A new in-vitro method for determination of Sun Protection Factor

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Synopsis

A new in-vitro SPF test method for determining the efficacy of sun care products was proposed and evaluated. The test method is based on UV (Ultra Violet) dosage cumulate response using Gafchromic EBT3 film. In-vitro SPF value of 2 sunscreen standards and 15 commercial products, including sunscreen lotions, BB creams, foundations and sprays with organic and/or inorganic UV filters, were measured with both new and current methods. The new method is more accurate, cost effective, reproducible, and does not employ UV spectrophotometer compared to the current test methods.

INTRODUCTION

Evaluation of the efficiency of sun care products has for a long time been assessed through the in-vivo sun protection factor (SPF) test (1). Minimal erythema dose (MED) is defined as the minimum dosage or time of radiation that produces skin erythema, while SPF is the dosage or time of UV radiation required to cause MED (sunburn/erythema) on skin with the sunscreen on, as a multiple of the dosage or time required without sunscreen (Equation 1). For example, if unprotected skin has a dosage to erythema (40J) and protected skin has dosage to erythema (640J), then SPF = 640/40 = 16. SPF equal to 1 means no UV protection.

$$SPF = \frac{Dosage_{MED}\ with\ Sunscreen}{Dosage_{MED}\ without\ Sunscreen} \qquad \text{Equation 1}$$

Current 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 (2). The transmittance of the sunscreen is the ratio of spectral transmittance through 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:

$$SPF = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda)*I(\lambda)*d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda)*I(\lambda)*10^{-A_0(\lambda)}*d\lambda} \qquad \text{Equation 2}$$

Where:

E(λ) = Erythema action spectrum (CIE-1987)

I(λ) = Spectral irradiance of the UV source (SSR or SPF testing)

A0(λ) = Mean monochromatic absorbance measurements per plate of the test product layer before UV exposure;

dλ = Wavelength step (1nm)

The mechanism of in-vivo SPF test is based on biological endpoint, skin erythema / sunburn, which is a UV dosage cumulate response. Although this method is similar to real sunscreen applications, it is expensive, time consuming and affected by skin types. It is also a subjective method depending on human observation of skin erythema. On the other hand, in-vitro SPF test is objective, cheap and fast method, with no human involved, but it is based on UV spectral transmission measurements, and has no UV dosage cumulating response. Many parameters will affect the results, including substrate materials, roughness, amount of sunscreen applied, and sunscreen photo-stability etc (3, 4). It is one of the reason that in-vivo SPF test is still required by all countries and regions for commercial sunscreens SPF claim.

In this article, a new in-vitro SPF test based on UV dosage cumulate response was proposed by using Gafchromic EBT3 film. EBT3 film has been developed to address the needs of the medical physicist and dosimetrist working in the radiotherapy environment (5). It is sensitive to UV, but not much to visible light, and the color of EBT3 film changed from green to dark under cumulative UV irradiation (6). Effective atomic number (Zeff) of film is 6.8, which is close to human skin (Zeff=7.4), where Zeff means the nature of radiation interaction with the medium.

EXPERIMENTAL

MATERIALS

15 different commercial sunscreen lotions, BB creams, foundations and sprays with organic and/or inorganic UV filters were purchased in the market. Two sunscreen standards were purchased from Cosmetech laboratories Inc., US were used in the in-vitro SPF test. Q-SUN test chamber (Q-lab, US), irradiation intensity 41w/m^2, temperature 40^{o}C, was used to irradiate Gafchromic EBT3 film (Ashland, US). The color change of the film was scanned by scanner (Epson 4990), and analyzed by FilmQA pro software (Ashland, US) to establish the calibration curve.

METHODS

EBT3 film was cut to rectangle shape (5.1cm*6.3cm), irradiated by Q-sun chamber with different time, and scanned by Epson 4990. Then the calibration curve can be obtained by the four points with FilmQA pro which is the function of irradiation energy and RGB color respectively. The average relationship between irradiation dose and response by scanner can be established by the following:

$$Film\ response = \frac{(a + bD)}{(D + c)} \qquad \text{Equation 3}$$

Where: D is UV dosage and a, b and c are constants

2.0 mg/cm^2 sunscreen was applied on skin in in-vivo SPF test. To consistent with that, 64.3 mg sample (2.0 mg/cm^2) was applied on EBT3 film, and then evenly distributed with gloved finger, from top to bottom and side-to-side with minimal pressure, avoid spotting on the edges. After 30 minutes UV irradiation, the irradiation dosage of EBT3 films with and without application of sunscreens was calculated by the calibration curve. The in-vitro SPF value was calculated by the following:

$$SPF = \frac{Dosage_{RECEIVED}\ without\ Sunscreen}{Dosage_{RECEIVED}\ with\ Sunscreen} \qquad \text{Equation 4}$$

The in-vitro SPF of samples was also tested by UV spectrophotometer (Varian, Cary 300) with Labsphere 1000 (UV DRA-CA 301) attachment. Both PMMA plate (Schönberg, 50mm*50mm, 2μm roughness) and 3M transpore tape were used as substrate materials. 18.8 mg sample (0.75 mg/cm^2) was applied with above method on the plate. PMMA plate with homogeneous layer of 15 mg glycerin was used as a reference. Minimal 3 plates were used for each sunscreen sample.

RESULTS AND DISCUSSION

EBT3 film is comprised of an active layer, nominally 27µm thick, containing the radiation-sensitive active component, marker dye, stabilizers and other additives. The active layer is between two, 120µm transparent polyester substrates. After exposed to UV light, the color of active layer changes from green to dark. The color change (RGB) will have defined relationship with cumulative UV irradiation dosage as described in Equation 3. The calibration curves of EBT3 films after 0, 10, 20, 30 minutes UV irradiation were shown in Fig. 1. All color parameters, R (Red), G (Green), B (Blue), could be used to calculate, but R (Red) parameter was chosen due to the highest sensitivity in our data range.

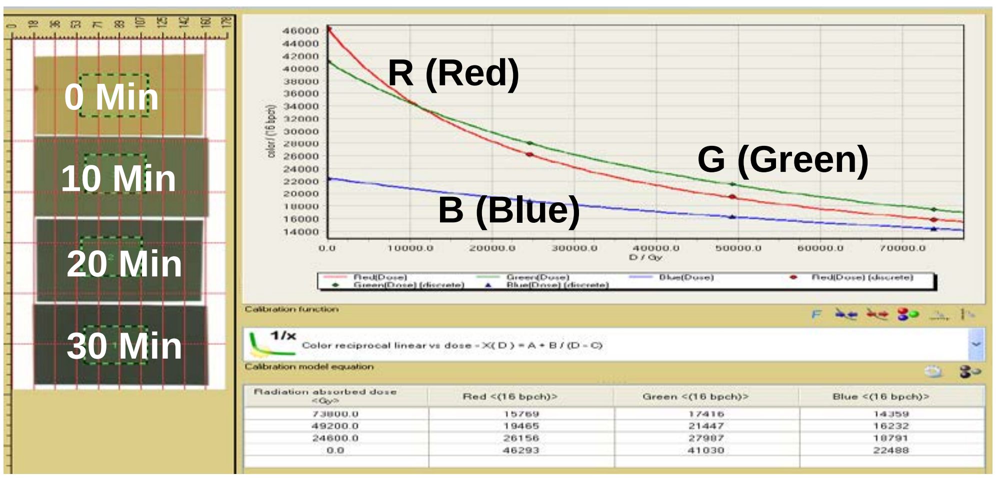

Figure 1. Calibration curve to set up the function between irradiation dosage and response

As an example for new in-vitro SPF test, a commercial sunscreen lotion (SPF30, PA++) was applied on EBT3 film. Both films with and without sunscreen were irradiated by UV for 30 minutes. The absorbed dosage of film without sunscreen is 73,800Gy, while the average calculated absorbed dosage of film with sunscreen is 2,477Gy. According to equation 4, the calculated SPF of sunscreen lotion is 29.8±3.2. The SPF result is very close to the labeled SPF value of 30, while SPF 30 – 40 will be labeled as SPF30 on the sunscreen bottle. When compare to the in-vivo SPF test (Equation 1), the new in-vitro SPF test (Equation 4) is also based on UV dosage cumulate response, and take sunscreen photo stability in the consideration.

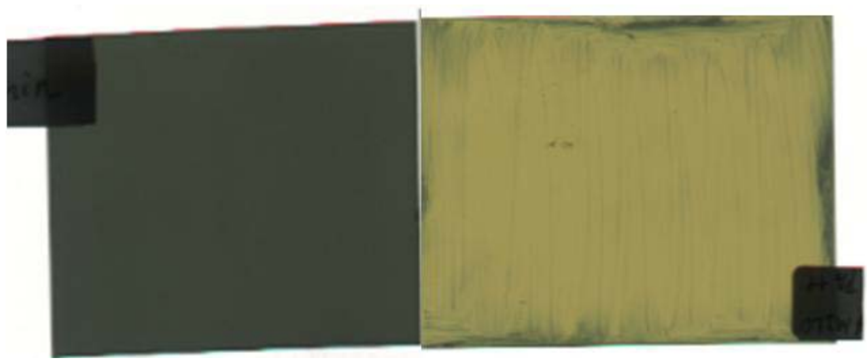

Figure 2. Comparison of EBT3 film with (Right) and without (Left) commercial sunscreen lotion (SPF30, PA++) after 30min UV irritation

To validate the new in-vitro SPF test, 15 commercial sunscreen products and 2 sunscreen standards were purchased in the market. The detailed UV filters in the products, and labeled SPF value on the bottle are shown in Table 1. The products include sunscreen lotions, BB creams, foundations and sprays with organic and/or inorganic UV filters. The SPF values labeled on the bottles vary from 15 to 50+ and no PA to PA++++. The in-vitro SPF value measured by UV spectrophotometer with 3M tape and PMMA plate was compared with the results by EBT3 film (Table 1).

Table 1. SPF test results comparison for commercial sunscreen products (1-8 products - organic UV filters only; 9-15 products - organic and inorganic UV filters)

Commercial Sample	Labeled SPF (In-vivo)	In-vitro SPF			UV Filters
		EBT-3 film	3M tape	PMMA plate	
1	FDA:14.0, ISO:16.2	3.2	17.1	13.2	BP-3, EHDP
2	Colipa:16	16.6	35.2	37.6	EHMC, OCR BMDM, BEMT
3	SPF15, PA++	14.6	41.2	15.0	EHMC
4	SPF30, PA+++	34.5	37.1	47.2	EHMC,MBBT,BEMT
5	SPF20, PA++	19.2	18.1	23.9	EHS,HMS,OCR,BMDM, Ensulizole, PBSA
6	SPF15, PA++	7.3	25.6	12.5	EHMC,BMDM,PBSA
7	SPF25, PA++	22.5	26.8	20.1	EHMC,OCR,EHT,DHHB,MBBT, BEMT
8	SPF50	51.7	44.7	46.6	BMDM, HMS, EHS, OCR, EHMC
9	SPF30, PA++	29.8	26.8	30.1	ZnO, EHMC, OCR, DHHB
10	SPF30+, PA+++	18.1	24.2	36.6	TiO2, EHMC, OCR, PBSA
11	SPF50+, PA++++	49.9	25.3	22.5	ZnO, EHMC, DHHB, OCR
12	SPF15, PA++	3.0	17.5	6.5	EHMC, BMDM, TiO2, MBC
13	SPF30, PA+++	31.5	4.2	12.7	HMS, PBSA, OCR, MBC, TiO2, BMDM, Neo Heliopan AP
14	SPF30+, PA++	34.2	30.7	33.2	EHMC, OCR, DHHB, TiO2, MBBT, PBSA
15	SPF50+, PA+++	61.8	34.9	36.2	EHMC, OCR, ZnO, TiO2
16	SPF30, PA++	30.5	8.8	8.9	ZnO
17	SPF30+	19.7	10.9	26.6	ZnO, TiO_2

For commercial products with organic UV filters only (Sample 1 – 8), the correlation of in-vitro results and labeled SPF value was illustrated in Fig.3. The trend line of EBT3 film data has R^2 of 0.9266, which means EBT3 film method is more accurate than 3M tape

(R^2=0.3174) and PMMA plate (R^2=0.5451) methods. In the best situation, the interception of the equation should be close to 0. The EBT3 film method could be improved by optimizing the test parameters, such as sunscreen amount, irradiation time, as well as the EBT3 film in the future, as long as the reproducibility is good.

Similar to in-vivo SPF test, EBT3 film method also considered the photo stability of sunscreens. If the sunscreen is degraded under the UV light, the SPF value measured will be lower than that measured by UV spectrophotometer.

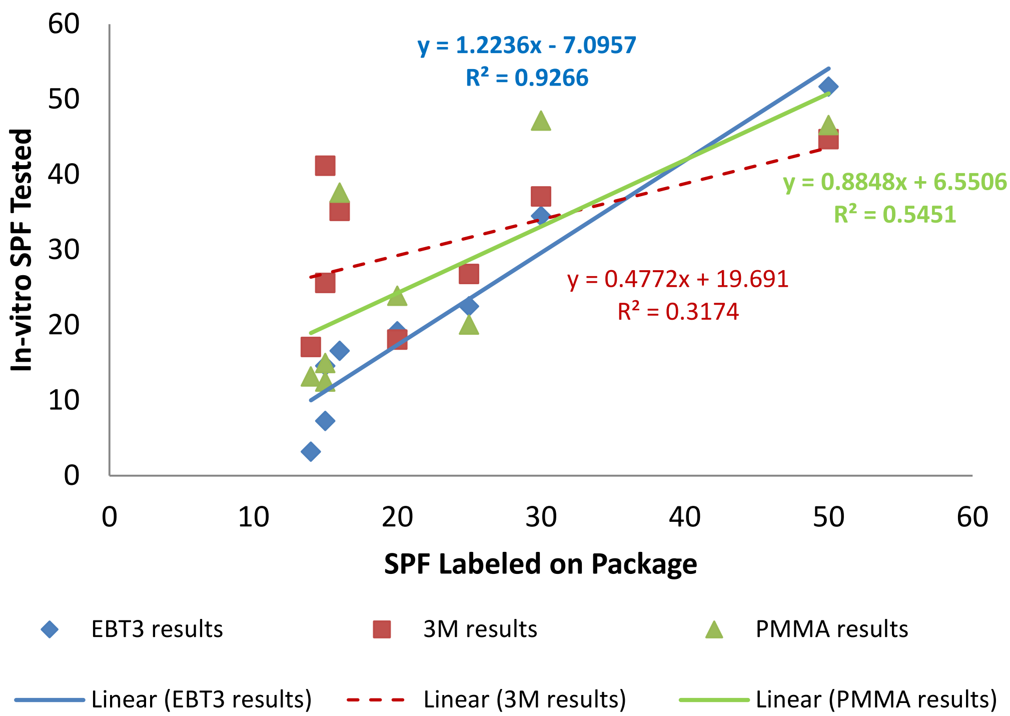

Figure 3. Correlation of in-vitro results and labeled SPF for products only with organic UV filters

Sunscreen products with inorganic UV filters are always difficult to measure accurately by in-vitro SPF test based on UV spectrophotometer. For commercial products with both organic and inorganic UV filters (Sample 9 – 15), the correlation of in-vitro results and labeled SPF value was illustrated in Fig.4. EBT3 film (R^2=0.8552) method is also more accurate than UV spectrophotometer method with 3M tape (R^2=0.2905) and PMMA plate (R^2=0.3152) methods.

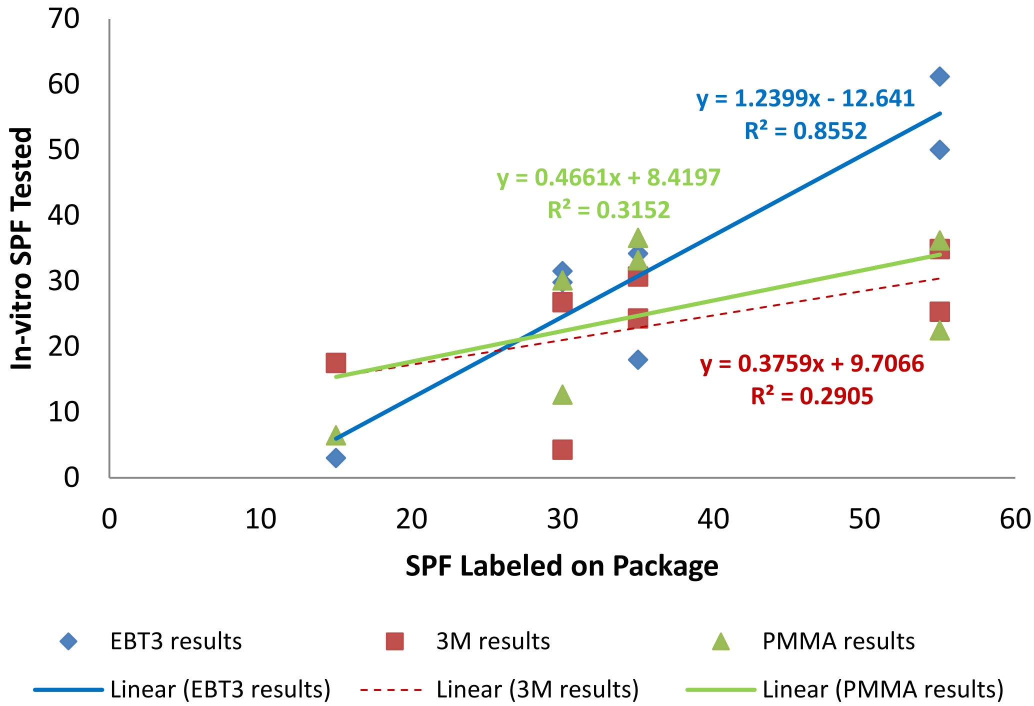

Figure 4. Correlation of in-vitro results and labeled SPF for products with both organic and inorganic UV filters

In-vitro SPF test based on UV spectrophotometer uses single UV beam to measure the spectral transmittance of a sunscreen on the substrate. Thus, sunscreens with high viscosity or inorganic UV filters are hard to measure SPF accurately due to the difficulty for homogeneous spreading. Instead of one spot, EBT3 film method uses an area to measure the SPF value, so the reproducibility of data will be significantly increased.

All products tested in this study were at least triplicate, and the results for triplicate tests were pretty similar (data not showed). We choose the following three products to run 7 parallel tests to show the reproducibility of EBT3 film method (Table 2). The coefficient of variation (COV) varies from 7.14% to 10.75%. For the in-vivo SPF test to be considered valid, the 95% confidence interval (CI) must fit within (COV) of 17% and, if not, the product must be tested on further subjects (up to a maximum of 20) until the 95% CI based on the data for all subjects does fit within COV of 17%. In-vitro SPF test based on EBT3 film has almost half of COV as compare to in-vivo SPF test, which indicates this new method has really good reproducibility.

Table 2. Reproducibility of in-vitro SPF test based on EBT3 film

In-vitro SPF Test (EBT3 Film)	Sunscreen 1 (SPF30, PA++)	Sunscreen 2 (SPF15)	Sunscreen 3 (SPF30, PA++)
Duplicate 1	32.95	17.26	33.90
Duplicate 2	35.06	13.91	28.26
Duplicate 3	29.59	14.36	28.51
Duplicate 4	28.00	13.19	28.92
Duplicate 5	26.81	13.83	32.11
Duplicate 6	29.59	14.72	32.12
Duplicate 7	26.41	15.14	29.85
SPF (Mean±Sd)	29.77±3.20	14.63±1.32	30.52±2.18
COV	10.75%	9.02%	7.14%

CONCLUSIONS

A new in-vitro SPF test based on UV dosage cumulate response was proposed by using Gafchromic EBT3 film. Based on the in-vitro SPF results tested from 17 commercial products, EBT3 film method is more accurate than UV spectrophotometer method with 3M tape and PMMA plate as substrates. EBT3 film method has almost half of COV as compare to in-vivo SPF test, which indicates this new method has really good reproducibility. This new method has potential to improve by optimizing the test parameters, such as sunscreen amount, irradiation time, as well as the EBT3 film in the future.

ACKNOWLEDGEMENTS

The authors would like to thank Shelley Shih (Ashland Inc.) and Xiang Yu (Ashland Inc.) for providing Gafchromic film, as well as valuable discussions.

REFERENCES

1. M. Pissavini and L. Ferrero, In vitro determination of sun protection factor, *Business Briefing: Global Cosmetics Manufacturing Reference Section,* 1-5 (2004)
2. D. Lutz and N. Cariou, Accurate, fast and quite easy way of sunscreen testing, *Focus on Sun Care – Supplement to Household and personal Care Today,* 3, 25-29 (2011)
3. L. Ferrero, M. Pissavini, A. Dehais, S. Marguerie, L. Zastrow, Importance of substrate roughness for in vitro sun protection assessment, *IFSCC Magazine,* 9(2), 97-108 (2006)
4. O. Dueva-Koganov, N. Shaath, Comparative evaluation of commercial SPF 100+ sunscreen products, *Happi,* 48-53 Dec. (2010)

5. V. Borca, M. Pasquino, G. Russo, P. Grosso etc., Dosimetric characterization and use of Gafchromic EBT3 film for IMRT dose verification, *J App Clinical Med Phys,* 14(2), 158-170 (2013)
6. E. Butson, T. Cheung, P. Yu, M. Buston, Measuring solar UV radiation with EBT radiochromic film, *Phys in Med and Biol,* 55, 487-493 (2010)