$ and of skin look similar (Figure 2a). After standardization, the relative spectrum is comparable to formerly published spectra (6) (Figure 2b), with exception of the UV range. It is obvious that reflections are missing (=absorption) in the range of fluorescent NADH (λ max 340–350 nm); in the UVB/C-range, where aromatic amino acids, especially tryptophan, are emitting fluorecence; and around 400 nm, where endogenous and bacterial porphyrins are efficient, fluorescent absorbers located at special skin sites (nose, upper back). Most of the observed absorption peaks can be related to definite endogenous epidermal or dermal chromophores (1).

REPRODUCIBILITY

The intraindividual variation coefficient determined from ten spectra gained within 15 minutes from the same skin site is approximately 2% from 300–650 nm, showing higher deviations especially in the deep red portion of the spectrum (Figure 3).

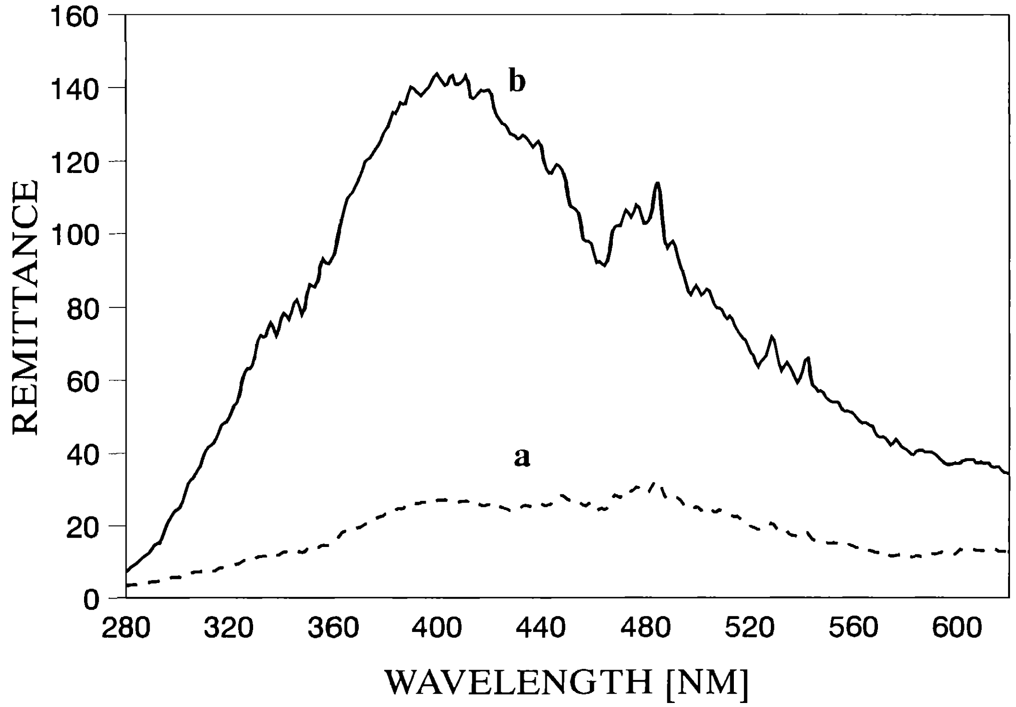

Figure 2a. Original reflection spectra of skin site (a) and of $BaSO_4$ standard (b).

Interindividual variations of untanned lower volar forearms of ten volunteers (date of recording: December) are larger (Figure 4) and wavelength-dependent.

A seemingly peculiar phenomenon is the high standard deviation at 320–340 nm (Figure 5), where one of the major absorption bands of NADH is located.

The Kolmogoroff-Smirnow test showed that the data at all three selected wavelengths (310, 340, and 480 nm) are distributed in a logarithmic mode.

REFLECTION SPECTRA OF DIFFERENT BODY SITES

Apparently the most transparent site is the postauricular area (PA), showing intense absorption by hemoglobin (420, 540, 580 nm) in contrast to the palm (Figure 6). The reflectivity of the PA is extremely high in the UV range too, probably on account of its lacking melanin. The palm shows low reflectivity in the UVB and UVA II range and the steepest increase in remittance with rising wavelengths. The reflectivity of the inner upper arm (UA), traditionally considered as a skin site where signs of photoaging are rarely visible, is very high throughout the spectrum from 300–700 nm. The differences between the back of the hand (large lifelong UV dose) and the UA (protected against sunlight) are especially big in the UVA range. Because the date of recording was summer, different levels of pigmentation will strongly influence the data.

REFLECTIVITY CHANGES CAUSED BY TAPE AND CYANOACRYLATE STRIPPING

The most striking observation is the steadily rising reflectivity in the range of 300–340

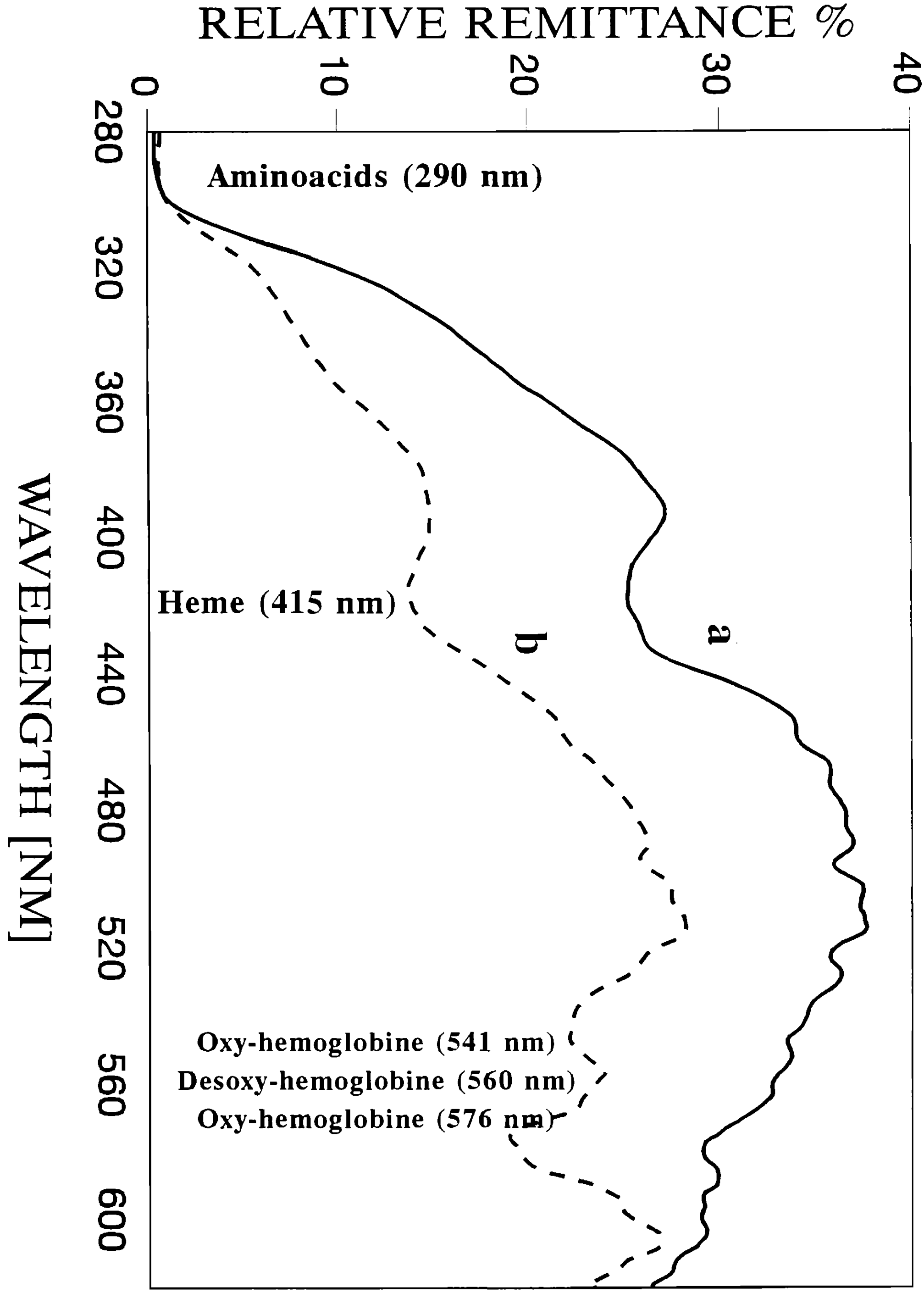

Figure 2b. Standardized spectrum of skin site (a, Figure 2a) and of a sunburned skin site (b) (approximate absorption wavelengths of main skin chromophores are indicated).

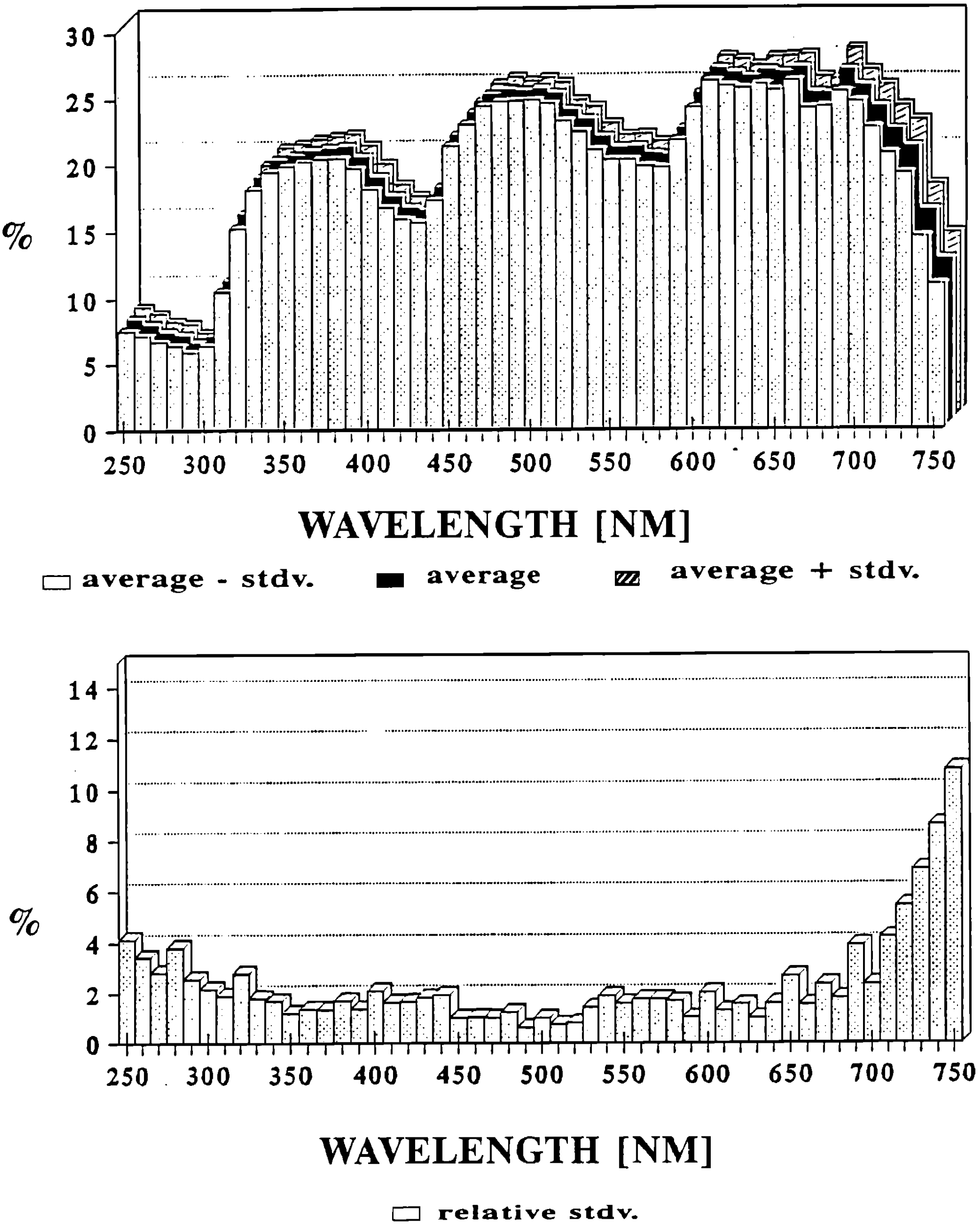

Figure 3. Intraindividual variation coefficient spectra recorded with the 1% transmission filter of the instrument (stdv. = standard deviation).

nm, parallel to increasing numbers of cyanoacrylate strips removed from skin. Reflectivity in the range of <300 nm is decreased less, and the decrease is barely visible in the range of >350 nm (Figure 7). An analogous behavior is observable after removal of tape strips.

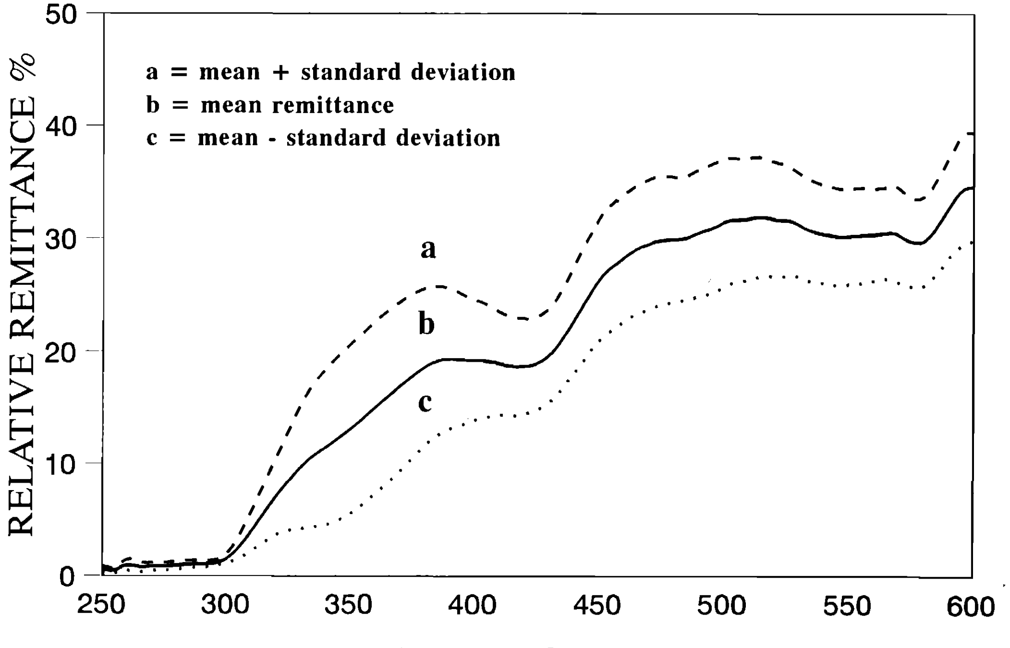

Figure 4. Interindividual variations of untanned lower forearms.

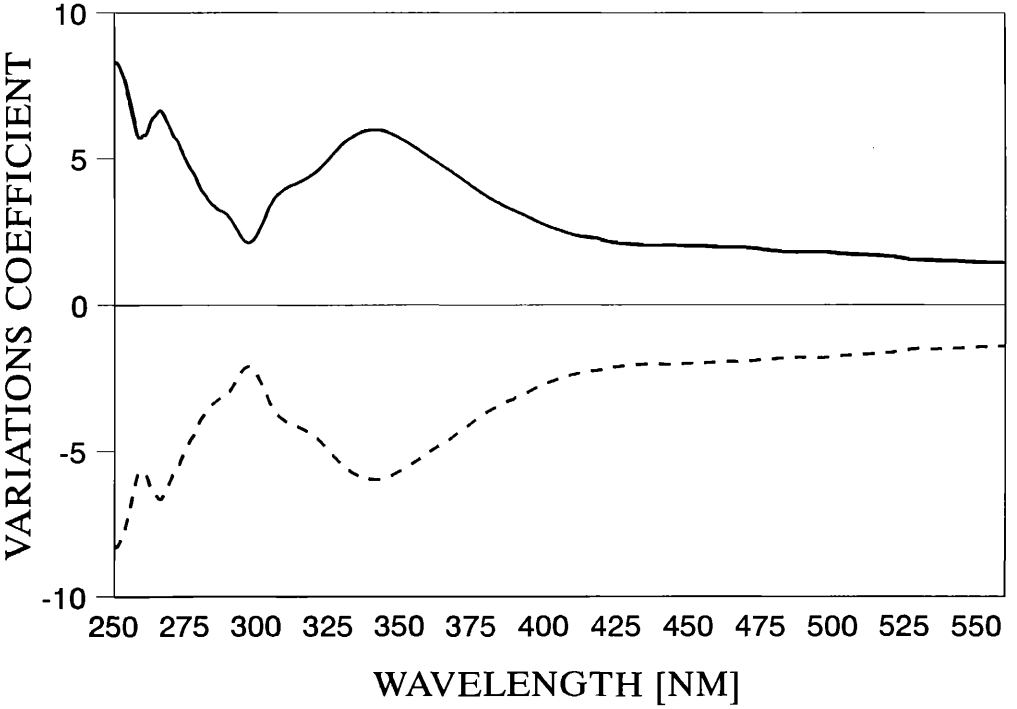

Figure 5. Variation coefficient of data illustrated in Figure 4 (assuming linear and normal distribution).

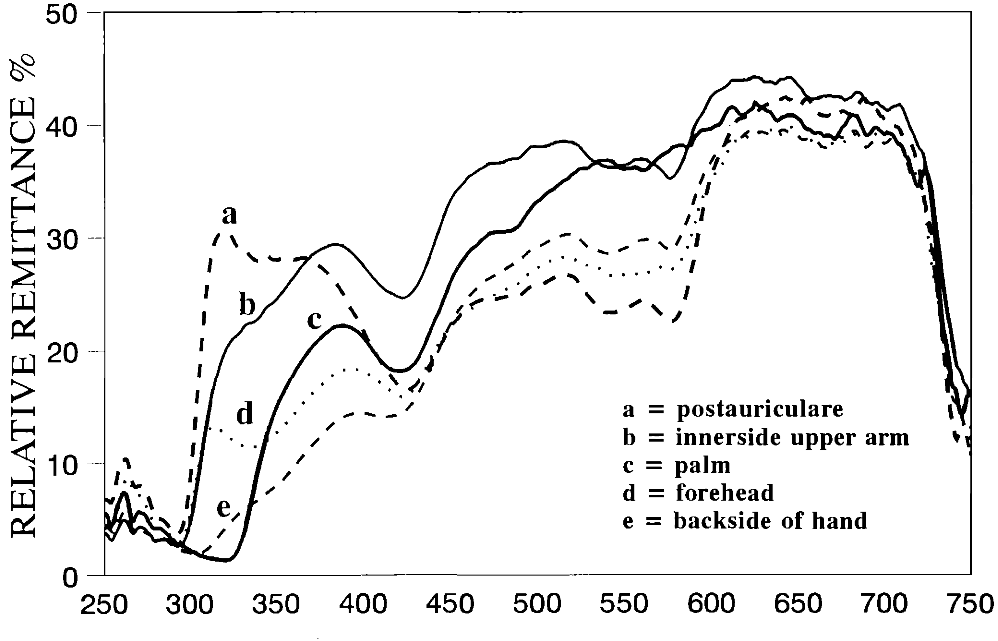

Figure 6. Standardized reflection spectra of different skin sites.

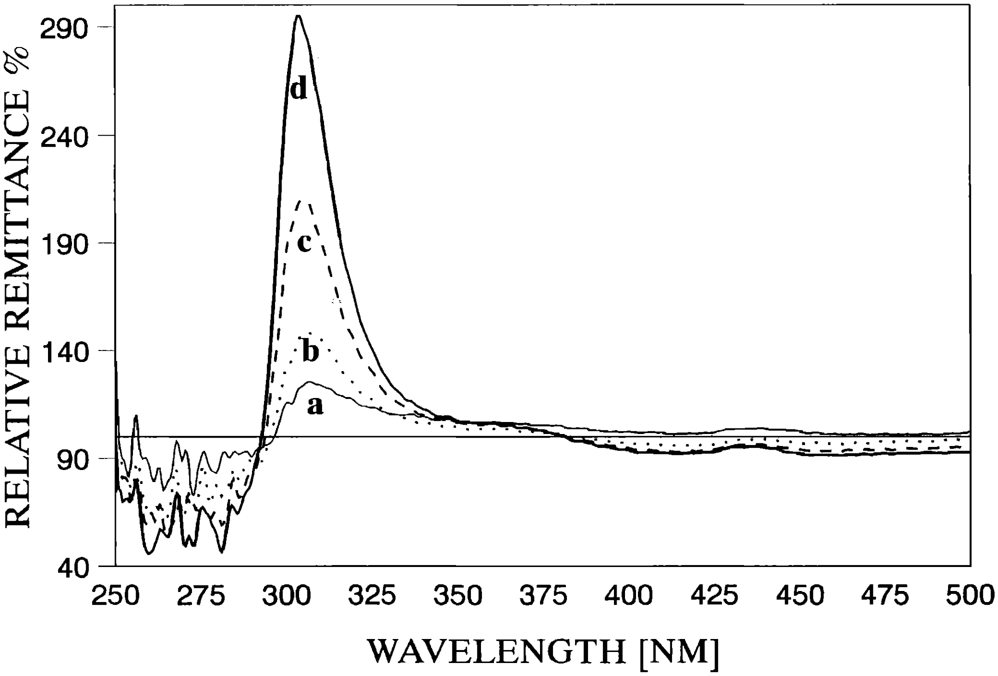

Figure 7. Normalized spectra of lower forearms after cyanoacrylate stripping (a–d = 1–4 strips removed).

OCCLUSION OF SKIN

Prolonged occlusion causes an increase of reflectivity comparable to the effects of stripping in the range of 300–340 nm and a decrease in the UVC range (Figure 8).

DRYING OF SKIN

Drying of skin efficiently increases reflectivity in the range 250–300 nm. After removal of silica gel, the normal reflectivity of skin is gradually restored (Figure 9).

REFLECTIVITY AND BLOOD FLOW

Erythema induced by nicotinic acid esters (Figure 10) does not differ much from pressure-induced increased blood flow (dermographism). The characteristic absorptions at 400–420 nm, 540 nm, and 580 nm are easily distinguishable and are caused by oxyhemoglobin (1). The absorptions at wavelengths >600 nm are occult concerning their origin. With the increasing endurance of erythema, further differentiation in the region of 400–420 nm takes place. Under venous occlusion, skin generally shows decreased reflectivity and absorption bands around 440 nm and 560 nm that are caused by desoxyhemoglobin. Within one minute after restoring normal blood flow, the regular absorption pattern is visible again (Figure 11).

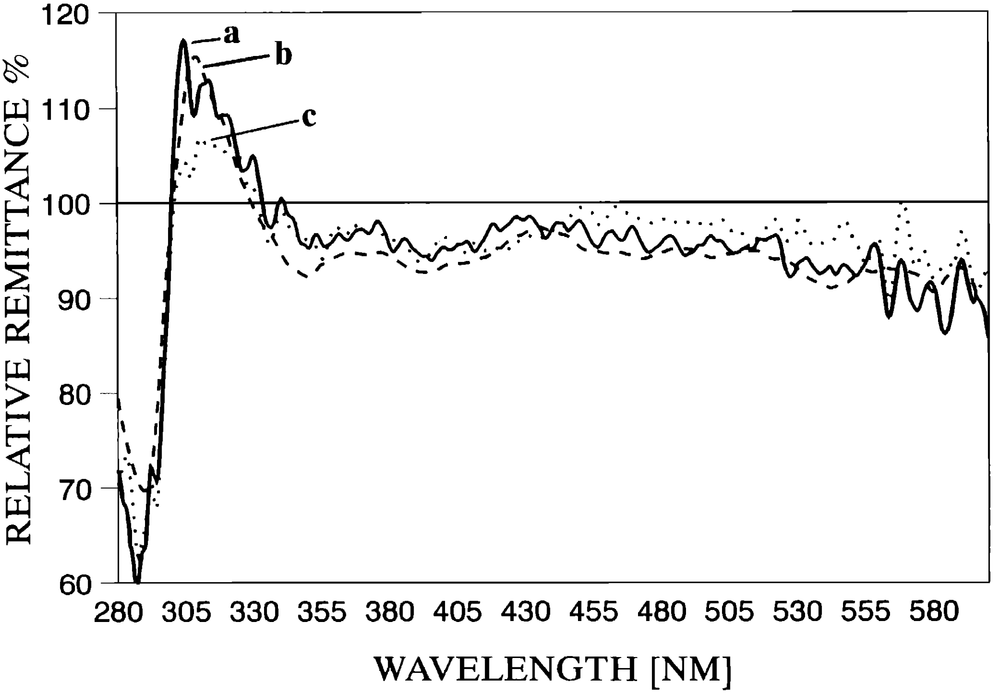

Figure 8. Normalized spectra of lower forearms after occlusion by Finn chambers (a, b, c, = 3, 6, and 12 minutes after removal of Finn chamber).

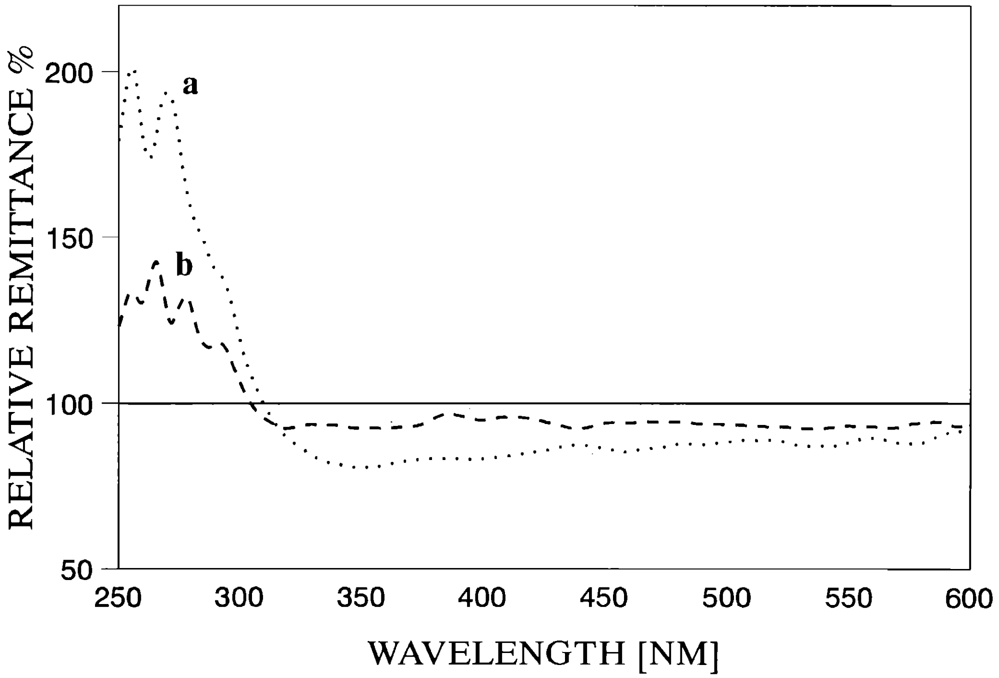

Figure 9. Normalized spectra after drying (a, b = 1 and 15 minutes after removal of silica gel).

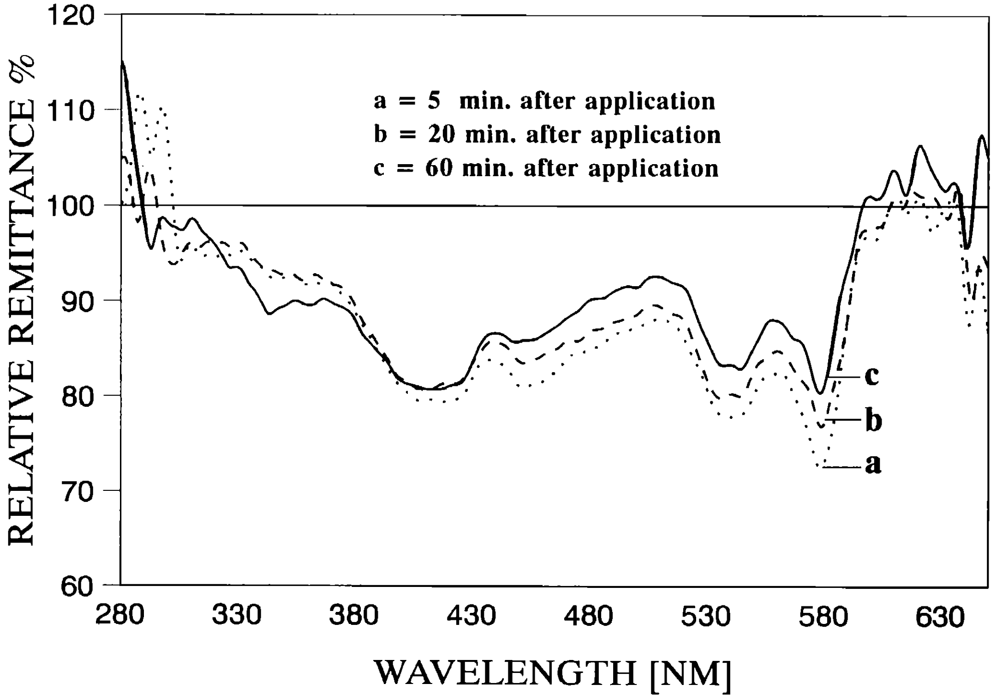

Figure 10. Normalized spectra of lower forearms treated with nicotinic acid ester (1%).

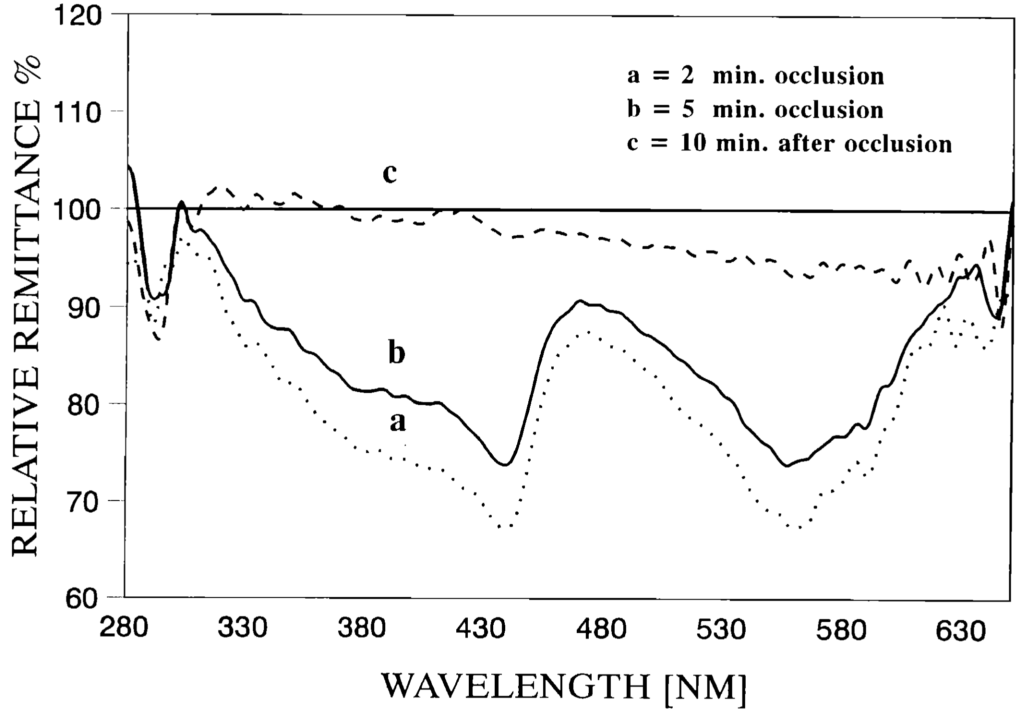

Figure 11. Normalized spectra of lower forearms after venous occlusion.

LOSS OF WATER-SOLUBLE ENDOGENOUS ABSORBERS DURING BATHING

The elutive power of surfactants can be estimated by considering Figures 12, 13, and 14. Reflectivity is increased in the range of 300–340 nm, falling steadily in an approximately exponential function after treatment with water. The primary loss of reflection in the range of 250–300 nm after bathing will be partially compensated in the case of treatment with water and within 40 minutes in the case of soap. Especially in the case of SDS an increase of reflectivity can be observed.

FREQUENT WASHING

Frequent daily washing for an extended period of time causes steadily rising reflectivity in the UVB/A-range (Figure 15).

DISCUSSION

Elimination of fluorescence from reflection spectra yields highly differentiated results in the UVB/C-range in comparison to published results (6, 10) and allows measurement of changing absorption of aromatic amino acids and NADH.

The reproducibility of the method is excellent between 300 and 650 nm, with a variation coefficient of 1–3% (Figure 4). Below 300 nm and above 650 nm the signal/

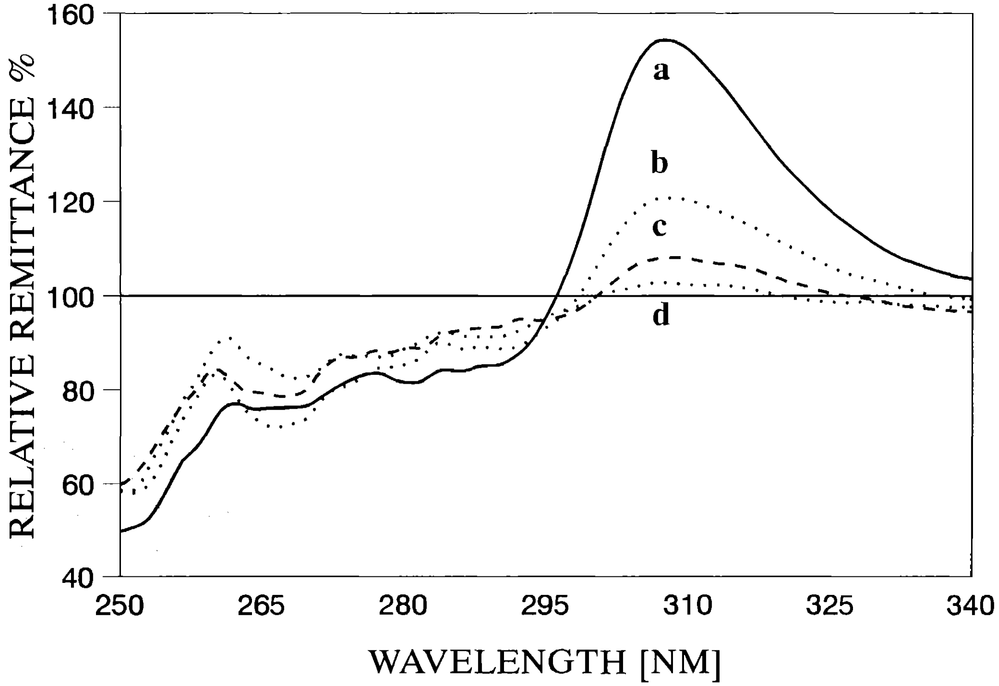

Figure 12. Normalized spectra of lower forearms after washing with tap water (a–d = 10, 20, 30, and 40 minutes after treatment).

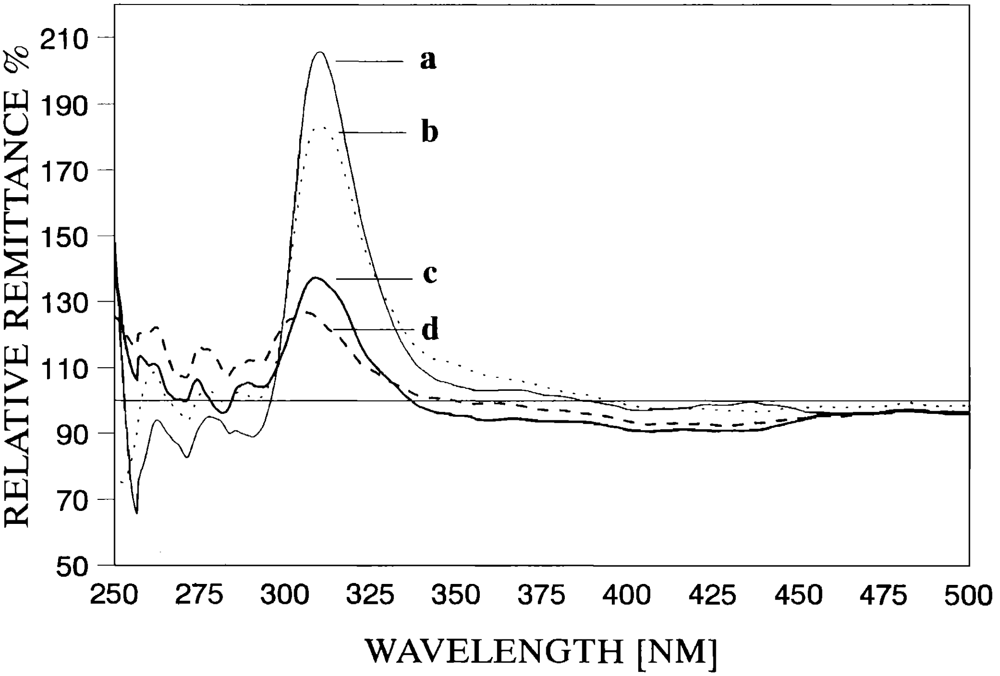

Figure 13. Normalized spectra of lower forearms after washing with soap (a–d = 10, 20, 40, and 80 minutes after treatment).

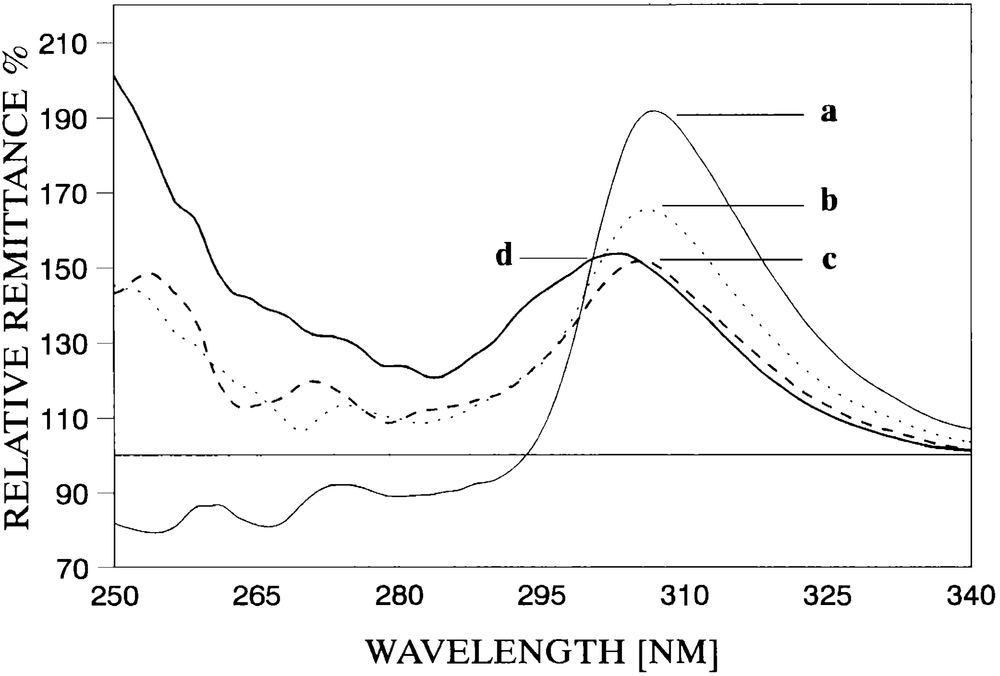

Figure 14. Normalized spectra of lower forearms after washing with SDS solution (a–d = 10, 20, 40, and 80 minutes after treatment).

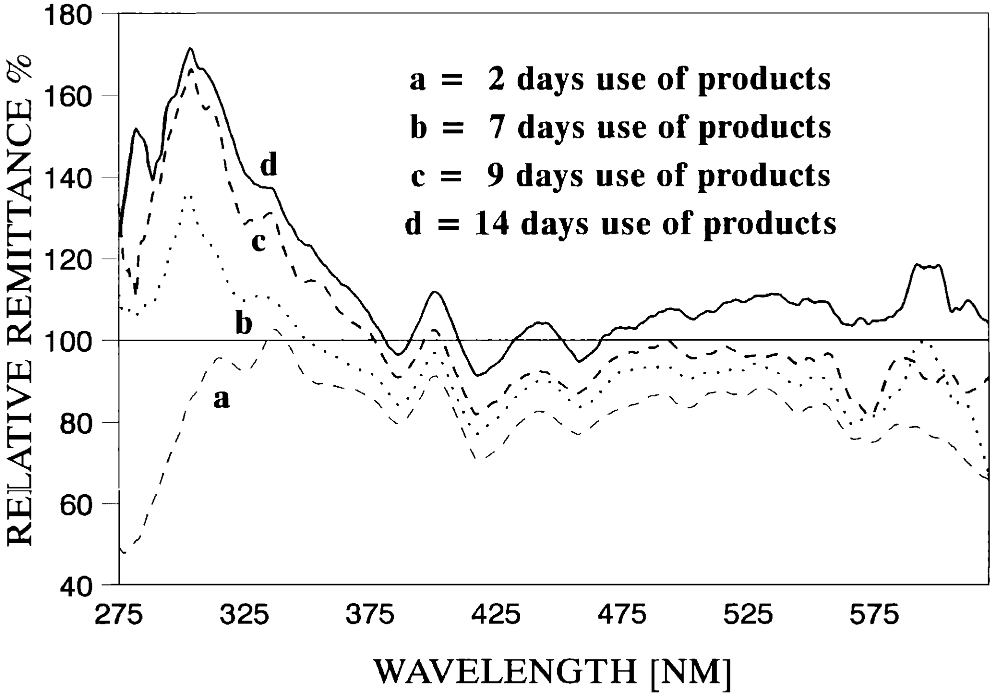

Figure 15. Normalized spectra after repeated, frequent washing with soap (a–d = 2, 7, 9, and 14 days of excessive treatment).

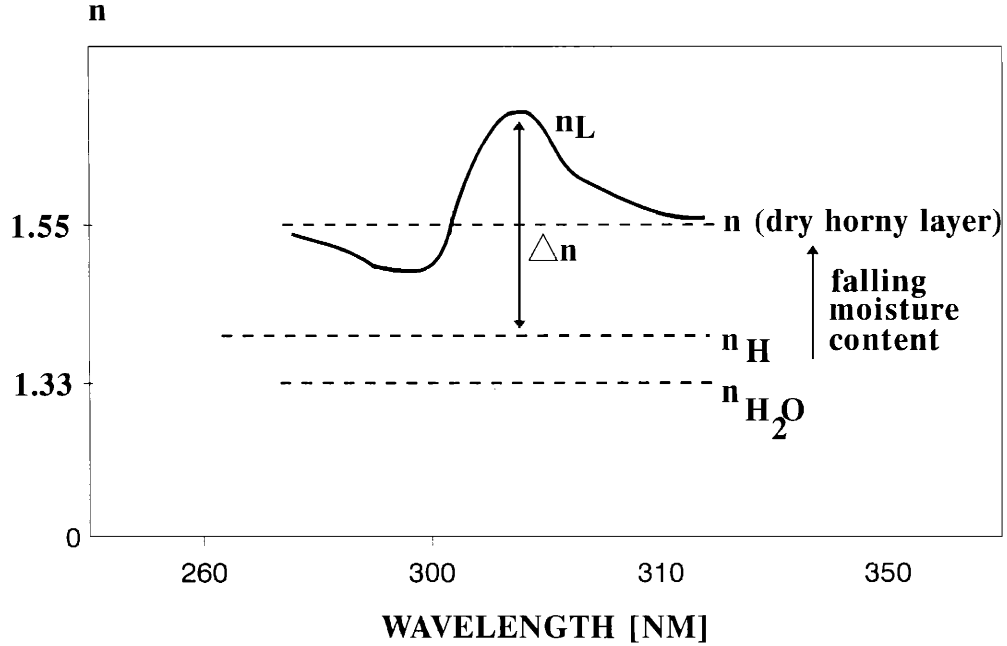

Figure 16. Hypothetical scheme of moisture-induced changes of refraction indices.

noise-ratio is lower on account of lower lamp power and the sensitivity of the photomultiplier. For improvement the attenuator used in the preceding study should be eliminated, increasing the signal intensity (<300 nm).

The wavelength-dependent intraindividual variation coefficient generally shows a maximum around 330–340 nm (Figures 5 and 6). Because this maximum is coincident with the main absorption of NADH, this could mean that changing metabolic states of skin are responsible for this phenomenon.

The individual intensity values irrespective of wavelength can be better described as logarithmic distributions than as linear ones evaluated by the Kolmogoroff-Smirnow test. This seems to fit into the idea that absorption processes play a major role in forming the general shape of the standardized spectra: Lambert-Beer's law relates the logarithm of light intensity to penetration pathlength and concentration of absorber. The low reflectivity below 310 nm is probably mainly determined by low-molecular-weight components of the horny layer.

Occlusion and stripping increase reflectivity between 300–340 nm and decrease it below 300 nm. The reason might be the anomalous dispersion of the refraction coefficient n (8) around main absorption peaks (λ max. ~ 295 nm) (Figure 16). Rising reflection may be caused by increased scattering, and rising differences at interfaces will increase reflection (Fresnel's law). Moisturization such as stripping removes dry levels of horny layer, leaving moist layers. Increasing water content ($n_{H_2O} = 1.33$) of corneocytes will decrease the n_H of hydrophylic cell content and leave constant n_L of lipophilic parts like membranes or hydrophobic proteins ($n_{SC} = 1.55$), resulting in rising $n = n_L - n_H$ with falling n_H. In the UVB/C-range, n after moisturizing is apparently smaller than in the drier state; therefore scattering will be less, optical paths will be longer, and the chance of absorption will be larger. Such phenomena will repeat at each absorption peak but will be negligible above 340 nm on account of increasing penetration depth and the resultant falling influence of moisturization of the upper horny layer.

Light-absorbing compounds very often show shifting absorption and fluorescence peaks, depending on the polarity of the solvent and the resulting stabilizing effects on ground and excited states (13). Adsorption of endogenous UV filters (such as aromtic amino acid or urocanic acid) to surfaces of corneocytes, modified by moisture content, might be another important factor influencing light-absorption characteristics of human skin (14,15).

Erythema either induced by UVB, pressure (dermographism), or nicotinic acid ester shows the normal O_2-hemoglobin absorption at 410 nm, 540, and 580 nm. The absorption at 460 nm is probably caused by bilirubin. Increased pressure during measurement decreases these absorptions. Skin with insufficient venous flow (the lower legs of elderly people) is characterized by intense absorptions at 440 and 560 nm, mainly the absorption of desoxyhemoglobin.

Water extracts low-molecular-weight ingredients from the horny layer. The spectra of Figures 13 and 14 show an overlapping of the increased reflectivity caused by increased H_2O content (300–340 nm) and caused by the increasing potential of soap and SDS to elute water-soluble substances absorbing in the spectral range of 250–300 nm. This is especially well recognized when the hydrated stratum corneum loses water (Figure 12) and the primarily lowered reflectivity is changing into increased reflectivity, caused by deficits of absorbing compounds (Figure 14).

Reflection spectroscopy, especially in the UV range, opens many new possibilities to answer unsolved questions referring to skin moisturization and to the content of low-molecular-weight compounds in the horny layer and their light absorption, which differ in the horny layer of changing moisture content from the well known optical behavior in definite solvents.

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