Automated In Vitro Analysis of UV Protection Performance of Powder Sunscreen Products

Ribeiro, Claudio[1]; Baroni, Karin[1]; Yeung, Joey[2]; Yao, Ge[2]; Jin, Suxin[3]*; **Chen, Ye[2]**

1 Lubrizol Life Science, Lubrizol, San Paulo, Brazil; 2 Lubrizol Life Science, Lubrizol Southeast Asia Pte Ltd, Singapore; 3 Lubrizol Life Science, Lubrizol Specialty Chemicals Manufacturing (Shanghai) Co. Ltd., Shanghai, China.

* Chen, Ye, 44 Tanjong Penjuru, Singapore, 609032, +65-66636316, ye.chen@lubrizol.com

Abstract

The performance evaluation of sunscreen products is crucial to understand the effectiveness of such products' protection against solar radiation. Traditionally, the effectiveness of sunscreens is assessed *in vivo*, which is expensive and has potential ethical issues. Hence, *in vitro* methods have been developed to address the concerns. Unfortunately, the *in vitro* methods acknowledged so far are not applicable to powder products, not to mention automation.

This work is about designing an effective automated *in vitro* method to evaluate the UV protection performance of powder sunscreen products. *In vivo* claims were used as references for method optimization to ensure correlation. SPF and UVA photoprotection were determined by assessing UV transmittance through a thin film of powder sunscreen sample through automated spreading on a roughened substrate, before and after exposure to a controlled dose radiation from a defined solar exposure source.

The results showed that the SPF, UVAPF, and critical wavelength tested *in vitro* can be well correlated to the *in vivo* claims. Proper sample treatment is necessary. The auto-application can give consistent and better correlated SPF values compared with manual application.

Keywords: sunscreen; UV protection; powder product; *in vitro*; automation.

Introduction.

As a highly regulated product category, sunscreen products are subject to *in vivo* sun protection factor (SPF) tests to assess their protection level against sun light and claimable SPF to consumers. The current efficacy tests acknowledged in most countries are *in vivo* based, and the new ISO 23675 (*in vitro* SPF testing) is still under development [1-3]. There is one in vitro test method developed for UVA (ultraviolet A) claims, ISO 24443, which is applicable to most of the product formats, however, excluding powder products [4].

On the other hand, with the increasing usage of sun care products, there are more occasions that consumers prefer products of solid formats such as powder sunscreens, which are easy to carry and use, with a dry and non-sticky feel.

Therefore, it's of significance to design an *in vitro* method that can produce accurate and repeatable results that are *in vivo* results correlated. With the help of automated spreading machine, the human error can be well mitigated, while consistency well improved. This study focuses on the method development based on data correlation with the *in vivo* claims.

Materials and Methods.

Materials: Liquid paraffin and glycerin were used as supply. Sandblasted (SB) PMMA plates were used for *in vitro* analysis.

Market samples

I: Claim: SPF 20

Active ingredients: Titanium Dioxide [Nano], Ethylhexyl Methoxycinnamate

II: Claim: SPF 50+ PA ++++

Active ingredients: Ethylhexyl Methoxycinnamate, Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, Titanium Dioxide, Ethylhexyl Triazone.

III: Claim SPF 32 PA +++

Active ingredients: Titanium Dioxide, Ethylhexyl Methoxycinnamate.

IV: Claim SPF 26 PA ++

Active ingredients: Ethylhexyl Methoxycinnamate, Titanium Dioxide, Zinc Oxide.

Sample treatment.

Three different approaches (A, B, and C) were conducted to examine if the sample treatment process is practical and analysis results show good correlation with in vivo results.

A) Direct application with puff: Powder sunscreen sample (1.2 mg/cm^2) is directly applied on a SB PMMA plate using a puff. (Puff provided by the market sample.)

A-1: Sample spread on the PMMA plate manually.

A-2: Sample spread on the PMMA plate by automatic robot arm.

B) With spatula: Powder sunscreen samples is taken out with a spatula and applied on a SB PMMA plate.

B-1: 0.0300±0.0005 g Sample spread on the PMMA plate manually.

B-2: 0.0300±0.0005 g Sample spread on the PMMA plate by automatic robot arm.

B-3: 0.0300±0.0005 g of sample spread on the PMMA plate by automatic robot arm.

C) Mixing with liquid paraffin: Powder sunscreen samples is taken out with a specular and mix well with liquid paraffin (1:1, 1:2, and 2:1) and applied on a SB PMMA plate.

C-1: 0.0300±0.0005 (0.015 g) of solution sample is applied on the PMMA plate and spread by automatic robot arm.

C-2: 0.0600±0.0005 (0.030 g) of solution sample is applied on the PMMA plate and spread by automatic robot arm.

Test Procedure for Method A and B:

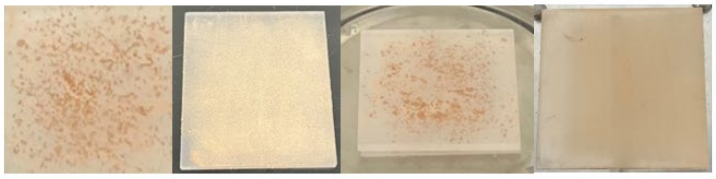
1. Used an air duster to blow the flint on the PMMA plate away.
2. Measured 0.03 g/0.06 g of sample on SB plate using respective methods using analytical balance.
3. Used manual or automatic robot arm application to apply 0.03 g/0.06 g of sample on the SB plate.
4. Measured the weight change after application using analytical balance.
5. Waited for 30 minutes before conducting pre-irradiation UV transmittance analysis.
6. Placed plate in solar simulator for stated exposure time in the software.
7. Conducted post-irradiation UV transmittance analysis after taking out plate.

Test procedure for Method C:

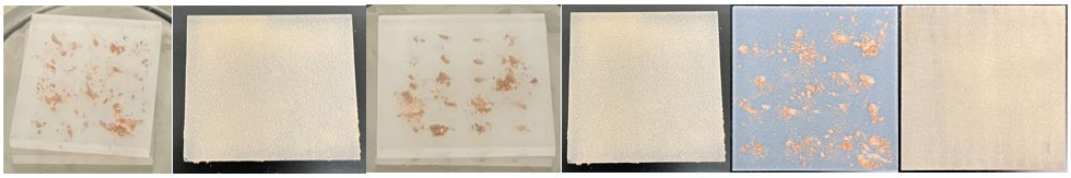
1. Measured powdered sunscreen with liquid paraffin in a 20 ml vial with different dilution levels.
2. Mixed the solution together with a magnetic stirrer at 500 rpm until homogenous.
3. Used air duster to blow the flint on the PMMA plate away.
4. Measured 0.03 g/0.06 g of solution on SB plate using micropipette using analytical balance.
5. Used automatic robot arm to apply 0.03 g/0.06 g of sample on the PMMA plate.
6. Measured the weight change after application using analytical balance.
7. Waited for 30 minutes before conducting pre-irradiation UV transmittance analysis.
8. Placed plate in solar simulator for stated exposure time in the software.
9. Conducted post-Irritation UV transmittance analysis after taking out the plate.

Results.

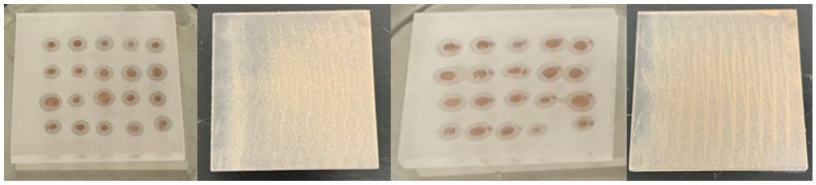
Market sample-I was used to test the effect of sample treatment. When the untreated sample was applied directly on the PMMA plate with a puff, it was difficult to ensure an even distribution of the sample on the plate (**Figure 1-a**). Moreover, powders are light and fluffy so they can easily come off when spread under force. Using a spatula can better control the amount put on the plate compared with the puff method (**Figure 1-b**). However, the same issue due to the fluffy nature of the product occurred during the spreading phase. Both puff and spatula methods gave a weight loss about or greater than 50% (**Table II**). When mixed with liquid paraffin, a fine liquid sample can be obtained and applied easily on the PMMA plates. The auto spreading can be utilized in a similar manner of standard liquid sunscreen samples (**Figure 1-c**).



(a) Puff method (A-1 & A-2)



(b) Spatula method (B-1, B-2, B-3)



(c) Mixing with liquid paraffin method (C-1, C-2)

Figure 1. Samples weighed on the PMMA plates and after spreading. (a) Using provided puff to apply samples on the plates and after spreading (A-1manual and A-2 auto). (b) Using spatula to take out sample and add on to the plates and after spreading (B-1 manual and B-2, 3 auto). (c) Using liquid paraffin to dilute the powder and after auto spreading.

The *in vitro* SPF and UVAPF results (in **Table II**) based on the transmittance show that the manual application gave lower SPF and UVAPF compared with auto application for puff and spatula methods. All SPF values obtained by puff and spatula methods are below claimed SPF value of 20. Coefficient of variation (COV) of SPF and UVAPF of auto application is greater than manual application for puff and spatula methods. Whereas, mixing the powder with liquid paraffin gave much smaller weight loss, narrower COVs for both SPF and UVAPF. The SPF reading of C-1 is 20.57, as claimed SPF of the product.

Table II. Test Results of Different Application Methods.

Method	**Puff**		**Spatula**			**Mixture**	
	A-1	A-2	B-1	B-2	B-3	C-1	C-2
Weight change (%)	53.95	48.14	51.50	50.66	74.34	8.91	22.35
SPF	6.02	12.11	4.64	7.86	9.61	20.57	32.57
SPF COV (%)	4.48	40.34	6.64	20.54	5.16	13.19	13.99
UVAPF	4.88	4.94	4.29	10.18	7.79	3.39	3.48
UVAPF COV (%)	0.81	24.19	2.11	23.55	5.03	3.62	2.32
$\lambda_{Critical}$ (nm)	385.00	383.50	383.90	383.90	383.90	376.20	376.50

Therefore, mixing powder product with liquid paraffin was selected for sample treatment before *in vitro* analysis. The dilution levels of 1:2 and 2:1 was also tested. With 2:1 dilution, the powder samples cannot be mixed well with good spreadability, whereas 1:2 sample was too liquid and showed phase separation overnight. Hence, 1:1 dilution ratio was selected as the best ratio for the test.

Four market samples were then analyzed with *in vitro* SPF method, 4 SB plates for each sample (0.0300±0.005 g liquid paraffin diluted sample per plate). The results in **Table III** show a

good correlation between tested SPF and UVAPF values and the claims of the commercial products.

Table III. Test Results of Market Samples.

Market sample	I	II	III	IV
In-Vitro SPF	19.18	48.59	30.21	49.23
In-Vitro SPF STD	1.41	7.70	4.17	6.66
In-Vitro SPF COV	7.35%	15.84%	13.79%	13.53%
SPF claimed	*20*	*50+*	*32*	*26*
UVAPF	3.97	5.33	9.25	7.67
UVAPF 95% CI	15.82%	3.32%	5.43%	5.60%
UVAPF claimed	*NA*	*PA ++++*	*PA +++*	*PA ++*
UVA balance	16%	9%	28%	14%
Critical wavelength (nm)	378.80	367.80	384.80	379.70
UVA/UVB ratio	0.458	0.379	0.641	0.502

Discussion.

The commonly used UV filters in powder sunscreens are Titanium Dioxide and Ethylhexyl Methoxycinnamate. Although not many UV filters are used in powder sunscreens, a variety of SPF and UVAPF claims can be achieved, covering low, moderate, and high protection. Dilution with liquid paraffin can greatly improve the weighing process of the powder sample and enhance the consistency of sample application (fulfilled by automated sample spreading). Moreover, liquid paraffin won't affect the reading of UV transmittance.

The total amount of sample applied on the PMMA plate is half the amount of liquid sample for *in vitro* method, which is sensible because the amount of powder applied on skin is not the same as liquid sample either.

Automated spreading procedure has proved its advantage over manual spreading in terms of repeatability, reproducibility, and reliability [5-7]. With puff and spatula method, the dry samples stuck on the roughened surface of the PMMA plate and made it difficult to spread. When spread manually, operators applied different forces to spread the sample over the plate. However, with constant pressure, force, and route, robot arm may not be able to adjust the force nor route

to intentionally spread the sample evenly. Hence, the COV values of automated spreading are higher than manual ones. On the other hand, when the sample can be evenly spread on the PMMA plate with constant force and fixed route, robot arm displayed narrower COV compared with manual spreading.

In vitro SPF and UVAPF values can be highly correlated with the *in vivo* claims when the samples were well distributed and spread on the PMMA plates. Thus, *in vitro* method can be applied to evaluate the UV protection performance of powder samples. With this method, the screening of sunscreen prototypes and assessment of formulation variable become feasible.

Conclusion.

The UV protection performance of powder sunscreen products can be evaluated by automated *in vitro* method. Dilution of powder sunscreen sample with liquid paraffin is necessary. The subsequent measurement of SPF, UVAPF, and critical wavelength can follow the *in vitro* method for standard liquid samples. The test method developed can be used as a reference for evaluating the effectiveness of powder sunscreen products, extending the use of non-invasive *in vitro* method and greatly facilitate the product development of powder sunscreens.

Acknowledgments.

The authors thank Lubrizol Life Science for support for this work. The authors gratefully acknowledge the assistance of Noelle Kung on the experimental work.

Conflict of Interest Statement.

NONE.

References.

1. Pirotta, G. I. U. L. I. O. (2015). An overview of sunscreen regulations in the world. *Household*

and Personal Care Today, *10*(4), 17-20.

2. Lodén, M., Beitner, H., Gonzalez, H., Edström, D. W., Åkerström, U., Austad, J., ... & Wulf, H. C. (2011). Sunscreen use: controversies, challenges and regulatory aspects. *British Journal of Dermatology*, *165*(2), 255-262.
3. Surber, C., Uhlig, S., Bertrand, C., Vollhardt, J., & Osterwalder, U. (2021). Past, present, and future of sun protection metrics. *Challenges in sun protection*, *55*, 170-187.
4. ISO 24443:2021 Cosmetics — Determination of sunscreen UVA photoprotection in vitro
5. Miksa, S., Lutz, D., & Guy, C. (2013). In vitro UV testing-robot vs. human spreading for repeatable, reproducible results. *Cosmt. Toil*, *128*, 742-752.
6. Miksa, S., Lutz, D., Guy, C., & Delamour, E. (2016). New approach for a reliable in vitro sun protection factor method–Part II: Practical aspects and implementations. *International Journal of Cosmetic Science*, *38*(5), 504-511.
7. Pissavini, M., Tricaud, C., Wiener, G., Lauer, A., Contier, M., Kolbe, L., ... & Matts, P. J. (2018). Validation of an in vitro sun protection factor (SPF) method in blinded ring-testing. *International Journal of Cosmetic Science*, *40*(3), 263-268