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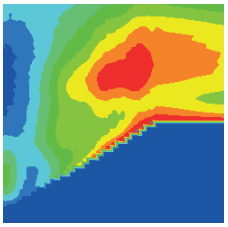
Fluorescence
Raman
Granulometry
PSA

Spectroscopic Methods for Sunscreens Characterization



Application Note

Cosmetics
FLUO RA PSA 02

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This Application Note outlines three different kinds of spectroscopic tools being used for the characterization of sunscreens, and discusses the obtained results. These include Fluorescence spectroscopy for photoactivity, Particle Size analysis for composition and Raman microscopy for formulation investigation.

Keywords: Sunscreen, Fluorescence, Raman, Particle size analysis, Spectroscopy, SPF, In-vivo

Introduction

Sunscreens protect the skin from the damaging effects of both UVA and UVB rays of natural light. They are characterized by the Sun Protection Factor (SPF). This term is adopted by many regulatory authorities, and by the cosmetics and pharmaceutical industries to define the ratio of the least amount of ultraviolet energy required to produce minimal erythema on sunscreen-protected skin to the amount of energy required to produce the same erythema on unprotected skin (Food & Drug Administration, 1978, 1993, 1998, 1999). It is popularly interpreted as how much longer skin covered with sunscreen takes to burn compared with unprotected skin (Health Education Authority, 1998).

In this work, we discuss the photoactivity, composition and formulation of two sunscreen products, namely SPF 20 and SPF 50 by using three techniques: Fluorescence spectroscopy, Raman microscopy and Particle Size Distribution Analysis (PSA).

Instruments

Fluorescence spectroscopy: The new Aqualog® is the only instrument which simultaneously measures absorbance spectra and fluorescence Excitation-Emission Matrices (EEMs). The Aqualog® (**Fig.1**) includes a special aberration-corrected double-grating excitation monochromator, a reference detector and absorbance detector (both Si photodiodes), and a unique emission detector comprised of a thermoelectrically cooled back-illuminated CCD and spectrograph. The system incrementally scans excitation from high energy to low energy and can collect the full emission spectrum at each excitation increment to rapidly and simultaneously generate absorbance spectra and fully corrected EEMs.

The spectrofluorometer is equipped with a bifurcated fiber bundle 6mm in diameter accessory which transmits light to the skin and returns the fluorescence emission to the detector. The tip of the fiber bundle is placed in contact with the volunteers skin with minimal pressure. Each spot before and after treatment was measured in triplicate using batch scanning.

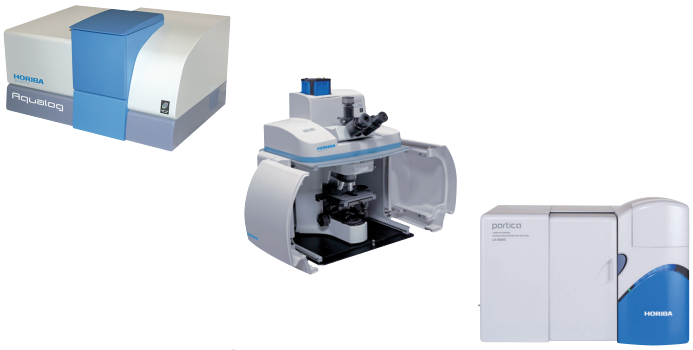

Fig.1 (Left) Aqualog® system, (Middle) XploRA™ PLUS Raman microscope, (Right) Particle Size Analyzer LA-960®

Particle size analysis: Particle size analysis is based on laser diffraction. The central idea in laser diffraction is that a particle will scatter light at an angle determined by its size. Larger particles scatter light at small angles, while smaller particles scatter light at wide angles. A collection of particles produces a pattern of scattered light defined by intensity and angle that can be transformed into a particle size distribution result. The system used here (LA-960®, **Fig.1**) combines the most popular modern sizing technique with state of the art refinements to measure wet and dry samples measuring 10 nanometers to 5 millimeters.

Both cream samples were pre-dispersed in “ultrapure” water. The cream slowly became more and more liquid by progressively adding water. This solution was directly introduced into the LA-960 tank containing “ultrapure” water at an optimal concentration. The tests were performed twice with a new solution. The refractive index chosen for each samples was 1.52 + 0i.

Raman microscopy: Raman microscopy combines Raman spectroscopy and optical microscopy. Raman spectroscopy is a non-destructive and non-invasive vibrational spectroscopy that provides information on molecular structures, crystal phases, polymorphism, and much more. Optical microscopy visualizes and captures images of particles based on static image analysis. An XploRA™ PLUS from HORIBA was used for Raman analysis (**Fig.1**).
In this study, we used respectively the 638nm and 532nm laser wavelengths for sunscreens SPF-20 and 50. The choice of the wavelength is related to the intrinsic fluorescence of the different creams. Acquisition times were set at 3 seconds and 5 accumulations.

Results

Photoactivity effect

In-vivo 2D contour plot of Excitation Emission Matrix (EEM) on forearm skin (before sunscreen application) of a volunteer is shown in **Fig.2**. The major skin fluorophores have been discussed in a separate HORIBA appnote (HORIBA AN FLSS41).

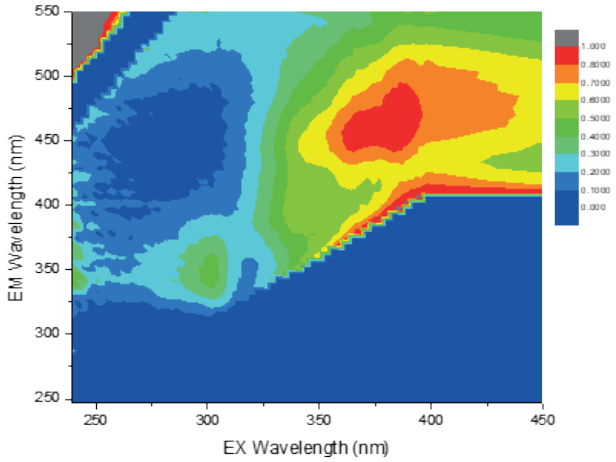

Fig.2 Normalized 2D-EEM contour plot of in-vivo forearm skin. Excitation wavelength range: 240-450nm.

The same area was then applied with the product following internationally agreed procedures (Food & Drug Administration, 1978; COLIPA, 1994) which defines protected skin as that to which a 2 mg/cm² layer of sunscreen has been applied. After air-drying for 20 minutes, the Fluorescence EEM spectra have been measured (**Fig.3**) showing the filter-effect (more intense for the SPF 50 in comparison to the SPF 20) of the applied sunscreens.

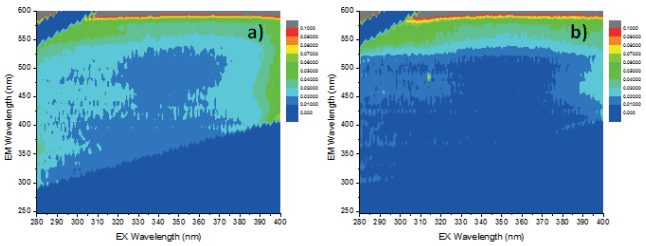

Fig.3 Normalized 2D-EEM contour plot of in-vivo forearm skin after a) SPF 20 and b) SPF 50 sunscreen application. Excitation wavelength range: 280-400nm.

The main photo-process occurring in sunscreen is absorption: sunscreens absorb into the outer skin layer (stratum corneum) and block the UV radiation from entering the inner skin layers by acting as optical filters, so the *in-vivo* fluorescence attenuation reflects the realized protective effects of the applied compounds. Although most of the high-energy UV photons are transformed, dispersed or absorbed by sunscreens, a certain amount of UV light will enter the epidermis. We have already shown how this technique can be used to diagnose damages (HORIBA AN FLSS41).

Composition control

Composition control is important in the sunscreen industry to explain the photoactivity and the skin absorption. Hence, particle size analyses based on laser diffraction were performed on the two creams. The obtained size distributions are displayed in **Fig.4**.

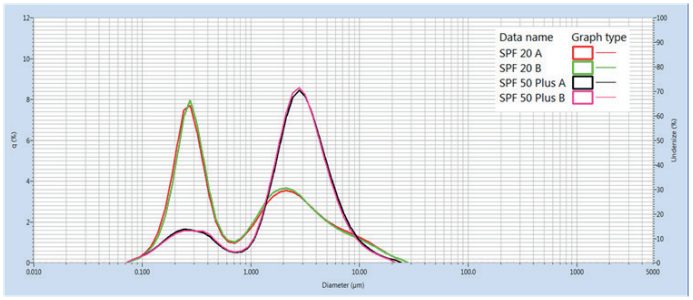

Fig.4 Size distribution of the two different sunscreens SPF 20 and SPF 50.

Both samples showed a common population centered on 270nm, certainly corresponding to sunscreens. Regarding the SPF 20 sample, a population from 80nm to 800nm represents 65% of the total volume of the sample against 16% for the SPF 50 plus sample.

Regarding those values, SPF 20 contains 4 times more filtering products than SPF 50, if this population can be associated with the filtering products.

The samples showed a second population that can be linked to the hydrating part. This population is centered at 2µm for the SPF 20 sample and 2.8µm for the SPF 50 plus sample. This second population is more heterogeneous with the SPF 20 sample. This may reflect a worse dispersion of this phase and may lead to a lower stability.

Formulation investigation

Raman micro-spectroscopy analysis may bring a valuable contribution for composition and formulation investigation of creams. Indeed, the Raman spectroscopy technique is able to provide a direct chemical and molecular identification of different compounds within a cream. Thus, the discrimination between several types of creams is possible by using the spectral fingerprint. We present here the Raman study of two different sunscreens with different sun protection factors (SPF) (respectively 20 and 50). The obtained Raman fingerprints shown on **Fig.5** were identified using spectral databases from KnowItAll® software.

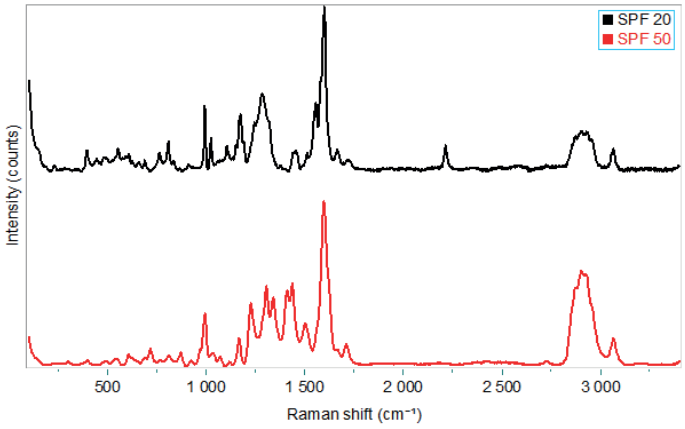

Fig.5 Sunscreens Raman spectra. Top: Cream with SPF-20. Bottom: Cream with SPF-50.

For each of these sunscreens, the comparison with spectral databases enables the investigation of the compounds and explains the spectral differences. The compositions are summarized in the table.

Table 1: Compound identifications based on Raman spectra

Sunscreen	Composition (% of presence)
SPF 20	• Avobenzone (33%) • β-Ionone (19%) • 4-Hydroxychalcone (18%) • Berrylium sulfate tetrahydrate (16%) • Benzoyl(2-hydroxybenzoyl)methane (14%)
SPF 50	• Cyabsorb UV-511 (26%) • Folinic acid (23%) • Polyamide EMO 67 HSP (22%) • Caffeic acid (19%) • Nitroxoline (11%)

Commercial products mainly result from complex formulas, generally remaining confidential. The exact and complete identification could be very difficult. That is why it is important to note that these identifications were simply based on a blind spectral comparison, without knowledge of the incomings.

Raman micro-spectroscopy was revealed as a good technique for composition and formulation investigation / control in the final product.

Conclusions

In this application, we presented how spectroscopic methods can help for sunscreen characterization in terms of photoactivity, composition and formulation, respectively based on Fluorescence spectroscopy, laser diffraction and Raman microscopy. These techniques are complementary and the results show that these systems could be useful tools for dermatological and cosmetic laboratories.

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