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Accurate, Fast and quite easy way of sunscreen testing

Compliant with in vitro global standard: COLIPA, BOOTS, FDA & ISO

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KEYWORDS: *In vitro Transmittance measurement, UV protection, UV testing, SPF, UVA, Sunscreen evaluation, PMMA substrate, roughness, control chart, critical wavelength, Boots Star.*

ABSTRACT: *In this article, the authors describe the main methods used for UV protection, evaluation and the most reliable instrument and substrate for such testing.*
They focus on the parameters which could affect the results and consequently the correlation with in vivo existing methods.

INTRODUCTION

It is now accepted that, in the long term, UVA (320-400nm) as well as UVB (290-320nm) radiation is potentially damaging. While UVB mainly causes damage such as burning and reddening of the skin, UVA radiation is known to cause more serious damage such as aging, DNA damage and even skin cancer. It has been established that a method to measure the protection provided by sunscreens in both UVB and UVA bands is required including the measurement of the stability of this protection under sun exposure.

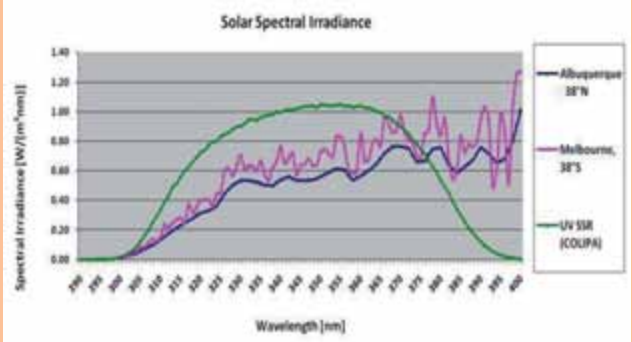

Figure 1. Solar spectrum irradiance.

IN VITRO SPF

The performance testing of sunscreens was originally performed on human volunteers. So-called in vivo testing involved the determination of the SPF (sun protection factor) based upon the delay in the onset of erythema (sun burn) on protected versus unprotected skin. If unprotected skin has a time to erythema (15 minutes) and protected skin has time to erythema (4 hours or 240mn) SPF = 240/15 = 16. SPF = 1 is no protection. In vitro SPF testing was developed in the early 1990s based upon the measurement of the spectral transmittance of a sunscreen applied to a transparent substrate material (1). The transmittance of the sunscreen is the ratio of spectral transmittance through a substrate material compared to the same material with a defined amount of sunscreen applied. The measured spectral power distribution is then weighted by the defined solar spectrum and erythemal effectiveness functions to yield the calculated in vitro SPF. The in vitro SPF is calculated as follows:

$$SPF = \frac{\int_{290nm}^{400nm} E_\lambda \cdot S_\lambda \cdot d\lambda}{\int_{290nm}^{400nm} E_\lambda \cdot S_\lambda \cdot T_\lambda \cdot d\lambda}$$

- Eλ is the CIE erythemal effectiveness function.
- Sλ is a solar simulated spectral irradiance.
- Tλ is the spectral transmittance of sunscreen.

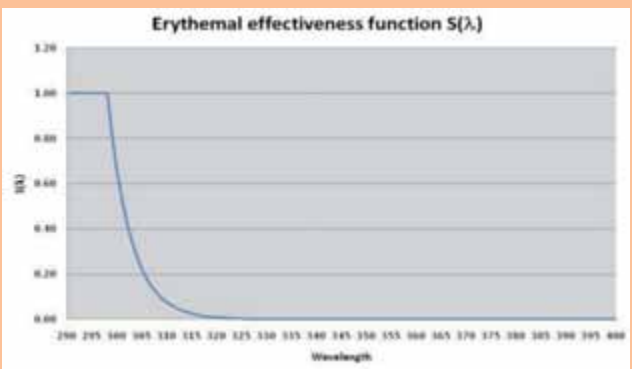

Figure 2. Erythemal effectiveness function.

Standardized functions for Eλ and Sλ, are illustrated in Figure 1 and in Figure 2. The "Albuquerque" or "Melboune" curves match the "standard Sun" and are used in the usual calculation of so called "in vitro SPF". The UV SSR curve has been introduced by Colipa in the UVA in vitro method. It matches the spectral irradiance of the solar simulator used for in vivo SPF testing.

IN VITRO UVAPF

An in vitro method has been described and validated (correlated with in vivo JCIA PPD UVA method) by Colipa (2) based also on transmittance measurement but using different weighting functions.

$$UVAPF = \frac{\int_{320nm}^{400nm} P_\lambda \cdot I_\lambda \cdot d\lambda}{\int_{320nm}^{400nm} P_\lambda \cdot I_\lambda \cdot T_\lambda \cdot d\lambda}$$

- P_λ is the UVA action spectrum correlated to PPD action spectrum.
- S_λ is the UVA spectral irradiance.
- T_λ is the spectral transmittance of sunscreen.
- P_λ and Sλ are shown in Fig. 3.

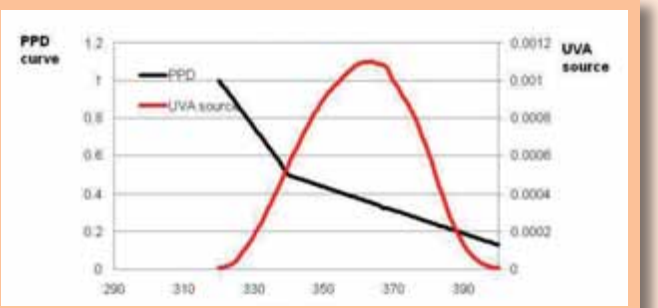

Figure 3. UVA spectral irradiance and UVA action spectrum.

The Colipa method includes a pre-irradiation step and an adjustment of the absorbance curve with a C coefficient based on the difference between in vivo and in vitro SPF measurements. The in vivo value is the measured value but it is also permitted to use the claimed value as a reference. The in vitro SPF value is the calculation of the SPF as described previously using the SSR curve as S_λ curve.

UVA/UVB RATIO

$$\frac{A_{UVA}}{A_{UVB}} = \frac{\int_{320nm}^{400nm} A_\lambda \cdot d\lambda}{\int_{290nm}^{320nm} A_\lambda \cdot d\lambda}$$

This system proposed by The Boots UK Ltd (3) is based on the ratio of the absorbance values in the UVA/UVB. Absorbance A is related to transmittance T by the following formula A = - log T or $T = 10^{-A}$.
The Boots ratio is calculated as:
The result is a coefficient between 0 and 1 that provides a rough guide to UVA protection – 0 equating to no UVA protection whereas 1 signifying equivalent protection in UVA and B. The coefficient is assigned a star value.

CRITICAL WAVELENGTH

$$R = \frac{\int_{290nm}^{\lambda} A_\lambda \cdot d\lambda}{\int_{290nm}^{400nm} A_\lambda \cdot d\lambda}$$

λc is the smallest value for λ where R ≥ 0.9
Critical wavelength (CW) has been described by B. Diffey (4). It has been proposed in the last 2006 EU recommendations (5) to be used to express the balance between UVA and UVB protection. However, this EU recommendation did not select or describe any method to measure this CW. It has been demonstrated that the shape of the curve may depend on the roughness of the substrate (7), so critical wavelength may depend on measurement conditions. Recently two modifications have been proposed by COLIPA (2). In the 2009 revision of its UVA method, the calculation of the critical wavelength has been included. In its second 2011 revision (2) COLIPA proposed to use 6 micron molded plates (HD6). It solves the problem of variation of critical wavelength with roughness by fixing the measurement conditions, and there is also a slight improvement in the UVAPF measurement when using this 6 µm roughness instead of the former 2 µm roughened plates. FDA (6) recently decided to consider the significant work done (with round robin tests) specifically by COLIPA and to finally introduce this critical wavelength measurement for claiming broad spectrum protection. But they referred to the former version of COLIPA and proposed to do this test on plates with roughness values between 2 to 7 microns and with a quantity of 0.75mg/cm². We could expect a certain harmonization in the future between the conditions of testing not only on 6 microns plates now recommended by COLIPA and very soon by ISO normalization but also on sunscreen quantity applied!

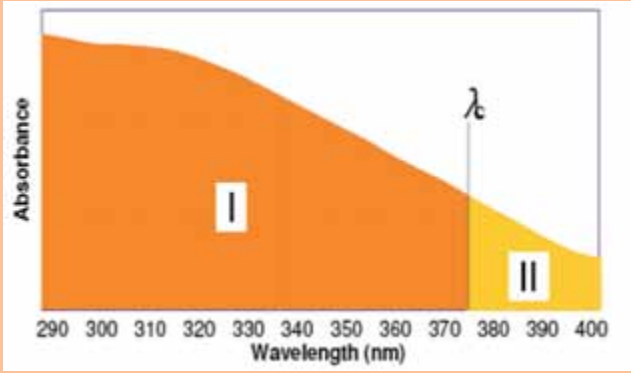

Figure 4. Critical wavelength definition.

PHOTOSTABILITY

Photostability is the expression of loss of performance in absorbing properties due to the proper UV exposure. The residual efficacy of protection is due to a photochemical process of degradation, so we have to consider the evolution of transmission curves without any weighting by efficacy action. In other words, it is not advisable to compare SPF before and after irradiation to express photo stability.

Photostability must be expressed for different parts of the curve (UVB / UVAI and UVAII) as long it depends on the filters. It is advisable to control possible thermo instability during testing. It is also very important to have results for several levels of irradiation to obtain not only an idea on the residual efficacy but also on kinetic behaviour as long it is a photochemical process with thermodynamic considerations. The pre-irradiation step is not photostability but a specific irradiation more or less related to the quantity of irradiation that the product would receive if tested in vivo. In vivo testing leads to a "residual" SPF and does not provide data on either SPF before irradiation or whether the sunscreen is degrading with irradiation. Access to such information is an advantage of in vitro testing. As a matter of fact it is a real challenge to enhance photo stability with such filters. The large number of patents demonstrate interest in this formulation. The average residual efficacy of a photo unstable product is obtained by using the average before and after irradiation transmissions in formula below:

$$\text{Residual efficacy \%} = \frac{\sum_{290}^{400} 1/T(\lambda)_t - 1}{\sum_{290}^{400} 1/T(\lambda)_0 - 1} \times 100$$

where T(λ)0 is the transmission before irradiation,
and T(λ)t is the transmission after irradiation.

MEASUREMENT – CRITICAL ASPECTS

All the in vitro methods: Boots, FDA, Colipa, ISO… are based on transmittance measurements of the sunscreen. One of the main concerns and controversial aspects is the ability to get a good correlation between in vitro SPF and in vivo SPF. The correlation is the goal as it is the way to confirm the reliability for all proposed modifications in the procedure of in vitro testing. But it is quite obvious there are still some reasons not to be well correlated which are not due to the reliability of in vitro method.

CALCULATIONS

It has been previously mentioned that beside the two curves based on "standard sun" (although defined in different conditions or localizations) with curves quite close to each other, there is a recent proposal from COLIPA (2) to use a quite different curve UV-SSR for the calculation of in vitro SPF in the UVA method. The in vitro SPF is calculated only to be compared with in vivo value and to be able to determine the correction coefficient C. This irradiance curve was introduced by Colipa to match the in vivo SPF conditions. The calculated SPF will differ depending on the solar spectrum and formulations used. It is interesting to note that, Helioscreen Lab has conducted a study over 250 random products calculating SPF, using either the Albuquerque sun or the Colipa sun as the sun irradiance curve. The results show that ¾ of the products have a difference of more than 20 percent in SPF between the 2 calculations.

Figure 5. Influence on solar irradiance on in vitro SPF.

INSTRUMENT

The instrument for spectral transmittance measurements includes:

- a source with a continuous spectrum (no peaks) emitting in the UV range from at least 290nm to 400nm. The source is collimated on the sample. Generally Xe lamps are used, continuous or flashed. It is recommended to stay under 0.2J/cm² to avoid degrading the sunscreen during measurement.
- an integrating sphere.

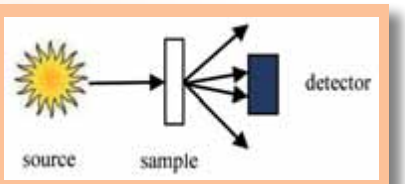

Figure 6. Set-up without an integrating sphere.

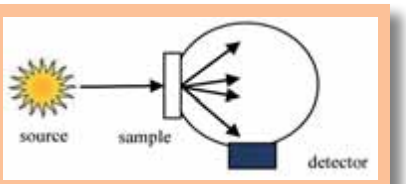

Figure 7. Set-up with an integrating sphere.

With a single detector, part of the light diffused by the sample is not detected leading to lower signal and higher SPF (see Figure 6). With an integrating sphere, all the light is collected. It is important that the sample is as near as possible to the integrating sphere port, so that all the light enters the integrating sphere (see Fig. 7).
a spectrometer which includes a diffraction grating to spread the light over the UV spectrum and a detector. There are two main types of spectrometers:

- monochromator with rotating grating: Figure 8.
  The grating is rotated in front of a detector to change from 1 wavelength to another.
- spectrograph: Figure 9. The detector is an array of detectors, each small detector seeing one wavelength.

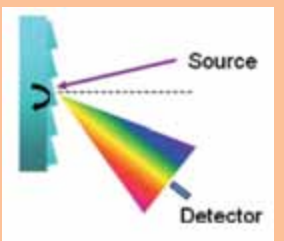

Figure 8. Monochromator based spectrometer.

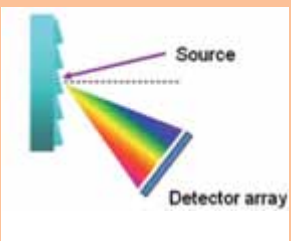

Figure 9. Spectrograph.

There are two different measurement configurations possible. The sample is illuminated with collimated light, the detector collects the total diffuse transmitted light with the integrating sphere. This configuration is called 0°/d. See Figure 10.
The sample is illuminated diffusely and the detector is collecting collimated light. This configuration is called d/0° and is equivalent to 0°/d. The advantage of this configuration is the ability to collect more light from the source; and everything being equal to have a better signal/noise ratio or better dynamic range. See Figure 11.

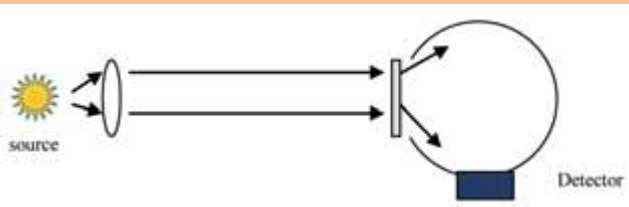

Figure 10. configuration 0°/d.

The spectral separation can be done at the level of the source, but it is only possible for configuration 0°/d, with a monochromator. The sample is illuminated by monochromatic light, one wavelength after the other. Or the spectral separation can be done after the sample at the level of the detector, using either a monochromator based detection system or a spectrograph.

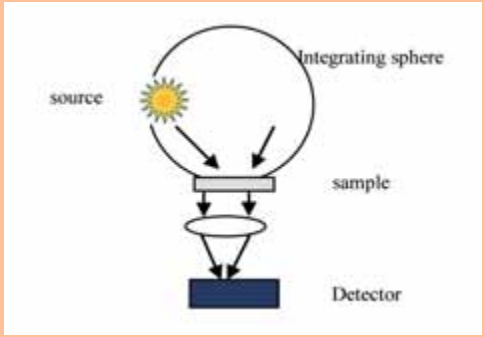

Figure 11. configuration d/ 0°

When the separation of light is done at the level of the source, an additional filter in front of the detector is needed to avoid fluorescence. When a material absorbs UV light, there is generally fluorescence induced in the visible. This means that in addition to the transmitted signal in the UV, there is a fluorescence signal at a higher wavelength. When the sample is lighted with monochromatic light, see Figure 12, the detector is not spectral and so is sensitive to flux, regardless of wavelength, and adds the fluorescence signal to the true signal, leading to higher transmittance and lower SPF. The solution is to add a filter in front of the detector which keeps signal below 400nm and blocks the signal in the visible. If the fluorescence occurs at wavelengths less than 400nm, it will react differently depending on the configuration and, in this specific case, can lead to different results depending on the configuration.

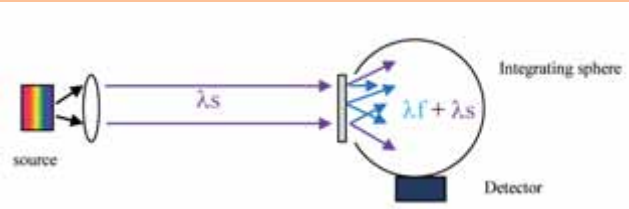

Figure 12. Monochromatic illumination and fluorescence.

The other important parameters to consider for a spectral system are the resolution/bandpass and the accuracy in wavelength. The bandpass is the width of a spectrum passed by a monochromator or a spectrograph. It is specified as FWHM or full width at half maximum. See Figure 13. The resolution is the instrument ability to resolve two consecutive spectral peaks. Wavelength accuracy is the ability for an instrument to display the correct wavelength. It can be checked very easily using either a Hg lamp with well-known peaks or a solution/material with well-known absorption peaks such as Holmium oxide. In the right hand curve of Figure 13, the wavelength is calculated during the calibration process as the centre wavelength of the curve, so even if the bandwidth is 4nm for example, it is possible to have wavelength accuracy of 1nm. During the measurement, the signal at each wavelength is more or less averaged on the bandwidth, so bandwidth specification is not so important for accuracy when measuring signal with low variation versus wavelength as sunscreen transmittance, but it is very important in the case of signal with peaks, as with this averaging, the curve of the peaks will be broadened and the peak max will be decreased. The dynamic range is the ability of an instrument to measure high SPF (low transmittance). The limitation is the noise on the detector which is the signal read by the detector with no light. Linearity is the ability of an instrument to measure correctly signals at different levels. It can be tested easily by measuring 2 substrates with absorbing filters separately and then together and verifying that the total absorbance of the 2 filters is the sum of the individual absorbance.

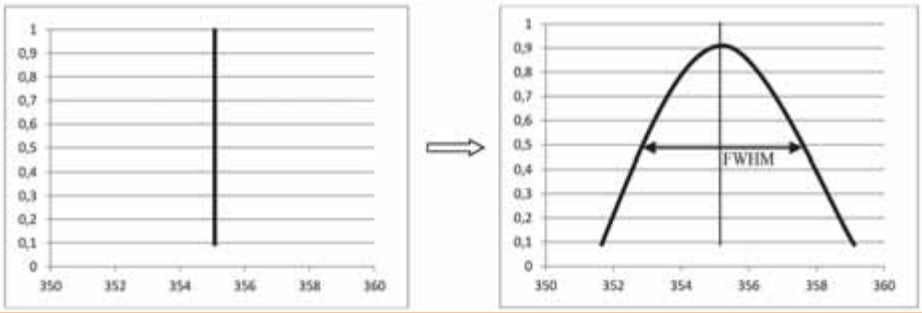

Figure 13. Bandpass

In conclusion, as a very quick statement, spectrograph based instruments allow quick measurements, where monochromator based instruments give the highest dynamic range. But there is a large range of instruments in both configurations, with a wide range of prices and specifications. The UV-2000S from Labsphere Inc was designed to fully comply with all current and proposed industry requirements. It is fully compliant with global in vitro testing methods; COLIPA UVA-PF, Boots-Star (2008 Revision), FDA-2011 and proposed ISO guidelines; and is capable of measuring high SPF products. The UV-2000S hardware is designed to meet recommendations for globally accepted in vitro testing methods and the software allows for obtaining results per the selected standard. The software eliminates time wasted exporting and cutting/pasting data to achieve results per a specific in vitro testing method as well as any chance of user error. The system is supplied with a validation kit which allows regular verification of instrument performance.

SUBSTRATE AND SUNSCREEN SPREADING

The substrate is the key parameter for reliability of in vitro testing. It must ensure reproducibility – as far as possible - of the T measurements of the spread product within the plates and between the plates. A very important parameter of in-vitro SPF determination is the thickness applied to the substrate (7). It is interesting to note that the critical wavelength and the Boots UVA/UVB ratio are relative measurements which should be less sensitive to applied quantity of sunscreen. Doubling the thickness will double the absorption. A sunscreen applied with a thickness t giving a calculated in vitro SPF of 13, will lead to a calculated SPF of 156 with a double thickness 2t. Clearly a given thickness is related to a specific roughness. There is no way to spray the right quantity of product reproducibly on substrates as Quartz / Transpore ™/ Vitroskin™ etc… as there is no way to control the roughness intra and inter plates. To ensure reliability on roughness regularity, it has been proposed to replace the PMMA sand blasted plates introduced in the 90s (8) by a specific substrate designed only for this purpose and introduced in 2008 (9).With the former PMMA plates (sand blasted) it was very difficult to ensure reproducible roughness intra and inter plates. The importance of controlled roughness of the new molded substrate for reproducible results has been demonstrated and published (10). The same document proves also that a higher controlled roughness of 6µm for this substrate (HD6 plates) not only makes the spreading easier but also considerably restricts variability of results (Figure 14).

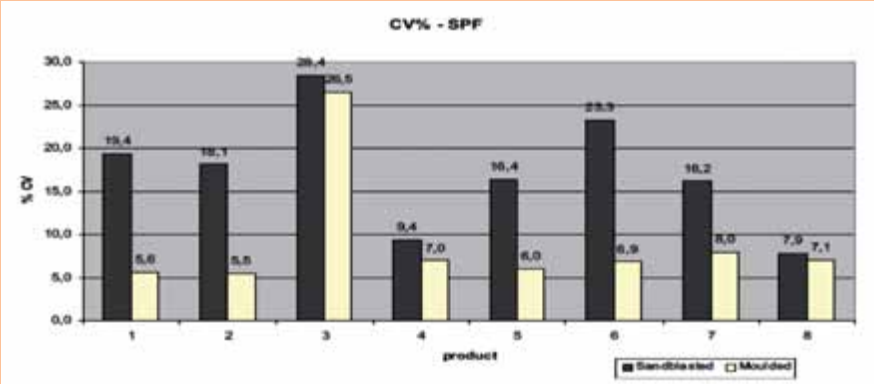

Figure 14. Beneficial effect of roughness homogeneity on SPF reproducibility.

This new substrate is totally controlled and guaranteed within specific ranges for several parameters (10) described in a control card. This improvement with its benefits on the reliability on the results has been recognized by COLIPA (2) and ISO which recommend these specifications. It has been also demonstrated a better correlation in the UVA Colipa method. In the state of these methods, any PMMA substrate designed for such testing will have to be normalized and should have surface topography characteristics that meet the following measurement targets and ranges:

Target roughness with upper and lower limits

Parameter	Ra	Rv	Rdq	Al	Ssc	Vvv
Target value	4.853	13.042	11.122	239.750	0.033	1.044E-6
Upper Limit	5.170	13.669	12.411	284.256	0.046	1.663E-06
Lower Limit	4.535	12.414	9.833	195.244	0.020	4.248E-07

It is also critically important that the optimum film thickness is achieved. The rough substrate has peaks and valleys. And the transmittance of the sunscreen is dependent on the film thickness regularity.

It is very important to apply the right amount of sunscreen the most uniformly possible manner. Unfortunately some products do not have the same affinity with the plates and good measurements still require to be done by a trained operator and daily practice on sunscreen spreading.

CONCLUSIONS

For ethical and cost reasons, in vitro methods are the preferred ones. But they still have to correlate with in vivo results. In vitro testing is already very helpful in research and development of new sun protective formulations. Comparative measurements of various formulations can be easily done in-house. The recent research and improvement led to the adoption worldwide of an in vitro method for the UVA protection. The next step is to work on an in vitro SPF method, which gives reliable results and good correlation with in vivo SPF results.

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