Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Table S1. Weighting used to calculate the sun protection factor by spectrophotometry.

Wavelength (nm)	**EE x I (normalized) – relative values**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total =	1

EE = erithemogenic effect obtained with monochromatic radiation at a wavelength λ.

I = solar intensity at wavelength λ.

Table S2. Fitzpatrick Scale used for the study.

Phototype	**Sunburn and tanning history**	**Constitutive color (unexposed buttock skin)**	**ITA°**
I	Burns easily, never tans	Ivory white	> 55°
II	Burns easily, tans minimally with difficulty	White	41° – 55°
III	Burns moderately, tans moderately and uniformly	White	28° – 41°

ITA°: Individual Tipology Angle.

Cosmetics Europe (formerly Colipa) SPF Reference Formula (P3).

Table S3. Ingredients for the SPF Reference Sunscreen.

Ingredient	**% (w/w)**
Phase 1	
Cetearyl Alcohol (and) PEG-40 Castor oil (and) Sodium Cetearyl Sulphate	3.15
Decyl Oleate	15.0
Ethyl Hexyl Methoxycinnamate	3.0
Butyl Methoxy Dibenzoylmethane	0.5
Propylparaben	0.1
Phase 2	
Water	53.57
2-Phenyl-Benzimidazole-5-Sulphonic Acid	2.78
Sodium Hydroxide (45% solution)	0.9
Methylparaben	0.3
Disodium EDTA	0.1
Phase 3	
Water	20.0
Carbomer (“Carbomer 934P”)	0.3
Sodium Hydroxyde (45% solution)	0.3

Manufacturing process

- Heat Phase 1 to 75-80 °C.
- Heat Phase 2 to 80 °C (if necessary boil until solution is clear and cool to 75-80 °C).
- Disperse Phase 3 carbomer in water by stirring with an Ultraturrax (rotor/stator disperser), then add Sodium Hydroxide for neutralization.
- Add Phase 1 into Phase 2 while stirring Phase 2.
- Add Phase 3 to Phases 1 & 2 while stirring and homogenize for about 3 minutes.
- Adjust pH with Sodium Hydroxide or Lactic Acid and stir until completely cool.
- Compensate for water loss and homogenize.

Theoretical background for UVAPF determination

The samples were accurately and quickly weighed (to reduce product evaporation and dryness) to satisfy the application rate of 1.3 mg cm^{-2} in each PMMA plate (actual quantity applied: 32.5 mg, determined by weighing the plates before and immediately after applying the products). They were directly weighed on the plate surface, applied as a large number of small droplets of approximately equal mass, and distributed in an even manner on the roughened surface of the plate. Then, the products were spread over the whole surface with a fingertip covered with a vinyl glove and pre-saturated with the product, to prevent possible losses of the amount weighed. The spreading was achieved in two steps: (i) quick distribution of the product, without pressure (20-30 seconds); and (ii) rubbing it into the rough surface using pressure (20-30 seconds too). For each product, three plates were prepared, which were kept protected from light exposure in a dark chamber at room temperature (≈ 20ºC) for 15 minutes, in order to facilitate the formation of a standard stabilized sunscreen film.

After this period, the plates containing the product were placed in the light-path of the UV-2000S Ultraviolet Transmittance Analyzer (Labsphere, USA). The transmission of UV radiation through the sample was measured from 290 to 450 nm at 1 nm intervals on 9 different sites of each plate (total measurement area = 2.0 cm^2). The blank was prepared using the HD6 plates covered with 15-µL of glycerin, because of its non-fluorescence and UV transparency.

Using the generated data, $SPF_{in\ vitro}$ was calculated using Eq. 1:

$$SPF_{\ in\ vitro} = \frac{\int_{\lambda = 290nm}^{\lambda = 400nm} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda = 290nm}^{\lambda = 400nm} E(\lambda) \times I(\lambda) \times 10^{\ -A_0(\lambda)} \times d\lambda} \quad (1)$$

where E(λ) is the erythema action spectrum, I(λ) is the spectral irradiance of the UV source, $A_0(\lambda)$ is the mean monochromatic absorbance measurements per plate of the test product layer before UV exposure, and dλ is the wavelength step (1 nm).

In order to generate the UVAPF value, the coefficient of adjustment "C" was calculated as shown in Eq. 2 and using the SPF label as the value generated by the UV-2000's software.

$$SPF_{\ in\ vitro,adj} = SPF\ label = \frac{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} E(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda) \times C} \times d\lambda} \quad (2)$$

Using the "C" value, initial UVAPF was calculated using Eq. 3, and the dose "D" of UV irradiation was determined by Eq. 4.

$$UVAPF_0 = \frac{\int_{\lambda=320nm}^{\lambda=400nm} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda=290nm}^{\lambda=400nm} P(\lambda) \times I(\lambda) \times 10^{-A_0(\lambda) \times C} \times d\lambda} \quad (3)$$

$$D = UVAPF_0 \times D_0 \quad (4)$$

where P(λ) is the PPD action spectrum[10] and D_0 = 1.2 J cm^{-2}.

The plates were inserted into a long-arc xenon SuntestTM insolator, type CPS+, UV irradiation source (Atlas, Germany) (temperature maintained below 40ºC) and then exposed to the calculated UV dose D. PMMA plates were supported firmly throughout the irradiation by a SunTray holder which also provided a dark background behind each plate to reduce the risk of any back exposure. After that, new transmission measurements of the sunscreen samples were conducted, for acquisition of the second UV spectrum. The final UVAPF was calculated according to Eq.5. If the coefficient of variation (CV) between the UVAPF's of the individual plates exceeded 20%, then further plates were measured until the CV threshold was achieved.

$$UVAPF = \frac{\int_{\lambda = 320nm}^{\lambda = 400nm} P(\lambda) \times I(\lambda) \times d\lambda}{\int_{\lambda = 290nm}^{\lambda = 400nm} P(\lambda) \times I(\lambda) \times 10^{-A(\lambda) \times C} \times d\lambda} \quad (5)$$

where A(λ) is the mean monochromatic absorbance of the test product layer after UV exposure.

For calculation of the Critical Wavelength Value (λ_c), a series of absorbance values were calculated for each of the three separate plates to which the samples were applied. Absorbance at each wavelength increment A(λ) was calculated using Eq. 6, and the λ_c using Eq.7.

$$A_\lambda = \log\left({C_\lambda}/{P_\lambda}\right) \quad (6)$$

where $C_\lambda = \sqrt[n]{(C_\lambda[1] \times C_\lambda[2] \times \ldots \times C_\lambda[n])}$ and $P_\lambda = \sqrt[n]{(P_\lambda[1] \times P_\lambda[2] \times \ldots \times P_\lambda[n])}$.

$$\int_{290nm}^{\lambda c} A\lambda \times d\lambda = 0.9 \int_{290nm}^{400nm} A\lambda \times d\lambda \quad (7)$$

Measurement uncertainty formulae

$$u(x_{prev}) = \sqrt{\frac{S^2}{b^2}\left(1 + \frac{1}{n} + \frac{(x_{prev} - \bar{x}_i)}{\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}\right)} \quad (1)$$

$$S = \sqrt{\frac{\sum_{i=1}^{n} (y_{calc} - y_{real\,i})^2}{n - 2}} \quad (2)$$

where x_{prev} = predicted value for the curve obtained by three individual spectrophotometric readings, n = number of points in the regression curve, x_i = actual values used to construct the calibration curve, b = angular coefficient, and S = standard deviation of the residues, calculated by the squared differences between the value calculated by the curve, y_{calc}, and the reference value obtained by reading the standard, y_{real}. Thus, there was obtained the standard uncertainty of the linear regression curve of the first degree of uncertainty as a component to "n - 2" degrees of freedom.

Total polyphenolic content

Gallic acid standard analytical curve

Equation: $y = 0.1145x + 0.0528$
$R^2 = 0.99746$

Table S4. Analysis of Variance of the analytical curve for determination of total phenolic content.

Source	Sum of Square (SS)	Degrees of freedom	Mean of Square (MS)	$F_{calculated}$	$F_{critical}$
Model	1.27	1	1.27	5098.94	4.67
Residual	3.23×10^{-3}	13	2.48×10^{-4}		
Lack-of-fit	1.56×10^{-3}	3	5.20×10^{-4}	3.13	3.71
Pure error	1.66×10^{-3}	10	1.66×10^{-4}		
Total	1.27	14	9.06×10^{-2}		

The test of significance of regression returned the value of $F_{calculated}$ (MS_{model}/ $MS_{residual}$) = 5098.94, greater than $F_{critical\ (1,\ 13)} = 4.67$, which confirms the existence of a significant linear relationship between the two variables, with 95% confidence. The test of lack of fit value returned $F_{calculated}$ ($MS_{lack\text{-}of\text{-}fit}$/ $MS_{pure\ error}$) = 3.13, smaller than $F_{critical\ (3,\ 10)} = 3.71$, which indicates no lack-of-fit, with 95% confidence (the statistical model for the regression adequately describes the relationship between the experimental factors and the response variable, i.e., it fits well for the purposes). This confirms that the model is suitable for the quantification of total polyphenolic constituents, calculated as gallic acid.

Total flavonoid content

Rutin standard analytical curve

Equation: $y = 0.015x + 0.0257$
$R^2 = 0.9944$

Table S5. Analysis of Variance of the analytical curve for determination of total flavonoid content.

Source	Sum of Square (SS)	Degrees of freedom	Mean of Square (MS)	$F_{calculated}$	$F_{critical}$
Model	3.44×10^{-1}	1	3.44×10^{-1}	2308.75	4.67
Residual	1.94×10^{-3}	13	1.49×10^{-4}		
Lack-of-fit	6.43×10^{-4}	3	2.14×10^{-4}	1.66	3.71
Pure error	1.29×10^{-3}	10	1.29×10^{-4}		
Total	3.46×10^{-1}	14	2.47×10^{-2}		

The test of significance of regression returned the value of $F_{calculated}$ (MS_{model}/ $MS_{residual}$) = 2308.75, greater than $F_{critical\ (1,\ 13)} = 4.67$, which confirms the existence of a significant linear relationship between the two variables, with 95% confidence. The test of lack of fit value returned $F_{calculated}$ ($MS_{lack\text{-}of\text{-}fit}$/ $MS_{pure\ error}$) = 1.66, smaller than $F_{critical\ (3,\ 10)} = 3.71$, which indicates no lack-of-fit, with 95% confidence (the statistical model for the regression adequately describes the relationship between the experimental factors and the response variable, i.e., it fits well for the purposes). This confirms that the model is suitable for the quantification of total flavonoids content, calculated as rutin.

Table S6. Caracterization of the volunteers for *in vivo* Sun Protection Factor determination.

Volunteer	Initials	Sex	Age	ITA (°)	Skin phototype
1	VHK	F	28	55.9	III
2	CFGO	F	53	55.2	I
3	SDLS	F	46	41.8	III
4	NFL	F	39	41.6	III
5	SHTS	F	41	46.5	III
6	MES	F	55	43.7	II
7	CCD	F	39	34.2	III
8	WCL	M	27	38.5	III
9	LFB	M	31	38.8	III
10	ICTO	F	47	40.2	III

ITA: Individual Tipology Angle.

Table S7. Individual results of MED and SPF for control and *L. sericea* sun-care systems.

Volunteer	MED_u (mJ cm^{-2})	Control		Product	
		MED_P (mJ cm^{-2})	SPF	MED_P (mJ cm^{-2})	SPF
1	33.6	537.6	16.0	268.8	8.0
2	25.2	403.2	16.0	252.0	10.0
3	23.5	376.3	16.0	150.9	6.4
4	37.3	477.1	12.8	191.3	5.1
5	42.0	537.6	12.8	215.6	5.1
6	46.7	747.6	16.0	299.0	6.4
7	46.7	747.6	16.0	373.8	8.0
8	33.6	537.6	16.0	268.8	8.0
9	33.6	344.1	10.2	336.0	10.0
10	38.3	490.6	12.8	306.6	8.0

MED_u: Mynimal Erithemal Dose – unprotected skin. MED_P: Mynimal Erithemal Dose – protected skin. SPF: Sun Protection Factor.

Table S8. Data regarding the protective factors before UV irradiation and calculated dose of UVA irradiation for the *L. sericea* sun-care system.

Plate	**$SPF_{in\ vitro}$**	**C**	**$UVAPF_0$**	**Dose UVA (J cm^{-2})**
1	4.3	1.399	2.90	3.48
2	5.8	1.156	2.97	3.57
3	5.2	1.233	3.08	3.69
4	5.9	1.142	3.02	3.63

C = coefficient of adjustment. $UVAPF_0$ = initial UVA Protection Factor.