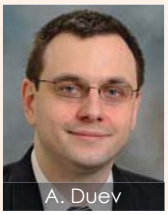
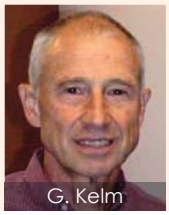
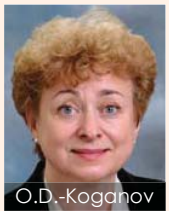
ARTYOM DUEV[1]*, GARY KELM[2], R. RANDALL WICKETT[2], OLGA V. DUEVA-KOGANOV[1]







*Corresponding author
1. AkzoNobel Surface Chemistry LLC, 23 Snowden Ave, Ossining, New York, USA
2. The James L. Winkle College of Pharmacy, 136 Health Professions Building, 3225 Eden Ave, Cincinnati, Ohio, USA

Are SPF and Critical Wavelength sufficient to measure efficacy of sunscreen products against sun induced skin damage?

KEYWORDS: Sun; Skin; SPF; CW; ROS; Sunscreen efficacy.

Abstract The SPF in vivo testing evaluates protection of sunscreen products against erythema induced by the light source that accurately simulates a small portion of sunlight spectrum. The FDA requirements of CW in vitro testing and 370 nm criterion for broad-spectrum protection extend the measurement of sunscreen effectiveness against UVA. However, this criterion provides no motivation for manufacturers to develop products with higher CW values; and there is ongoing debate whether current CW threshold might be revised. ROS are the significant damaging factors associated with sun exposure, and action spectrum for their production extends into VIS and IR. Continuing research whether antioxidants could mitigate sunlight induced ROS generation and the utilization of testing methodologies mimicking end-usage conditions will foster the development of better sunscreen products.

INTRODUCTION

According to the International Commission on Illumination (also known as the CIE) natural sun light that reaches the earth has different amounts of Ultraviolet (UV, 290-400 nm) ~ 6%, Visible (VIS, 400-800 nm) ~ 55%, and Infrared (IR 800-2450) ~40 % radiation (1). Sunscreen products are used to protect skin against damage from the sun's rays and in the USA sunscreen products are OTC drugs regulated by the Food and Drug Administration (FDA). In 2011 the FDA issued a Final Rule "Labeling and Effectiveness Testing; Sunscreen Drug Products for Over-the-Counter Human Use" stating that that complete measure of broad-spectrum protection provided by a sunscreen product can be determined by criteria of Critical Wavelength (CW), measuring breadth of UVB and UVA protection and SPF value measuring magnitude of UVB and UVA protection; and sunscreen products must have CW value of at least 370 nm to be allowed a broad-spectrum protection label claim (2). The FDA new regulations likely helped to increase the sun protection awareness in the USA and also globally. The sunscreen market in the USA is one of the largest in the world; and the sales of sunscreen products in the mass-market outlets in this country rose to $ 1.2 billion in 2012 (3). However, in order to maintain consumer confidence in the category, sunscreen finished goods manufacturers need to continue developing more effective products based on the on-going research and new scientific discoveries.

NATURAL SUNLIGHT IRRADIATION SPECTRA

Sun light that reaches the earth is composed of UVB, UVA, VIS, and is extended further to IR radiation (1) – as illustrated in Figure 1.

MED (MINIMAL ERYTHEMAL DOSE)

The Minimal Erythemal Dose (MED) is the smallest UV dose that produces perceptible redness of the skin (erythema) with clearly defined borders at 16 to 24 hours after UV exposure (2). According to the Solar Light Company, Inc.,
a manufacturer of solar simulators, MED can be also presented as a time under the specific irradiation conditions to induce the erythemal response (3). Fitzpatrick stated that the MEDs are as follows for the respective skin types:
Skin type I = 200-300 J/m^2-eff; Type II = 250-350 J/m^2-eff;
Type III = 300-500 J/m^2-eff; Type IV = 450-600 J/m^2-eff;
Type V-VI = 600-2,000 J/m^2-eff (4).

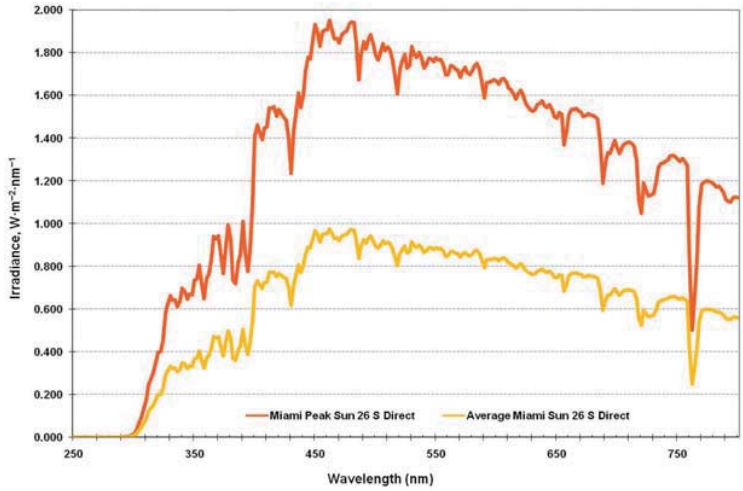


Figure 1. Sunlight Irradiance in Miami Florida, USA – based on the data from Atlas Material Testing Solutions

To illustrate the MED concept in relation to the natural sunlight, authors calculated the approximate times to induce the erythemal response in different Fitzpatrick skin types during outdoor exposure in Miami Florida, USA (25°47′16″N 80°13′27″W) on June 19, 2013; calculations were based on the following information:

- Fitzpatrick averaged MEDs for different skin types (4);
- Earth Networks company data that **UV Index** in Miami Florida, USA (25°47′16″N 80°13′27″W) on June 19, 2013 at Solar Noon was **11** (5);
- The interpretation of UV readings provided in the Application Note of Davis Instruments indicating that **UV Index 11** corresponds to dose-rate of approximately 4.7 MED per hour or 1 MED = 13 minutes (6).

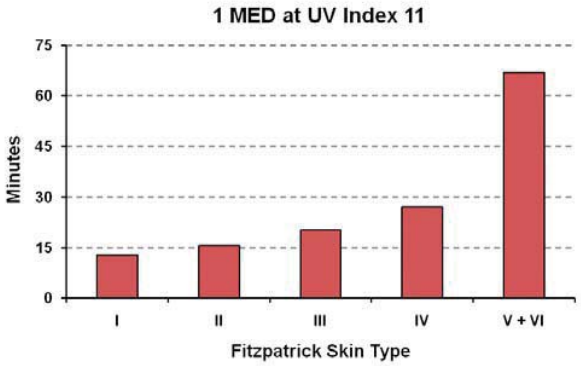


Figure 2. The approximate times (minutes) to induce the erythemal response in different Fitzpatrick skin types during outdoor exposure at **UV Index 11**

THE SUN PROTECTION FACTOR (SPF) *IN VIVO* TESTING

SPF *in vivo* tests are conducted under controlled irradiation conditions to measure sunscreen protection against simulated sunlight, not the natural sunlight. SPF is calculated as the ratio of the least amount of ultraviolet energy required to produce a minimal erythema on skin protected by sunscreen (MEDp) to the amount of energy required to produce the same erythema on unprotected skin (MEDu): SPF= MEDp/MEDu (2). In order to reduce the exposure time during the SPF *in vivo* tests without over-heating the skin - the simulated sunlight used for these tests has much higher contribution of the short wavelength UVB irradiance relative to UVB irradiance under the natural sunlight - as illustrated in Figure 3 below.
The Model 601 Multiport® Solar Simulator is used specifically for SPF *in vivo* testing and dermatological studies; it accurately simulates the UV spectrum of the sun light in the region of 290 to approximately 370 nm; this system has an intensity of up to 4 MED's per minute for each irradiation aperture (port), which represents approximately 20 times the intensity of the sun in this particular wavelength region. It should be noted that the MED under accelerated irradiation conditions is reached within about 15 sec. (3). In contrast, the MEDs under real sunlight are 10-20 min for fair-skinned individuals, depending on geographical location and season – according to Sayre and Dowdy (8), and the calculations presented above. Thus, the SPF *in vivo* testing evaluates predominately the UVB protection of sunscreen products against skin erythema induced by the artificial light source that accurately simulates the ultraviolet spectrum of the sun light only in UV region of 290 to about 370 nm, which represents a rather small portion of natural sunlight spectrum.

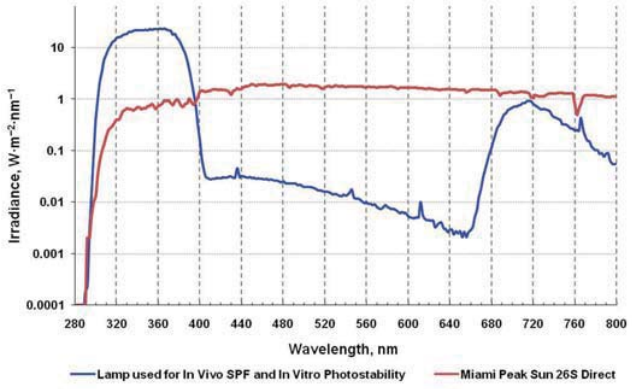


Figure 3. Irradiance Spectra of Natural and Simulated Sunlight (7)

CRITICAL WAVELENGTH (CW)

The scientific term and metric of Critical Wavelength (CW) were developed by Diffey in 1994 (9). The CW is defined as the wavelength at which the area under the absorbance curve represents 90 percent of the total area under the curve in the UV (290-400 nm) region. CW *in vitro* determination requires substrate spectroscopy measurements - when sunscreen products are applied and spread uniformly on the surface of substrate that is transparent in 290-400 nm area. Figure 4 below illustrates this measurement. CW measurement is expressed mathematically as:

$$\int_{290}^{\lambda_c} A(\lambda)d\lambda = 0{,}9 \cdot \int_{290}^{400} A(\lambda)d\lambda$$

In this expression, A(λ) is the mean absorbance at each wavelength, and dλ is the wavelength interval between measurements.
Sunscreen products offering primarily UVB protection would have CW less than 320 nm, whereas those providing both UVB and UVA protection would have CW between 320-400 nm.

Parameters	FDA Final Rule (2)
Pre-Irradiation Dose	4 MED (800 J/m²-eff)
Irradiation Flux	Limits provided
Application Dose	0.75 mg/cm²
UVA Efficacy parameter	CW, nm
Substrate	PMMA (polymethyl methacrylate) plates; roughness parameter (Sa) between 2 and 7 micrometers;
Application	Two phase spreading: 60 sec total

Table 1. CW Test Parameters

CRITICAL WAVELENGTH (CW) *IN VITRO* TESTING

Recently the FDA adopted CW metric to measure broad-spectrum protection (or breadth of UVB and UVA protection) provided by a sunscreen product; the FDA also requires that CW is determined after pre-irradiation with 4 MED, a fixed (universal) dose used for all sunscreen products (2). Test parameters are presented in Table 1.

CW STUDIES OF COMMERCIAL SPF 15 SUNSCREEN PRODUCTS

As the FDA Final Rule is a subject of great interest in the industry and consumers, authors tested a selection of commercially available sunscreen products according to the protocol described above.
Seven commercially available SPF 15 products were randomly selected, purchased in a US drug store, and tested for their CWs. All test products were US origin; five were branded and two were private label sunscreen products. Products were applied to PMMA plates; 0.75 mg/cm² were dispensed onto each plate

using an analytical balance. Test products were then applied in two stages using a finger cot and a stopwatch: 30 seconds slow spreading followed by 30 seconds of intense rubbing. The plates were air-dried for 15 minutes and subjected to 4 MED pre-irradiation using the setup that includes filtered 16S-300-002 (Air Mass 1.5) xenon arc lamp, XPS 400 precision current source; and PMA2100 Radiometer in conjunction with PMA2101 Detector (all from SolarLight Company, PA USA). Figure 4 the absorbance spectra of the test articles - measured after 4 MED pre-irradiation doses. CWs values are marked as squares of color matching the curves. A dashed vertical line shows the 370 nm cutoff criterion for broad-spectrum protection claims.

ACTIVES, %	Test Articles						
	A	B	C	D	E	F	G
Avobenzone	3	3	2	2	1		
Homosalate			10	10	6		
Octisalate		2	5	5			
Octocrylene	10			5	0.8		
Oxybenzone			3		2		5
Ensulizole						1	
Octinoxate		2				6	7.5
CW, nm	**378**	**376**	**378**	**377**	**374**	**334**	**352**

Table 2. Sunscreen actives concentrations in the test articles and their CWs

Table 2 contains the information regarding sunscreen actives in the tested products and their respective CW values.

Test results showed that five sunscreen products (A, B, C, D and E) all containing Avobenzone have exceeded the FDA's criterion of CW 370 nm. Avobenzone is a notable sunscreen filter that can help to achieve broad-spectrum protection. Remaining products (F and G) without Avobenzone demonstrated lower CWs (less than 340 or 360 nm, respectively) – while making broad-spectrum protection label claims. Therefore, these claims are potentially misleading to the consumer and would not be allowed under the FDA Final Rule. Thus, on a positive note, the FDA's CW standard will prevent some unscrupulous marketers from bringing to the market products with unsubstantiated UVA/UVB broad-spectrum claims; and even a less than perfect broad-spectrum protection standard is better than its absence. Our experimental data also showed that CW 370 nm can be easily met with low concentrations of Avobenzone; for example, Product E with just 1% of Avobenzone demonstrated CW of 374 nm.

These experimental data are in agreement with Diffey's statement that "by setting bar too low, it is not difficult to exceed the 370 nm threshold, especially in Europe where a wider range of UV filters are permitted than in the United States" (10). Arguably, the threshold of 370 nm provides no effective motivation for sunscreen manufacturers in the USA to develop products with higher CW values because 370 nm is already achieved by majority of sunscreen products currently marketed in the USA. In addition, a relatively weak CW standard makes it difficult or impossible for consumers to distinguish better broad-spectrum sunscreen products from the less effective ones.

Possible solutions were proposed to address the potential problems associated with rather low CW threshold.

According to the Critical Wavelength Testing Company, if the FDA allowed sunscreen manufacturers to put the exact CW value on the label, it would help consumers to differentiate between sunscreen products - because higher CW value equals better UVA protection. Since 2007, the American Academy of Dermatology (AAD) has asked the FDA to require placement of an absolute CW value on sun protection products to help them select the best products for their patients with photo-sensitivities. Therefore, consumers should be able to start looking for a product's CW value as one factor to help them select the most appropriate product for any given occasion (11).

Diffey commented that the way forward might be either to introduce a new metric that more closely reflects the performance of modern sunscreens or, more simply, raise the bar by revising the threshold CW to 380 nm (10).

PROTECTION AGAINST SUN LIGHT INDUCED OXIDATIVE STRESS – A LOOK BEYOND SPF AND CW

The natural sunlight predominantly consists of ~55% VIS (400-800 nm) and ~40% IR (800-2,450 nm) radiation; while UV (290-400 nm) contribution is just ~ 6% (1). However, in the Final Rule the FDA concluded that "UV radiation in the range of 370 – 400 nm is not very harmful based on the available action spectra for sunburn and skin cancer; and most of the harmful effects from the sun are caused by UV radiation in the range of 290 – 370 nm" (2).

It should be noted that the FDA is referring to the UV wavelength range that is similar to the one simulated by the lamp used for SPF *in vivo* testing (Figure 3).

However, the FDA's focus on the narrow 290 – 370 nm region as the predominant cause of the harm associated with skin sun exposure is being contradicted by the ongoing research and relevant discoveries in the field; some of these findings are discussed in the literature overview presented below. According to Kamath and Ruetsch, skin photodamage can be caused by full spectrum solar radiation in the range of 290-800 nm and involves free-radical mechanisms; free radicals are generated by the interaction of radiation with the substrate and diffuse into subsurface region causing damage (12). Gracy, Talent, Kong *et al* stated that cytotoxic, radical and non-radical, reactive oxygen species (ROS) are generated by a variety of sources from the environment, e.g. photo oxidations and emissions (13). Rodgers and Snowden discovered that singlet oxygen is responsible for much of the physiological damage caused by ROS and its lifetime is sufficiently long to permit significant diffusion into and through cells and tissues (14). Svobodova, Walterova and Vostalova pointed out that UVA generates more oxidative stress than UVB (15). Hanson, Gratton and Bardeen demonstrated that 60 minutes after application, the sunscreen filters octocrylene, octinoxate and oxybenzone can enhance UV-induced ROS generation determined by fluorescence

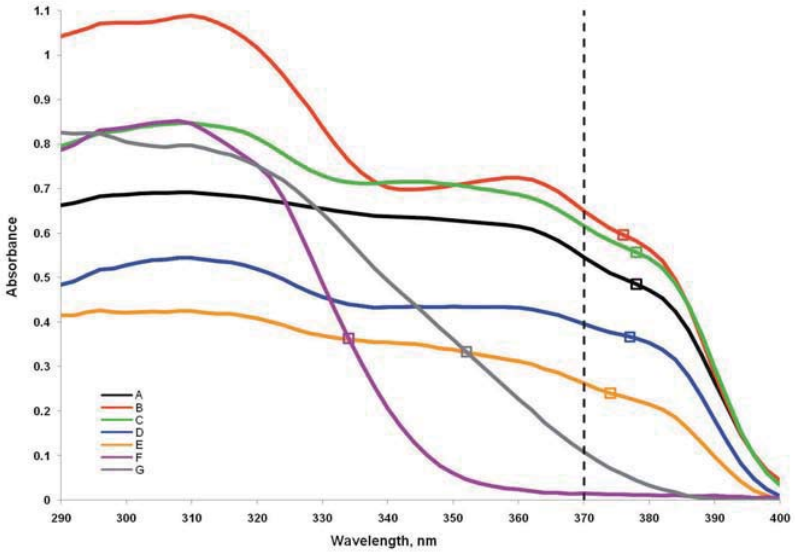


Figure 4. Absorbance Spectra and CW of Commercial SPF 15 Products

in epidermal skin model (15). Hanson and Simon showed that singlet oxygen is linked with the *in vivo* UVA action spectrum, which is considered responsible for skin photoaging (17). Berneburg, Grether-Beck, Kürten *et al.* identified a previously unrecognized biological function of singlet oxygen by demonstrating that oxidative stress is indeed responsible for the generation of large scale deletions of mitochondrial DNA in human cells that have been exposed to UVA radiation, which induces tissue aging under normally occurring conditions. These findings suggest that it is possible that the generation of singlet oxygen in human skin is of central importance for photoaging and singlet oxygen quenching may thus represent an effective strategy to protect human skin from sun induced photoaging (18).

Zastrow, Groth, Klein *et al.* discovered that the free radical formation is occurring in epidermis and dermis at all UV, VIS and near-IR wavelengths over all sun spectrum (19, 20); authors mentioned that VIS and IR parts of the sun spectrum have received little attention concerning their possible contribution to skin damage; however, the convolution of the action spectrum with sunlight spectral irradiance showed that 50% of the total skin oxidative burden was generated by VIS light. The formation of excess free radicals by near-IR radiation was also evidenced; in that case, free radical generation does not depend exclusively on the dose, but also on the skin temperature increase initiated by near-IR light (20). Zastrow, Groth, Klein *et al.* also emphasized that sunscreens should be designed with antioxidants or radical scavengers in order to ensure sufficient radical protection (19). Dueva-Koganov and SaNogueira demonstrated that the incorporation of very small amounts of carotenoids, for example only 0.011 wt. % of lutein (a known antioxidant) to the sunscreen formulation resulted in a SPF enhancement (boost) as compared to the formulation with equal amounts of sunscreen actives and no carotenoid (21). According to Baptista, the amount of light necessary to maintain normal functionality of the dermis without harming the skin is dependent on the skin characteristics inherent to each individual. Therefore, besides protecting the skin from sun light by using sun-blocking agents, it is important to consider other strategies including processes that aim to facilitate maintenance of the redox balance (22). In addition, it was recently discovered by Dueva-Koganov, Koganov and Duev that higher diffuse reflectance of lighter skin color types increases the probability for more photons to be reflected, not absorbed and to participate in the ROS generation (23). Therefore, the evaluations of the antioxidant potency of topical products against sunlight induced ROS using relevant testing methodologies that mimic end-usage irradiation conditions and the photo damage processes in various skin types could further increase an understanding of the effects of full spectrum sunlight radiation on skin and help to develop more effective anti-aging sunscreen products (23, 24); and continuing research of the effects that sunlight radiation exerts on human skin and whether antioxidants could effectively mitigate sunlight induced ROS generation will foster the development of antioxidants and bioactive agents that can be used in combination with sunscreen filters to provide better photo protection (16, 25).

CONCLUSIONS

The SPF *in vivo* testing evaluates protection of sunscreen products against skin erythema induced by the artificial light source that accurately simulates the UV region from 290 to approximately 370 nm that represents a small portion of natural sunlight spectrum. The FDA requirements of CW *in vitro* testing and 370 nm criterion for broad-spectrum protection claim extend the measurement of sunscreen effectiveness against UVA portion of sunlight. However, this criterion provides no motivation for manufacturers to develop products with higher CW values because the 370 nm threshold is already achieved by majority of sunscreen products marketed in the USA. There is an ongoing debate in the industry whether current CW threshold might be revised. Recent findings in the field indicate that ROS are the significant damaging factors in sun exposure, and action spectrum for their production extends into VIS and IR range. The continuing research whether antioxidants could effectively mitigate sunlight induced ROS generation will increase an understanding of the effects of full spectrum sunlight radiation on skin. The utilization of relevant testing methodologies that mimic end-usage irradiation conditions and the photo damage processes in various skin types could help to develop better sunscreen products.

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