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EBT3 Gafchromic[®] film as a new substrate for *in vitro* determination of the sun protection factor

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There are no conflicts to declare.

ABSTRACT

OBJECTIVE: In this study, we aim to develop a new *in vitro* method for the determination of the Sun Protection Factor (SPF) value of a sunscreen using EBT3 Gafchromic® film as a substrate.

METHODS: The color of EBT3 Gafchromic® film changes when it is exposed to UV. Films were covered by sunscreen preparations of different SPF values ranging from zero to 50. Uncovered and covered films were exposed to different solar light energies and their color change was compared. Absorbance spectra of films was measured at 633nm using a UV-VIS spectrophotometer apparatus.

RESULTS: The color of EBT3 Gafchromic® films darkens when ultraviolet (UV) energy increases, which means that their absorbance increases with exposure time. However, when films are covered by sunscreens, their color change is less visible and their absorbance significantly decreases when the SPF value increases. **CONCLUSION:** There is a linear correlation between the absorbance of EBT3 Gafchromic® film and the SPF value of the sunscreen that is covering the film. Statistical method validation shows that we can predict the SPF value of a sunscreen using EBT3 Gafchromic® film as a substrate. It is the first time that an *in vitro* method is developed based on the color change of a substrate and that takes into consideration the exposure time which make it closer to real life simulation. The film tanning properties and its reaction to the sunscreen coverage makes it close to the skin sun-exposure reaction. All these parameters make our method a reliable *in vitro* method for the SPF testing.

KEY WORDS: Sunburn protection factor, Ultraviolet radiation, Gafchromic® EBT3, Film dosimetry, Skin protection.

1. INTRODUCTION

Solar spectrum is a mixture of ultraviolet (UV), visible and infra-red (IR) radiations ¹. The spectrum of the ultraviolet light (100 – 400nm) is divided into three types: UVA (315 – 400nm), UVB (280 – 315nm) and UVC (100 – 280nm) ². UVC and a part of UVB radiations are blocked by ozone layer ³. Only UVA and around 5% of UVB reach the earth ⁴. Prolonged and frequent solar exposure can lead to skin ageing, solar elastosis and in worst cases, skin cancer ^{5,6}. In fact, UVA radiations are mainly responsible of skin photo damage causing wrinkles and lack in firmness and elasticity ⁷, while UVB radiations cause a de-organization of the epidermis and are known to be carcinogenic ^{1,7}. For all these reasons, one should always be protected from sunlight by wearing sunscreen. A sunscreen is a UV filter containing product that protects from the harmful effects of UV radiations. The application of a sunscreen prevents skin burns, decreases the risks of skin cancer and decreases the signs of aging ^{8,9}.

The efficiency of a sunscreen is indicated by the sun protection factor (SPF) value. According to the regulations of the European Commission and to the ISO norm 24444:2010, the SPF should be determined by *in vivo* testing. Volunteers are exposed to a Xenon arc lamp under different energies and the SPF is “a ratio calculated from the energies required to induce a minimum erythema response with and without sun product applied to the skin of human volunteers, using ultraviolet radiation usually from an artificial source” ^{10,11}. Prior to the *in vivo* testing, preliminary *in vitro* SPF calculations are usually realized by spectrophotometric measurements, which are easier, faster and cheaper to conduct. Many methods have been developed and currently, the most common *in vitro* method requires the use of PMMA plates. PMMA plates do not absorb in the UV range. Sunscreen is applied on the plate at 2 mg cm⁻² and the SPF is based on the erythema action spectrum, the spectral irradiance received from the UV source and on the measurement of the spectral transmittance of the sunscreen between 290 and 400nm ¹²⁻¹⁶. However it is still not an official method.

Although *in vivo* testing is the international recommended method for the evaluation of the SPF and the closest process to real life simulation, it remains a method that requires 10 to 20 human volunteers exposed to UV rays until they develop skin erythema ¹¹.

In this study, we develop a new method to determine the SPF value of a sunscreen using EBT3 Gafchromic® film as a substrate. It is a film that consists of an active layer of 28 µm of thickness coated on both sides by a protective polyester layer of 100 µm ¹⁷. The active layer is a patented mixture of active component, marker dye, stabilizers and other components ¹⁸. The color of a virgin

film is light green and becomes darker when exposed to several types of radiations such as X-rays, electron beams¹⁷ and UV light¹⁹.

Gafchromic® films have different applications in several fields, mainly in the dosimetry of radiotherapy treatments²⁰⁻²². In addition, they are used in the measurement of solar ultraviolet dose. In order to assess the risks of hazardous solar exposure, to evaluate the variation of UV doses in shaded areas or to assess UV-excessive or dangerous geographical zones, UV-dosimetry is indispensable and was widely measured using Gafchromic® films^{19,23-26}.

In this study, we were able to establish for the first time an *in vitro* method for the evaluation of the SPF value of a sunscreen using EBT3 Gafchromic® films as a substrate. This new approach is based on the color change of the substrate and not on the spectral transmittance of the product itself which presents the advantage to be experimentally close to real life simulation. Another main advantage is that our new method takes automatically into consideration the photo-instability of the UV filters during UV-exposure which has a very important influence and is similar to real-life simulation.

Our results pave the way to a promising and reliable method that could be conducted prior to *in vivo* testing.

2. MATERIALS AND METHODS

2.1 Preparation of sunscreens with different SPF values

Eight formulations of gel sunscreen standards are prepared. The SPF of each formulation was determined by *in silico* calculation according to BASF sunscreen simulator²⁷.

Gel sunscreens were prepared as per tables 1 and 2.

Table 1 - The formulations of the gel sunscreens.

	INCI	Percentage (w/w) in formulation
Phase A	Paraffinum Liquidum	q.s. 100
	UV Filters	(Ref. Table 2)
Phase B	Silica	5.9%
	Silica Sylilate	2.7%

Table 2 - The percentages of UV filters in the gel sunscreen formulations. EHMC: Ethylhexyl Methoxycinnamate, EHT: Ethylhexyl Triazone, OCR: Octocrylene, BEMT: Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine.

UV FILTER	Zero Protection	SPF 6.1	SPF 10.3	SPF 15.3	SPF 20.1	SPF 25.3	SPF 30.1	SPF 41.4	SPF 50.3
EHMC	0.0%	3.5%	7.5%	9.0%	10.0%	10.0%	10.0%	10.0%	10.0%
EHT	0.0%	0.0%	0.0%	3.5%	5.0%	5.0%	5.0%	5.0%	5.0%
OCR	0.0%	0.0%	0.0%	0.0%	2.1%	6.0%	10.0%	10.0%	10.0%
BEMT	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.0%	2.3%

Procedure: Ingredients of phase A were mixed together. Ingredients of phase B were dispersed one after the other in phase A while mixing and stirred until homogeneous gel is formed. In case the formulation contains Ethylhexyl Triazone, phase A was heated until totally melt then procedure was continued normally. Paraffinum liquidum was supplied from Mosselman (Ghlin, Belgium), silica and silica silylate from Evonik (Hanau-Wolfgang, Germany) and UV filters from BASF (Grenzach, Germany).

2.2 SPF verification by UV-spectrophotometric measurement

In 1986, Mansur et al. determined a UV-spectrophotometric method for the calculation of the SPF value²⁸. We applied the method in order to verify the SPF values of our sunscreens.

0.1g of each formulation is weighed in a 10mL volumetric flask and diluted to volume with ethanol absolute. Solution is then ultra-sonicated for 5 minutes and filtered rejecting the first 2mL. 200µL aliquot is then transferred to a 10mL volumetric flask and completed to volume with ethanol absolute. The absorbance spectra of the above solution are measured between 290 and 320nm, every 5nm wavelength interval with ethanol absolute as blank. Three measurements for each are repeated and the SPF is calculated according to equation 1 determined by Mansur et al.²⁸.

Equation 1 - Mansur et al. equation of the SPF calculation by UV spectrophotometry

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where: EE (λ) is the erythemal effect spectrum; I (λ) is the solar intensity spectrum; Abs (λ) is the absorbance of sunscreen product and CF is the correction factor (= 10).

$EE(\lambda) \times I(\lambda)$ values are constants determined by Sayre et al.²⁹ and are presented in table 3:

Table 3 - Normalized $EE(\lambda) \times I(\lambda)$ values used in the calculation of the SPF

$EE(\lambda) \times I(\lambda)$ (normalized)	0.015	0.0817	0.2874	0.3278	0.1864	0.0839	0.018
Wavelength (nm)	290	295	300	305	310	315	320

2.3 Preparation of EBT3 Gafchromic® films

The EBT3 Gafchromic® radiochromic film (lot number 05161703, Ashland Specialty Ingredients, NJ, USA) is available in sheets of 20.32 x 25.4 cm². Samples were cut into 5 × 4 cm² rectangles before exposure. This size is regular and common in the literature and is wide enough to avoid uncertainties during exposure and reading¹⁷. Films were handled with latex gloved hands in order to avoid stains and fingermarks that can affect reading results. Whenever not in use, un-exposed and exposed films were stored all together in envelopes in the dark to avoid thermal differences.

2.4 Exposure of EBT3 Gafchromic® films to full spectrum sunlight and determination of the absorbance

Films were displayed in a Q-SUN® Xenon test chamber (model: Q-Sun® Xe-1 from Q-Lab corporation, Saarbrücken, Germany) and exposed to a full spectrum sunlight (Xenon arc lamp with day light Q filter). An integrated irradiance of 765 W m⁻² between 300 nm and 800 nm throughout the test, exposure energies ranging from zero to 1.84E+06 J m⁻² and a constant temperature of 22°C were applied. After exposure, films were wiped and their absorbance was read with a spectrophotometer apparatus (Multiskan GO from thermo fisher scientific corporation, Vantaa, Finland) at λ_{\max} 633 nm²³. Readings were conducted at least 24h hours after exposure in order to ensure the color stabilization of the films [11]. To account for repeatability, every experiment was repeated three times and absorbance measurements of each film were performed at twelve different spots of the film. To limit variability due to the distribution of sunscreens on the films, mean absorbance values were presented.

As previously mentioned, EBT3 Gafchromic® films have the distinctive feature to darken when they are exposed to UV radiations thanks to their active layer. The more they are exposed, the more they darken. When the color of the film is dark, it means its absorbance is higher [13]. Indeed, Figure 1

shows the color change of Gafchromic® EBT3 films when they are exposed to different solar light energies. This series of films was designated as the control in the rest of our study.

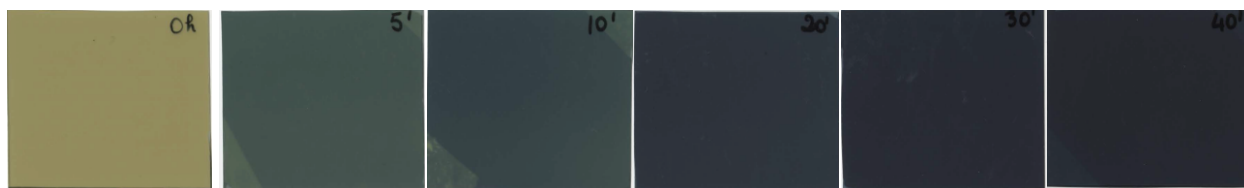


Figure 1 - From left to right: the darkening effect of EBT3 Gafchromic® films when exposed to solar light energies of 0, 2.3E+05, 4.59E+05, 9.18E+05, 1.38E+06 and 1.84E+06 J m⁻².

As a summary, films were exposed to a solar simulator (300 nm – 800 nm). Twenty four hours after exposure, the color of the films was assessed by their absorbance in the visible spectrum at λ_{\max} 633 nm²³.

The same experiment was repeated with films covered by the sunscreens prepared according to Table 1 and Table 2. Sunscreen standards were applied on the surface of the EBT3 films at 2 mg cm⁻² ± 2.5% - as recommended by the European Commission for *in vivo* testing¹⁰. Product was distributed evenly on film by movements from right to left and vice versa with gloved finger, same procedure of *in vivo* testing where the finger cot is used to spread the product on skin¹⁰. The homogeneity of the product's distribution was checked using an elcometer.

2.5 Statistical analysis for method validation

The analysis of variance (ANOVA) was performed to compare differences between the mean values of absorbance of EBT3 Gafchromic® films covered with sunscreens. 1% and 5% levels of significance were used, and results are plotted as Mean ± Standard Deviation³⁰.

To assess the validity of the use of EBT3 Gafchromic® films for SPF measurement, we estimated the repeatability (standard deviation (SD) and the relative standard deviation (RSD%)), the accuracy (relative error of the estimated absorbance compared to the true value) and the linear relationship between theoretical SPF values and measured absorbance on EBT3 films (F-test of dependency and R²)³¹.

Root mean square relative error of calibration (RMSREC) was calculated to evaluate the relevancy of calibration model (linear correlation between SPF values and absorbance)^{32,33}. RMSREC is given by Equation 2.

Equation 2 – Equation of the root mean square relative error of calibration. y is the measured absorbance, y_{estim} is the estimated absorbance obtained from the linear model and n is the number of different SPF values.

$$RMSREC = \sqrt{\frac{\sum \left(\frac{y - y_{estim}}{y} \right)^2}{n}}$$

To validate the robustness of the proposed method, leave-one out cross validation (LOOCV) was used. For each iteration, one SPF value was excluded from the calibration set and was used for the validation. Root mean square relative error of prediction (RMSREP) was calculated to estimate the relevancy of SPF predictive model and is given by Equation 3.

Linear relationship between the predicted and theoretical SPF values was then evaluated^{32,33}.

Equation 3 - Equation of the root mean square relative error of prediction. SPF is the theoretical SPF value, $SPF_{predict}$ is the predicted SPF value and n is the number of different SPF values.

$$RMSREP = \sqrt{\frac{\sum \left(\frac{SPF - SPF_{predict}}{SPF} \right)^2}{n}}$$

3. RESULTS & DISCUSSION

As it has been previously mentioned and shown in Figure 1, the higher the exposure energy is; the darker the EBT3 film becomes.

In the results below, we first show how EBT3 Gafchromic® films covered by sunscreens are responding when they are exposed to different solar energies. Their color change is linked to their absorbance. Afterward, we establish the correlation between the absorbance of the films and the SPF values of the sunscreens.

3.1 Effect of solar light exposure energy on EBT3 Gafchromic® films covered by sunscreens with various SPF

EBT3 films were covered by the sunscreens of various SPF values prepared according to Table 1 and Table 2 and were exposed to the solar light simulator with exposure energies ranging from zero to $1.84E+06 \text{ J m}^{-2}$. Figure 2 shows the variation of the absorbance of exposed EBT3 Gafchromic® films - covered by sunscreens of SPF values ranging from zero to 50 - as a function of exposure energy.

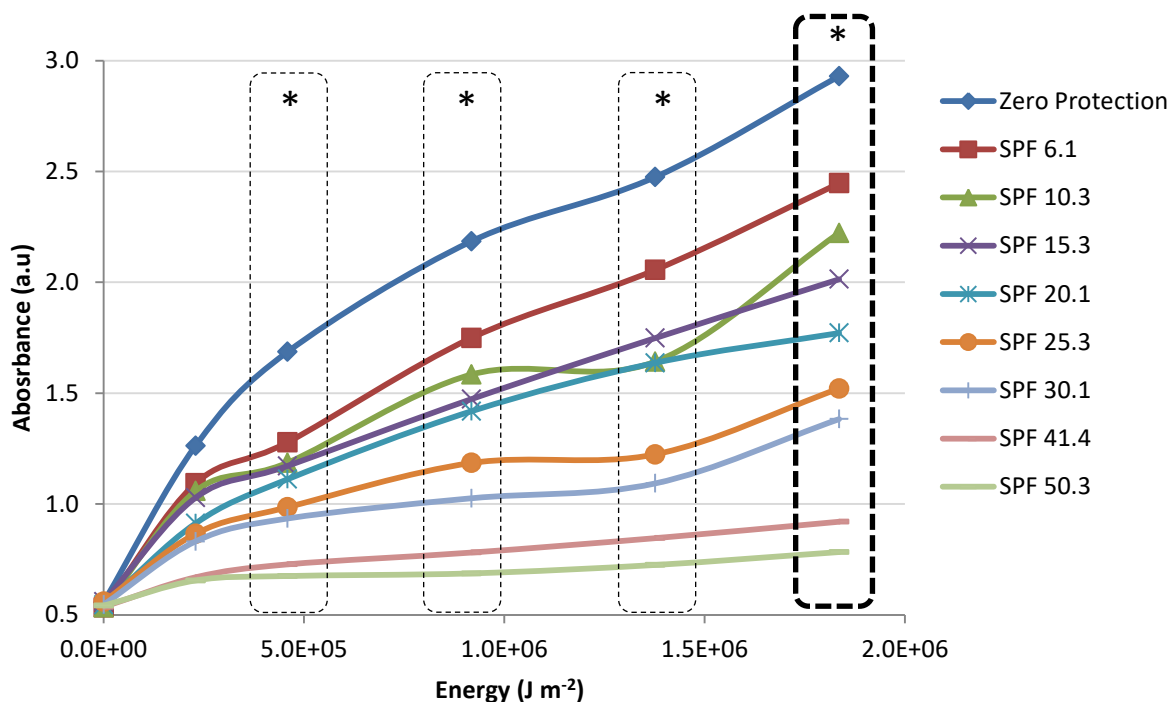


Figure 2 - Variation of the absorbance as a function of the solar light exposure energy. EBT3 films are covered by sunscreens of zero protection and SPF 6.1, 10.3, 15.3, 20.1, 25.3, 30.1, 41.4 and 50.3. Error bars that correspond to the standard deviations are smaller than the size of the symbols. Statistics: Mean \pm SD on 3 replicates - ANOVA single factor test: (*) p-value<0.001.

For a given SPF, the absorbance value significantly increases when exposure energy increases. This variation is consistent with results shown in Figure 1 and means that EBT3 film further darkens when exposure energy increases.

While comparing effects of various SPF values, Figure 2 shows that at a given exposure energy, the absorbance of EBT3 exposed films decreases when the SPF value increases. This means that the more the film is protected, the less it darkens. This effect is the most visible and clear at exposure energy of $1.84E+06 \text{ J m}^{-2}$ (highlighted in bold red dashed rectangle in Figure 2).

Figure 3 shows a scan of the films covered by sunscreens and exposed to energy of $1.84E+06 \text{ J m}^{-2}$.

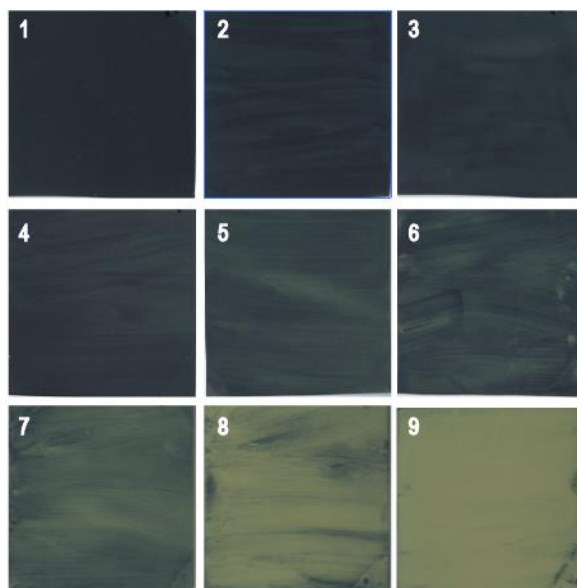


Figure 3 - From 1 to 9: scan of the films covered by sunscreens of zero protection, SPF 6.1, 10.3, 15.3, 20.1, 25.3, 30.1, 41.4 and 50.3 respectively and exposed to solar light simulator energy of $1.84\text{E}+06 \text{ J m}^{-2}$.

3.2 Method validation

In order to show the correlation between the absorbance values of EBT3 Gafchromic® films and the SPF values of the sunscreens, we fixed the exposure energy to $1.84\text{E}+06 \text{ J m}^{-2}$.

According to the European Commission recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto, section 2 related to UVA/UVB protection, claims, precautions for use and usage instructions, it is recommended to “re-apply sunscreen frequently to maintain protection, especially after perspiring, swimming or toweling [34]. And according to the U.S. Food and Drug Administration (FDA), it is recommended to renew the sunscreen application after a maximum of 2 hours exposure to sunlight [35]. Therefore, we have decided to choose our exposure energy in the Q-sun® chamber to $1.84\text{E}+06 \text{ J m}^{-2}$ which corresponds to an average of 1.5 to 2.5 hours in the real sun [36,37].

Results at exposure energy of $1.84\text{E}+06 \text{ J m}^{-2}$ are presented in Figure 4.

Figure 4 plots the absorbance of the films as a function of the *in silico* SPF values. Dependency F-test ($p\text{-value} < 0.001$) and R^2 (0.975) confirmed the linearity of the model. The linear regression function was computed (Equation 4) and was used to calculate the estimated absorbance values.

Repeatability was determined by calculating the standard deviation (SD) and the relative standard deviation (RSD%) for the measured absorbance values (Table 4).

Accuracy between the estimated and the measured absorbance was lower than $\pm 10\%$ for all SPF values. Only SPF 50 showed an accuracy value at 23%. Root mean square relative error of calibration (RMSREC) was calculated at 0.088 (8.8%) and validated the relevancy of calibration model.

To evaluate the quality of SPF prediction, the relevancy of the predictive model was evaluated using leave-one out cross validation (LOOCV). The accuracy between the theoretical and the predicted SPF values (shown in Table 4) was lower than $\pm 11\%$ for all SPF values. The Root mean square relative error of prediction (RMSREP) was calculated at 0.072 (7.2%) showing good relevancy of prediction.

Figure 5 shows the linear relationship between the theoretical and predicted SPF values with a slope equal to 0.996 and a R^2 equal to 0.942. These values and the low RMSREC and RMSREP validate the feasibility to deliver accurate SPF value using EBT3 Gafchromic® films.

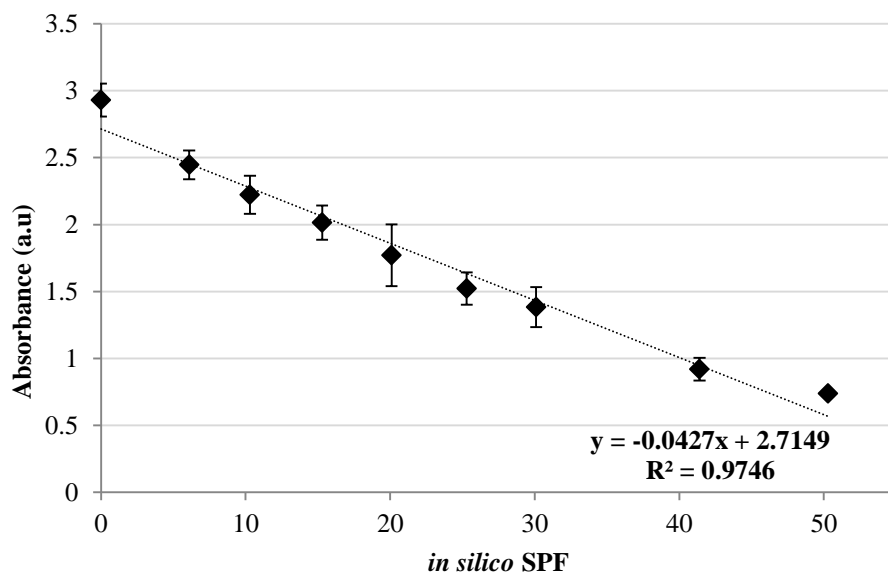


Figure 4 - Calibration set: absorbance of EBT3 Gafchromic® films as a function of the *in silico* SPF values of sunscreens. Films are exposed to solar light energy of $1.84E+06 \text{ J m}^{-2}$ and absorbance is read at 633 nm. Error bars correspond to the standard deviations.

Equation 4 - Equation of the linear regression function

$$y = -0,0427x + 2,7149$$

Table 4 - The mean, standard deviation (SD), relative standard deviation (RSD%), the estimated absorbance and the accuracy for SPF values ranging from 0 to 50.3.

Theoretical SPF values	Measured absorbance values			Estimated absorbance values	Accuracy%
	Mean	SD	RSD%		
0	2.93	0.12	4%	2.71	-7%
6.1	2.45	0.11	4%	2.45	0%
10.3	2.22	0.14	6%	2.28	2%
15.3	2.01	0.13	6%	2.06	2%
20.1	1.77	0.23	13%	1.86	5%
25.3	1.52	0.12	8%	1.64	8%
30.1	1.38	0.15	11%	1.43	3%
41.4	0.92	0.08	9%	0.95	3%
50.3	0.74	0.02	3%	0.57	-23%

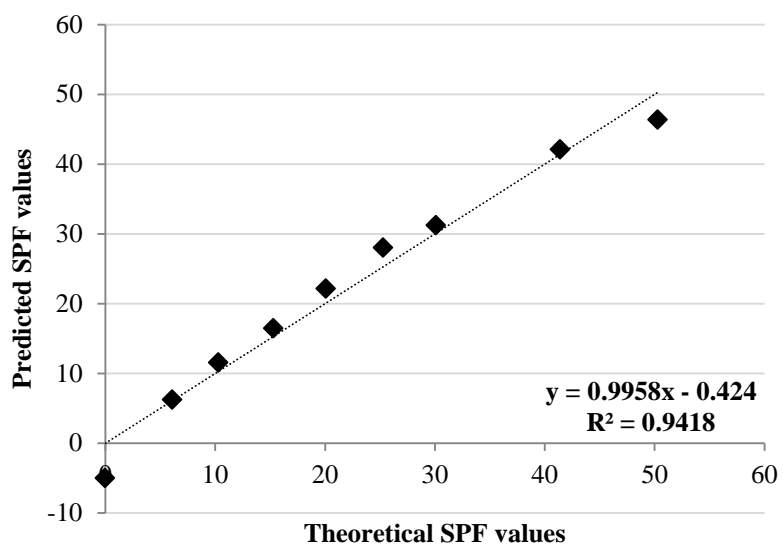


Figure 5 - Linearity between EBT3 predicted and *in silico* theoretical SPF values.

In silico simulation is a reliable, accurate and reproducible method that accounts for irregularity of the sunscreen film on the skin and for photo-instabilities of UV filters. The accuracy is based on model parameter adjustments on *in vivo* results [38].

Figure 5 shows that predicted SPF values by EBT3 method are significantly correlated to theoretical *in silico* values with regression coefficient R-squared (R^2) equal to 0.94 which makes our method an accurate one.

3.3 Correlation between *in silico* and *in vitro* SPF values

As a last step verification, we correlated the SPF values of the *in silico* method with the SPF values determined by *in vitro* (Mansur et al.) and *in vitro* (EBT3) methods (Figure 6).

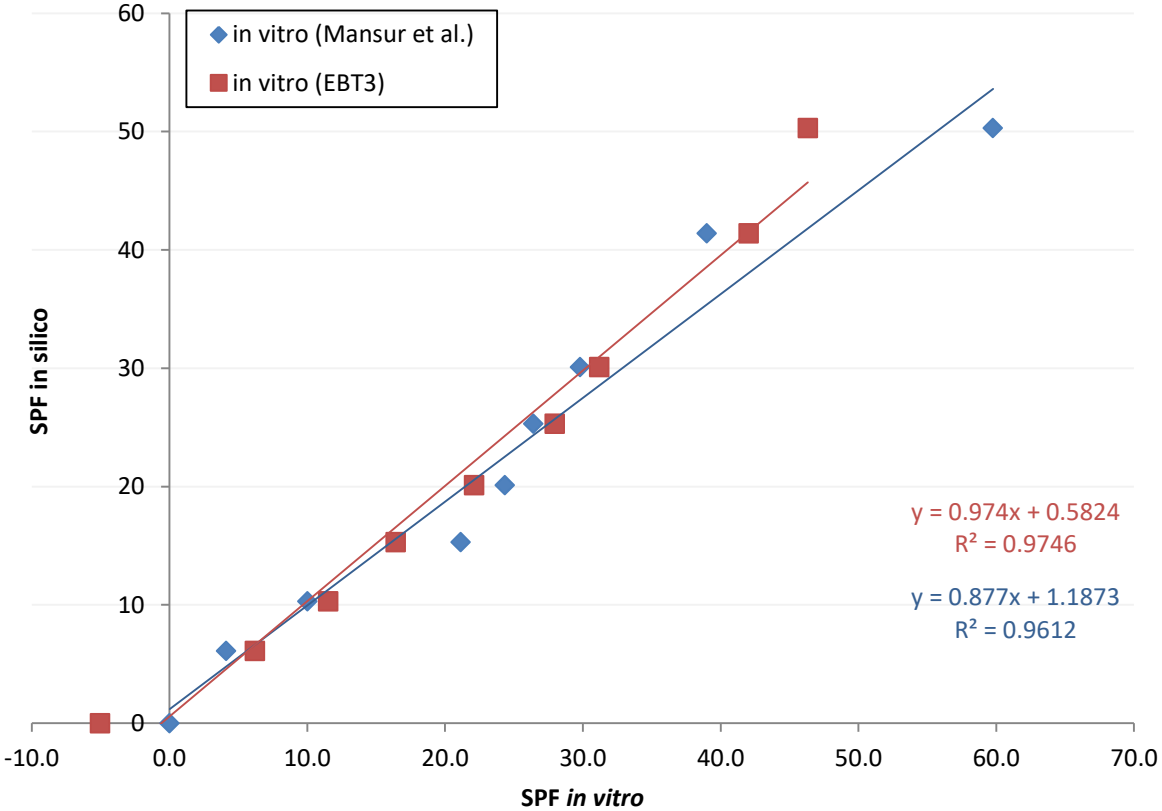


Figure 6 - Correlation between SPF *in silico* and SPF *in vitro* (Mansur et al.) and SPF *in vitro* (EBT3).

Figure 6 shows good correlation between the *in silico* and *in vitro* Mansur et al. & *in vitro* EBT3 methods with regression coefficient R-squared (R^2) equal to 0.96 and 0.97 respectively.

In our study, the color change of the films is represented by their absorbance. According to Figure 2 and Figure 3 and according to the statistical results, at fixed energy of $1.84E+06 \text{ J m}^{-2}$, the absorbance of EBT3 Gafchromic® films significantly depends on exposure energy. Furthermore, the absorbance of the films and the SPF values of the sunscreens that were covering the films during exposure are significantly dependent. The absorbance significantly decreases when the SPF value increases. Indeed, the higher the SPF value is, the more it protects the film and the less the film darkens.

On another note, we have demonstrated a linear relationship between the absorbance of the films and the SPF values of the sunscreens. LOOCV confirmed the feasibility of SPF estimation based on measuring the absorbance value of an EBT3 Gafchromic® film covered with a sunscreen with unknown SPF.

4. CONCLUSION

EBT3 Gafchromic® films darken when exposed to several types of radiations and more specifically to UV and sunlight. They are widely used for UV dosimetry and for the quality control in the medical field and are considered a reliable method in therapeutic dosimetry.

The behavior of EBT3 Gafchromic® film when exposed to solar light and the effect of a sunscreen application on it can be highly linked to the skin. In fact, there are many similarities between EBT3 Gafchromic® films and the human skin and their reaction to sun exposure. Same as for the EBT3 films, the color of the skin changes when exposed to solar light and darkens further with exposure time. This color change for both skin and EBT3 films is visible to the naked eye. In addition, when sunscreen is applied to skin, it protects it from tanning and the higher the SPF is, the more the skin is protected and the less it darkens. Same response is detected when applying sunscreen to EBT3 films and exposing them to solar light.

To the best of our knowledge, our method is the first *in vitro* method for the evaluation of the SPF using EBT3 Gafchromic® films as a substrate. It is as well the first *in vitro* method based on the color change of the substrate and not on the absorbance spectra of the sunscreen (which is the PMMA case). In addition, it is the first *in vitro* method to take into consideration the exposure time during SPF evaluation. The main advantage and the novelty of our method are its close similarity to real life simulation and to *in vivo* evaluation procedure: the product is applied at a density of 2 mg cm^{-2} , the substrate is exposed to solar light energy, the product is removed, the substrate is allowed to rest for 24 hours and the SPF is then evaluated according to the color change of the substrate.

The reliable and trustworthy application of EBT3 Gafchromic® films in the dosimetry medical field, makes our method a reliable and novel one for the determination of the SPF value of a sunscreen.

5. REFERENCES

1. Johnson BE. The influence of radiation on the skin and the basis of protection. *Int J*

- Cosmet Sci.* 1983;5:131-139. doi:10.1111/j.1467-2494.1983.tb00334.x
2. Mbanga L, Mulenga M, Mpiana PT, Bokolo K, Mumbwa M, Mvingu K. Determination of Sun Protection Factor (SPF) of Some Body Creams and Lotions Marketed in Kinshasa by Ultraviolet Spectrophotometry. *Int J Adv Res Chem Sci.* 2014;1(8):7-13. www.arcjournals.org.
 3. Gupta D. UV Absorbing Properties of Some Plant Derived Extracts. *Rjces.* 2013;1(2):34-36.
 4. Alexandra Amaro-Ortiz, Betty Yan JAD. Ultraviolet radiation, aging and the skin: prevention of damage by topical cAMP manipulation. *Molecules.* 2015;19(5):6202-6219. doi:10.3390/molecules19056202.Ultraviolet
 5. Cenizo V, André V, Reymermier C, Sommer P, Damour O, Perrier E. LOXL as a target to increase the elastin content in adult skin : a dill extract induces the LOXL gene expression. *Exp Dermatol.* 2006;15:574-581. doi:10.1111/j.0906-6705.2006.00442.x
 6. Matts PJ, Fink B. Chronic sun damage and the perception of age , health and attractiveness. *Photochem Photobiol Sci.* 2010;9:421-431. doi:10.1039/b9pp00166b
 7. Gonzaga ER. Role of UV Light in Photodamage , Skin Aging , and Skin Cancer Importance of Photoprotection. *Am J Clin Dermatol.* 2009:19-24.
 8. Couteau C, Couteau O, Alami-El Boury S, Coiffard LJ. Sunscreen products : What do they protect us from ? *Int J Pharm.* 2011;415:181-184. doi:10.1016/j.ijpharm.2011.05.071
 9. Palm MD, O'Donoghue MN. Update on photoprotection. *Dermatol Ther.* 2007;20:360-376. doi:10.1016/S1578-2190(10)70696-X
 10. European Commission. *Standardisation Mandate Assigned to CEN Concerning Methods for Testing Efficacy of Sunscreen Products.*; 2006.
 11. ISO24444:2010. *Cosmetics — Sun Protection Test Methods — In Vivo Determination of the Sun Protection Factor (SPF).* Vol 2010.; 2010.
 12. Diffey BL, Robson J. A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. *J Soc Cosmet Chem.* 1989;40:127-133.
 13. Pissavini M, Ferrero L, Alard V, et al. Determination of the in vitro SPF. *Cosmet Toilet.* 2003;118(10):63-72.
 14. Bielfeldt S, Klette E, Rohr M, et al. Multicenter methodology comparison of the FDA and ISO standard for measurement of in vitro UVA protection of sunscreen products. *J Photochem Photobiol B Biol.* 2018;189:185-192. doi:10.1016/j.jphotobiol.2018.10.018
 15. Sohn M, Herzog B, Osterwalder U, Imanidis G. Calculation of the sun protection factor of sunscreens with different vehicles using measured film thickness distribution - Comparison with the SPF in vitro. *J Photochem Photobiol B Biol.* 2016;159:74-81. doi:10.1016/j.jphotobiol.2016.02.038

16. Ferrero L, Pissavini M, Doucet O. How a calculated model of sunscreen film geometry can explain in vitro and in vivo SPF variation. *Photochem Photobiol Sci.* 2010;9:540-551. doi:10.1039/b9pp00183b
17. Farah N, Francis Z, Abboud M. Analysis of the EBT3 Gafchromic film irradiated with 6MV photons and 6MeV electrons using reflective mode scanners. *Phys Medica.* 2014;30(6):708-712. doi:10.1016/j.ejmp.2014.04.010
18. Welch D, Randers-Pehrson G, Spotnitz HM, Brenner DJ. Unlaminated Gafchromic EBT3 film for ultraviolet radiation monitoring. *Radiat Prot Dosimetry.* 2017;176(4):341-346. doi:10.1093/rpd/ncx016
19. Tajuddin MA, Omar AF. Measuring Solar Ultraviolet Exposure Dose on EBT3 Film Through the Application of Visible Absorbance Spectroscopy. In: *9th International Conference on Robotic, Vision, Signal Processing and Power Applications.* Vol 398. ; 2017:639-646. doi:10.1007/978-981-10-1721-6
20. Bahreyni Toossi M., Khorshidi F., Ghorbani M., Mohamadian N. DD. Comparison of EBT and EBT3 RadioChromic Film Usage in Parotid Cancer Radiotherapy. *J Biomed Phys Eng.* 2016:1-12.
21. Nakano M, Hill RF, Whitaker M, Kim J. A study of surface dosimetry for breast cancer radiotherapy treatments using Gafchromic EBT2 film. *J Appl Clin Med Phys.* 2012;13(3):83-97.
22. Cheung T, Butson MJ, Yu PKN. Multilayer Gafchromic film detectors for breast skin dose determination in vivo. *Phys Med Biol.* 2002;31(47):31-37.
23. Aydarous A, Al-Omary EA, El Ghazaly M. Characterization of Gafchromic EBT3 films for ultraviolet radiation dosimetry. *Radiat Eff Defects Solids.* 2014;169(3):249-255. doi:10.1080/10420150.2013.848446
24. Butson MJ, Cheung T, Yu PKN, Abbati D, Greenoak G. Ultraviolet radiation dosimetry with radiochromic film. *Phys Med Biol.* 2000;45:1863-1868.
25. Butson ET, Yu PKN, Butson MJ. Solar ultraviolet radiation response of EBT2 Gafchromic, radiochromic film. *Phys Med Biol.* 2013;58(287-294). doi:10.1088/0031-9155/58/21/N287
26. Tsang PY, Chan PM, Yu KN. Measuring diffuse ultraviolet exposures using Gafchromic EBT3 films. *Results Phys.* 2017;7:1492-1493. doi:10.1016/j.rinp.2017.04.016
27. Herzog B, Osterwalder U. In silico determination of topical sun protection. *Cosmet Sci Technol.* 2011:62-70.
<http://cosmeticsciencetechnology.com/articles/samples/2471.pdf%5Cnhttp://www.skin-care-forum.basf.com/en/articles/home/a-review-of-cosmetics-regulations-in-asia/2012/11/87a437df-d785-4304-8a6c-018fb2b2298b/in-silico-determination-of-topical-sun-protection>.
28. Mansur JS. Determinação do fator de proteção solar por espectrofotometria. *An Bras*

- Dermatol.* 1986;61(3):121-124.
29. Sayre et al. A Comparison of In vivo and In vitro Testing of Sunscreening Formulas. *Photochem Photobiol.* 1979;29:559-566. doi:10.1111/j.1751-1097.1979.tb07090.x
 30. Quatela A, Miloudi L, Tfayli A, Baillet-Guffroy A. In vivo Raman microspectroscopy: Intra- and intersubject variability of stratum corneum spectral markers. *Skin Pharmacol Physiol.* 2016;29(2):102-109. doi:10.1159/000445079
 31. De Bleye C, Dumont E, Rozet E, et al. Determination of 4-aminophenol in a pharmaceutical formulation using surface enhanced Raman scattering: From development to method validation. *Talanta.* 2013;116:899-905. doi:10.1016/j.talanta.2013.07.084
 32. Miloudi L, Bonnier F, Barreau K, et al. ATR-IR coupled to partial least squares regression (PLSR) for monitoring an encapsulated active molecule in complex semi-solid formulations. *Analyst.* 2018;143(10):2377-2389. doi:10.1039/c8an00547h
 33. Miloudi L, Bonnier F, Bertrand D, et al. Quantitative analysis of curcumin-loaded alginate nanocarriers in hydrogels using Raman and attenuated total reflection infrared spectroscopy. *Anal Bioanal Chem.* 2017;409(19):4593-4605. doi:10.1007/s00216-017-0402-y