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Assessment of Functional Stability of Photoprotective Formulations Containing Rutin Succinate

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Abstract: The aim of this study was to evaluate physicochemical and functional stability of two different self-emulsified oil/water (O/W) systems. Each system contained 0.4% *w*/*w* rutin succinate, which was associated or not with the photo-unstable chemical (7.5% *w*/*w* of 2-ethylhexyl 4-methoxycinnamate and 3.0% *w*/*w* of 2-hydroxy-4-methoxybenzophenone) or physical filters (3.0% *w*/*w* titanium dioxide). A Normal Stability Test was carried out with the formulations containing rutin succinate (**S**) associated either with sunscreens (**MS**) or not (**M**) for 90 days. The formulation systems were assessed for organoleptic, functional, physicochemical and rheological behavior parameters. The **MS** formulation was found to be homogenous and had no significant alterations of pH, hysteresis area, antiradical activity or Sun Protection Factor values. Such stability was mainly observed when the formulation was incorporated into base cream A. The ability of chemical filters to resist degradation caused by UV radiation in the presence of rutin succinate preventing lipid peroxidation by entrapment of initiator radicals is a mechanism that might explain the results. The combination of rutin succinate to chemical filters improved formulation functionality, as it led to a more stable formulation which maintained the effectiveness of the added sunscreens. Consumer acceptance could be improved, considering that film formation and rheological spreadability characteristics of the tested formulation are better than those of traditional formulations.

Keywords: rutin succinate; in vitro SPF; formulation/stability; sunscreen

1. Introduction

Ultraviolet radiation (UVB and UVA) promotes photochemical reactions on the body which lead to skin tanning, development of inflammatory processes and even severe burns [1,2]. These reactions can also trigger oxidative stress in cells by promoting formation of reactive oxygen species and initiating carcinogenesis by causing damage to DNA, proteins and lipids; such reactions are thus responsible for structural and functional disorders [1,3,4].

As concern for prevention of the harmful effects caused by sunlight exposure, which can trigger skin cancer, increases the interest in development of photoprotective formulations of proven efficacy and safety of use has increased as well. Research on new photostable molecules has focused on the use of substances of botanic origin in cosmetic and pharmaceutical preparations. In these formulations, the use of such substances can reduce the use of chemical filters, which, although being effective photoprotectors, are also constantly associated with adverse effects such as skin irritation and photoallergic reactions caused by topical use [5]. Rutin (3-o-rutinoside, quercetin) and

its derivatives belong to the flavonoid chemical group; its similarities of structural formula and of UV absorption spectra with those of chemical filters suggest it might have potential photoprotective actions in addition to its powerful antioxidant properties [6,7].

This study aimed to evaluate the physicochemical and functional stability (antiradical activity and photoprotective efficacy) of two different self-emulsified oil/water (O/W) systems containing rutin succinate either associated or not with chemical (2-ethylhexyl 4-methoxycinnamate and 2-hydroxy-4-methoxybenzophenone) and physical filters (titanium dioxide). The developed cream bases were: **A**—Cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate + Cetearyl alcohol (and) polysorbate 60 (and) cetearyl glucoside (and) stearyl alcohol; and **B**—acryloyldimethyl-taurate Ammonium/VP copolymer (and) rapeseed oil sorbitol esters (and) trilaureth-4 phosphate (and) mineral oil (and) isopropyl palmitate.

2. Materials and Methods

2.1. Photoprotective Formulations Studied

The O/W emulsion systems tested (Table 1) were prepared using (**a**) Cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate + Cetearyl alcohol (and) polysorbate 60 (and) cetearyl glucoside (and) stearyl alcohol and (**b**) ammonium acryloyldimethyl-taurate/VP copolymer (and) rapeseed oil sorbitol esters (and) trilaureth-4 phosphate (and) mineral oil (and) isopropyl palmitate. The formulation associations were as follows: (**S**) rutin succinate 0.4% (*w*/*w*); (**M**) 2-ethylhexyl 4-methoxycinnamate 7.5% (*w*/*w*) + 2-hydroxy-4-methoxybenzophenone 3.0% (*w*/*w*) + 3% titanium dioxide (*w*/*w*); (**MS**) 2-ethylhexyl 4-methoxycinnamate 7.5% (*w*/*w*) + 2-hydroxy-4-methoxybenzophenone 3.0% (*w*/*w*) + titanium dioxide 3.0% (*w*/*w*) + rutin succinate 0.4% (*w*/*w*).

2.2. Normal Stability Test (NST)

The six different formulations were prepared in replicates (n = 2) and tested under three different temperature and humidity conditions after a 24 h resting period (t0), in order to assess the completion of the emulsification process [8]. Formulation samples stored at room temperature protected from light and moisture (22.0 ± 2.0 °C) were considered as standard samples, as their organoleptic, physical, physico-chemical and chemical characteristics should suffer negligible or at the very least only minor changes [9]. Experimental conditions and pre-established days of analysis were as follows:

- **t0 (day 0)**—24 h after preparation of the six formulations at room temperature (22.0 ± 2.0 °C);
- **t15, t30, t60 and t90** (days 15, 30, 60 and 90)—set amount of days and trialed under conditions of: (a) 45.0 ± 0.5 °C and 75% relative humidity (RH); (b) 25.0 ± 2.0 °C under indirect sunlight and influence of UV radiation; (c) and 5.0 ± 0.5 °C.

Variations of parameters considered acceptable for approval of formulation samples were as follows [9,10]:

- **Appearance, color and odor:** normal (N) when stored under direct or indirect sunlight at room temperature. Slight modifications (SM) were considerable acceptable for formulations stored at higher temperatures;
- **pH value:** variability range up to 0.2 pH units;
- **Apparent viscosity and hysteresis area:** percentage changes of ±20% and ±25%, respectively;
- **SPF value (Sun Protection Factor):** percentage changes of ±5%; considering the coefficient of variation obtained by reading 9 different points on the plate, acceptance was ±5%;
- **Critical wavelength value (λ_C):** percentage changes of ±2%;
- **UVA/UVB ratio value:** percentage changes of ±5%;
- **Value of antiradical activity, flow behavior index and consistency index:** percentage changes were not considered.

The analysis of percentage variation of the above parameters was carried out by comparing the formulations at time zero (t0) and after 90 days (t90), with the exception of the parameters antiradical activity, flow behavior index and consistency index.

Table 1. Composition of the formulations under study.

Components	Formulations					
	Percentage of Components in Each Formulation (% *w*/*w*)					
	S		M		MS	
	A	B	A	B	A	B
Distilled water qs. to	100.0		100.0		100.0	
Dissodium EDTA	0.2		0.2		0.2	
Propyleneglycol	3.0		3.0		3.0	
Rutin succinate	0.4		-		0.4	
BHT	0.2		0.2		0.2	
Cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate	5.0	-	5.0	-	5.0	-
Cetearyl alcohol (and) polysorbate 60 (and) cetearyl glucoside (and) stearyl alcohol	3.0	-	3.0	-	3.0	-
2-hydroxy-4-methoxybenzophenone	-		3.0		3.0	
2-ethylhexyl 4-methoxycinnamate	-		7.5		7.5	
Ammonium acryloyldimethyl-taurate/ VP copolymer (and) rapeseed oil sorbitol esters (and) trilaureth-4 phosphate (and) mineral oil (and) isopropyl palmitate	-	3.0	-	3.0	-	3.0
Vinyl dimethicone crosspolymer (and) C12-14 pareth-12	1.0		1.0		1.0	
Cyclopentasiloxane	1.0		1.0		1.0	
Sodium polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG-PPG-18/18 dimethicone	1.0		1.0		1.0	
Caprylic/capric triglyceride	3.0		3.0		3.0	
Dimethicone (and) trimethylsiloxysilicate	2.0		2.0		2.0	
Titanium dioxide (and) manganese oxide	-		3.0		3.0	
Phenoxyethanol (and) methylparaben (and) butylparaben (and) ethylparaben (and) propylparaben (and) isobutylparaben	0.5		0.5		0.5	
Neutralizing agent qs. to	pH 6.0–6.5		pH 6.0–6.5		pH 6.0–6.5	
Fragrance	qs		qs		qs	

2.3. Statistical Analysis

The results were statistically analyzed using Statistica® 7.0 software (Stat SoftTM, Tulsa, OK, USA). Initially, homogeneity of variances were assessed by means of a Hartley test ($\alpha = 0.05$) [10]. Results obtained for apparent viscosity, SPF, critical wavelength, UVA/UVB ratio, antiradical activity, hysteresis area, flow behavior index and consistency index were analyzed by a non-parametric test. Mean values for different treatments were tested using univariate analyses of variance (one-way ANOVA) and were compared using Fisher LSD tests at a 5% probability [10]. These analyses were performed by comparing all data placed in the same row in the table by taking into account a formulation sample and the conditions to which each sample was subjected during the 90-day period of analysis.

2.4. Physicochemical and Functional Parameters of the Photoprotective Formulations

2.4.1. Determination of pH

For pH assessment, 10% *w*/*v* dispersions were prepared in three separate replicates using freshly distilled water, which were then homogenized and had their pH evaluated at room temperature (22.0 ± 2.0 °C) [8,11].

2.4.2. Assessment of Antiradical Activity by DPPH· (2,2-Diphenyl-1-picrylhydrazyl) Method

The formulations were evaluated for antiradical activity by the DPPH· spectrophotometric method. Initially, formulation samples were dispersed in ethyl alcohol at a concentration of 20 mg/mL (1:50) and then centrifuged at 3000 rpm for 15 min. An aliquot of 0.5 mL of the supernatant was added to 2.5 mL of DPPH· (100 uM); the resulting solution was stirred vigorously and incubated under no light for 30 min at room temperature (22.0 ± 2.0 °C). After this period, reduction of the free radical DPPH was measured in duplicates by reading absorbance using an UV-VIS spectrophotometer at a wavelength of 517 nm. A solution containing only 2.5 mL of DPPH· (100 µM) and 0.5 mL of ethyl alcohol was used as a negative control. Pure ethanol was used as a blank. Results were expressed as percentage of inhibition of DPPH· radical scavenging activity according to the equation below [12]:

$$\%\ \text{Inhibition of DPPH}\cdot = (\text{Negative control absorbance} - \text{sample absorbance}) \times 100/(\text{Negative control absorbance})$$

2.4.3. In Vitro Photoprotective Efficacy

Formulation samples were collected with disposable syringes, weighed to an amount of 2 mg.cm^2 and applied to 25 cm^2 polymethylmethacrylate (PMMA) plates. Samples were manually applied to the plates using a finger protected with a finger cot-presaturated with a small amount of formulation itself in circular movements (horizontal and vertical) applying similar pressure on the whole plate for 30 s, so an uniform film would be formed on each plate. Next, the plates were dried out under controlled temperature of 22.0 ± 1.0 °C for 15 min. Immediately after, the absorbance of the film was measured using a Labsphere® equipment (North Sutton, USA); light within a spectrum ranging from 290 to 400 nm was emitted over nine different areas of the plate [13,14]. Results were determined as transmittance versus wavelength curves; SPF values, λ critical and UVA/UVB ratio were calculated from these data. As a blank reference representing 100% light transmission, an intact, not coated, PMMA plate was used [13,14].

2.4.4. Determination of Rheological Profiles

Flow curves (upwards and downwards on the shear rate scale) were obtained in order to analyze rheological profiles, including hysteresis areas. Ascendant flow curves were assessed according to the equation:

$$\tau = K \cdot \gamma^n$$

which allowed determination of the consistency index (K) and flow behavior index (n).

Readings were carried out with a Paar Physica MCR-300 rheometer equipped with a Peltier plate for control of temperature, which had a ±0.05 °C variation. Equipment setup employed in all experiments was plate/plate, 49.00 mm diameter. Shear rates applied scanned a range from 0.1 to 200 s^{-1}. In the ascendant curve, 60 points of 5 s/point were collected. That was followed by an interval of 5 points at 5 s/point at a constant shear rate of 200 s^{-1} before the descendant curve began, in which similar conditions to the ascendant curve were applied (60 points at 5 s/point). Apparent viscosity was set at 200 s^{-1} for following analyses as this shear rate value seems to approach the natural spreading condition of semi-solid cosmetic formulations on the skin [15,16].

Formulation samples studied in the rheological experiments weighed approximately 0.5 g. Prior to any measurements, formulation systems rested on the Peltier plate (TEK 150P-C, Anton Paar,

VA, USA) of the rheometer for a required time (usually around 2 min) in order to achieve thermal equilibrium at the desired temperature, 22.00 ± 0.05 °C.

3. Results and Discussion

3.1. Organoleptic Characteristics and Determination of pH

The overall appearance of all formulations, labeled **S**, **M** and **MS** (for both **A** and **B** cream bases), remained unchanged throughout the 90 days of testing, there being no phase separation or any sort of precipitation.

Regarding stability of color and smell, it was found that after 60 days of testing, sample **M**, for both cosmetic emulsions, suffered only minor changes due to temperature variations or exposure to sunlight.

The pH range of all tested formulations remained between 5.9 and 6.3; thus, the pH of the formulations was considered not detrimental and suitable to applying to epidermal tissues since human skin has a physiological pH that ranges from 4.6 to 5.8 [11].

Formulations prepared with both cream bases (**A** and **B**) had pH variations of no more than 0.2 units at the three temperature study conditions of 5.0 ± 0.5, 45.0 ± 0.5 and 25.0 ± 2.0 °C throughout all 90 days of testing, the only exception being the **S** formulation incorporated into cream base B. This formulation, when incubated at 45.0 ± 0.5 °C, had its pH increased from 6.2 (t0) to 6.6 (t90), a total of 0.4 pH units. Such pH variation is understandable, as it only demonstrates the impactful effects of higher temperatures and humidity on the formulation; considering current legislation and comparing the effects on pH variation with those observed on the formulations tested under normal temperature conditions (which did not vary significantly at all), such pH variation can be considered acceptable [8].

The **MS** formulation suffered less impactful variations on organoleptic parameters and pH values under the analyzed conditions, demonstrating that rutin succinate associated with sunscreens (**MS**) improves stability of formulations prepared with both cream bases (**A** and **B**).

3.2. Analysis of Functional Features: Antiradical Activity and Photoprotective Effectiveness

Regarding physicochemical functional parameters (antiradical activity), samples **S** and **MS** showed a DPPH· radical inhibition interval that ranged from 32.0 to 36.0% (t0). Analysis of variance homogeneity (ANOVA F-test) indicated that **MS** formulation samples suffered no variations throughout the 90-day testing period, especially when considering only formulations made with cream base (**A**). However, cream base (**B**) formulations allowed for significant changes of antiradical activity when kept under refrigeration (5.0 ± 0.5 °C), starting from day 15 of storage, and also when kept in incubators (45.0 ± 0.5 °C), starting from day 60 (Table 2). The DPPH method was used to assess antiradical activity of photoprotective formulations because of the hydrophilic properties of rutin succinate and due to the solubility of chemical filters in alcohol and interactions by hydrogen donation.

Significant variations of antiradical activity were found for **S** formulations under different conditions of analysis throughout storage time, regardless of the cosmetic base used. Stability of the antiradical potential of these formulations improved due to association of rutin succinate with sunscreens (**MS**).

Rutin succinate is a phenolic compound derivative of rutin, capable of scavenging oxidized species and thus decreasing damage caused by UV radiation. In comparison with rutin alone (log P = 0.85 ± 0 .05), the insertion of carboxylate groups on hydroxyls of the rutin disaccharide increases its water solubility by 80 times (log P = −1.13 ± 0.02). However, its antioxidant activity and ability to prevent lipid peroxidation (as determined by assessing formation of malondialdehyde, with IC50 = 13.46 μM) [17] remains intact.

Table 2. Analysis of antiradical activity as assessed by Normal Stability Test of formulations developed with cream bases **A** and **B** with addition of chemical and physical filters (**M**), rutin succinate (**S**) or rutin succinate + chemical and physical filters (**MS**).

A						
Time (Days)		**t0**	**t15**	**t30**	**t60**	**t90**
Formulation	**Condition**	**% Radical DPPH· ± DP Inhibition**				
S	**G**	36.78 ± 1.72 [a]	36.80 ± 1.72 [a]	32.39 ± 1.26 [a]	34.88 ± 1.19 [a]	31.97 ± 0.97 [a]
	E		45.13 ± 2.34 [c]	32.28 ± 0.11 [a,b]	29.08 ± 0.22 [b]	28.45 ± 0.55 [b]
	RT		42.38 ± 3.04 [b]	36.66 ± 0.6 [a,b]	31.24 ± 1.05 [a,c]	27.30 ± 2.84 [c]
M	**G**	11.59 ± 0.89 [a]	11.59 ± 0.89 [a]	8.72 ± 1.03 [a]	11.76 ± 1.56 [a]	8.95 ± 1.19 [a]
	E		9.32 ± 0.59 [a]	9.50 ± 1.80 [a]	12.91 ± 0.58 [a]	10.71 ± 0.62 [a]
	RT		9.82 ± 0.88 [a]	8.28 ± 0.65 [a]	11.39 ± 1.02 [a]	9.85 ± 1.66 [a]
MS	**G**	36.21 ± 0.59 [a]	36.19 ± 0.58 [a]	36.67 ± 1.19 [a]	37.30 ± 0.51 [a]	36.83 ± 0.97 [a]
	E		37.30 ± 1.87 [a]	35.81 ± 0.80 [a]	36.07 ± 0.93 [a]	36.12 ± 0.14 [a]
	RT		36.97 ± 0.30 [a]	36.95 ± 0.23 [a]	36.02 ± 0.34 [a]	35.69 ± 0.48 [a]
B						
S	**G**	32.51 ± 0.7 [a]	33.88 ± 0.77 [a]	29.46 ± 0.23 [b]	28.23 ± 0.20 [b]	33.76 ± 0.63 [a]
	E		30.97 ± 0.1 [b]	30.80 ± 0.24 [b]	29.62 ± 0.22 [c]	32.86 [a]
	RT		33.85 ± 0.14 [a]	34.94 ± 0.27 [a]	26.51 ± 0.20 [b]	31.17 [c]
M	**G**	12.64 ± 0.98 [a]	11.50 ± 0.88 [b]	8.47 ± 0.16 [c]	6.22 ± 0.05 [d]	3.31 ± 0.33 [e]
	E		12.27 [a]	15.26 ± 0.24 [d]	7.48 ± 0.58 [c]	3.51 ± 0.32 [b]
	RT		12.3 [a,b]	12.06 ± 0.19 [b]	5.88 ± 0.14 [c]	2.31 ± 0.35 [b]
MS	**G**	35.16 ± 0.89 [a]	36.43 ± 0.19 [b]	35.73 [a]	36.27 [a,b]	34.81 [c]
	E		35.02 ± 0.03 [a]	35.45 ± 0.12 [a]	34.30 ± 0.20 [b]	34.76 ± 0.94 [b]
	RT		35.48 ± 0.27 [a]	35.18 ± 0.76 [a]	34.56 ± 0.34 [a,b]	34.05 [b]
P (ANOVA) *		<0.05	<0.05	<0.05	<0.05	<0.05

Legend: G: 5.0 ± 0.5 °C; **E**: 45.0 ± 0.5 °C, 75% relative humidity; **RT**: 25.0 ± 2.0 °C, indirect lighting. **DPPH·**: 2,2-diphenyl-1-picrylhydrazyl. Mean ± standard deviation (n = 2). Different superscript letters in the same row represent statistically different as assessed by Fisher LSD test ($p < 0.05$). * Probability values obtained by one-way ANOVA.

The association of rutin succinate with sunscreens in formulations (**MS**) increased by nearly 70% their antiradical activity when compared to formulations which contained sunscreens only (**M**), as shown in Table 2. Evaluation of this parameter is vital for proper assessment of photostability of the sunscreens incorporated in the formulations, as antiradical compounds can scavenge reactive species and prevent photodegradation.

Thus, the chemical filters 2-hydroxy-4-methoxybenzophenone and 2-ethylhexyl 4-methoxycinnamate, which are capable of filtering UVA and UVB radiation, respectively, were chosen for association with rutin succinate in this study. These filters are widely used in commercial sunscreens. However, they do tend to suffer photodegradation, limiting their use in broad-spectrum products. When chemical filters lose photostability, structural changes take place and the filters may interact with other molecules of the formulation, decreasing their ability to absorb UV radiation, leading to a reduced photoprotective efficacy.

The 2-ethylhexyl 4-methoxycinnamate filter can undergo photodegradation, in which isomerization occurs decreasing molar extinction coefficient from 23,300 mol^{-1} cm^{-1} (λ = 311 nm) to 12,600 mol^{-1} cm^{-1} and also decreasing absorption wavelength to a maximum of 301 nm [18]. The 2-hydroxy-4-methoxybenzophenone filter, with maximum absorption in two spectral regions, UVB (λ = 288 nm, $\in$ = 14,000 mol^{-1} cm^{-1}) and UVA II (λ = 325 nm, $\in$ = 9400 mol^{-1} cm^{-1}), can undergo oxidation when in its triplet state, which can easily react with phospholipids found in cosmetic formulations, resulting in the formation of peroxyl radicals [19].

Evaluation of the photoprotective efficacy of the studied formulations (Table 3) showed there was no significant variation of SPF, UVA/UVB ratio and critical wavelength values (>370 nm) for the **MS** formulation throughout the 90-day testing period under the three studied conditions. Such results are due to the association of chemical filters with rutin succinate in the formulation, as it

caused the chemical filters to resist UV radiation-mediated degradation. As a flavonol, rutin succinate prevents lipid peroxidation by scavenging initiator radicals such as singlet excited oxygen, hydroxyl radicals and superoxide ions [6,7]. Formulation **M**, on the other hand, proved to be unstable, as SPF values for formulations prepared with cosmetic base **A** when studied at 25.0 ± 0.5 °C decreased 48.5% throughout the 90-day period; this is a case for concern, as RT conditions are precisely the conditions of use of the average consumer. Still, from day 15 to day 30, there was an increase of FPS values determined for formulation **M** when incorporated into cream base **A**, which further reinforces the fact that such chemical filters (2-hydroxy-4-methoxybenzophenone and 2-ethylhexyl 4-methoxycinnamate) are relatively unstable and susceptible to photodegration, as already mentioned above.

Formulations prepared with incorporation of sunscreens in cream base **A** (Table 3) showed SPF values approximately 50% higher when compared to those formulations incorporated in cream base **B** (Table 4), which were the formulations **M** (19.19; 10.31) and **MS** (20.25; 9.73), respectively. Rheological behavior and pH values also did not change significantly throughout the study, and these data can be correlated with the data for SPF values, especially when formulations were prepared with cream base **A**, which was found to allow preparation of a more stable formulation. Despite the fact that both cream bases used share structural similarities, the formation of micelle-based structures in cream base **A** seems to be more robust than in cream base **B**.

3.3. Analysis of Rheological Behavior of Formulations

Current research shows great interest on the study of the rheological behavior of emulsions, as it is closely related to properties of formulations that define their stability. This study was thus carried out in order to investigate whether rutin succinate could be used in sunscreen formulations as an auxiliary component which could improve the efficacy of photodegradable sunscreens. The ability of these formulations to be applied to skin and variations on FPS and UVA/UVA ratio values, consistency and spreadability were also assessed.

The rheological behavior of the formulations was assessed by analysis of apparent viscosity, flow curve and hysteresis area. After formulation samples have undergone heat and light stress at pre-established periods of time, their resulting rheological behaviors allowed for detection of changes that destabilize the sunscreen formulation, and such changes are not always perceived by organoleptic analyses [15,16].

The tests mentioned above were carried out only with the formulations incorporated in cream base **A**, as the formulations prepared with this cream base showed more favorable results compated with formulations incorporated in cream base **B** for all other tests previously described.

Both self-emulsifying bases (**A** and **B**) are systems with phosphate anionic O/W characteristics; they were chosen for this study as they are good alternative bases for the incorporation of many cosmetic actives, including sunscreens, while also having biomimetic properties, as they have characteristics similar to those of skin phospholipids [20].

Only cream base **A** possesses two phosphoric esters linked to chemical structures of both high and low degree of ethoxylation (dicetyl phosphate and ceteth-10 phosphate), which allowed association with sorbitan monostearate 20 EO (nonionic emulsifier). This combination induces an electrosteric stabilization mechanism, which decreases repulsion of negative charges and consequently reduces the size of micelles [20,21].

Emollients used in the studied formulations were chosen according to their ability to properly spread when applied on skin, covering more area and protecting more skin tissue, and also according to their sensory properties, which should be pleasant to the user, such as the capability of the formulation to cause a dry and velvety feeling when applied. All of these characteristics can be found in volatile silicones (cyclomethicones) associated with other thickener/stabilizer silicones that stabilize micelles (PEG-PPG 18/18 dimethicone and vinyl dimethicone crosspolymer) and with polysiloxanes, responsible for improving the formulation resistance to water.

Table 3. Analysis of photoprotective efficacy as assessed by Normal Stability Test of formulations developed with cream base **A** with addition of chemical and physical filters (**M**), rutin succinate (**S**) or rutin succinate + chemical and physical filters (**MS**).

A													
Time (Days)		**t0**				**t15**				**t30**			
Formulation	**Environmental Condition**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**
S	**G** (5.0 ± 0.5 °C)	1.00	1.80	383 ^{a}	1.005 ^{a}	1.00	2.35	371 ^{b}	1.354 ^{e}	1.00	2.00	381 ^{a,b}	1.227 ^{d}
	E (45.0 ± 0.5 °C)					1.00	2.60	378 ^{b}	1.235 ^{c}	1.00	2.45	377 ^{b}	1.286 ^{b}
	RT (25.0 ± 2.0 °C)					1.00	1.60	380 ^{d}	1.048 ^{b}	1.00	2.75	373 ^{b}	1.062 ^{c}
M	**G** (5.0 ± 0.5 °C)	19.19 ^{a}	16.00	373 ^{a}	0.507 ^{a}	14.76 ^{b}	7.30	369 ^{b}	0.474 ^{b}	20.91 ^{a}	7.55	369 ^{b}	0.462 ^{c}
	E (45.0 ± 0.5 °C)					14.13 ^{b}	13.20	369 ^{b}	0.465 ^{c}	19.89 ^{a}	15.65	369 ^{b}	0.476 ^{b}
	RT (25.0 ± 2.0 °C)					11.44 ^{b}	7.05	357 ^{d}	0.355 ^{e}	20.33 ^{a}	3.00	370 ^{b}	0.488 ^{b}
MS	**G** (5.0 ± 0.5 °C)	20.25 ^{a}	11.00	369 ^{a}	0.449 ^{a}	20.20 ^{a}	11.50	369 ^{a}	0.449 ^{a}	20.00 ^{a}	2.45	369 ^{a}	0.449 ^{a}
	E (45.0 ± 0.5 °C)					20.25 ^{a}	11.10	369 ^{a}	0.450 ^{a,b}	20.73 ^{a}	7.40	368 ^{a}	0.449 ^{a}
	RT (25.0 ± 2.0 °C)					20.22 ^{a}	8.45	369 ^{a}	0.449 ^{a}	20.55 ^{a}	9.55	369 ^{a}	0.449 ^{a}

Time (Days)		**t60**				**t90**				**t0 a t90**		
Formulation	**Environmental Condition**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**% SPF**	**% λ_c**	**% UVA/UVB Ratio**
S	**G** (5.0 ± 0.5 °C)	1.00	1.07	384 ^{a}	1.061 ^{c}	1.00	1.44	373 ^{b}	0.569 ^{b}	-	2.6	43.4
	E (45.0 ± 0.5 °C)	1.00	1.36	385 ^{a}	1.080 ^{d}	1.00	3.24	370 ^{c}	0.359 ^{e}	-	3.4	64.3
	RT (25.0 ± 2.0 °C)	1.00	1.53	384 ^{a}	1.085 ^{d}	1.00	1.76	376 ^{c}	0.634 ^{e}	-	1.8	36.9
M	**G** (5.0 ± 0.5 °C)	13.22 ^{b}	8.30	368 ^{b}	0.426 ^{d}	14.07 ^{b}	13.40	367 ^{b}	0.431 ^{d}	26.7	1.6	15.0
	E (45.0 ± 0.5 °C)	14.00 ^{b}	11.36	368 ^{b}	0.444 ^{d}	12.61 ^{b}	11.53	366 ^{c}	0.415 ^{d}	34.3	1.9	18.1
	RT (25.0 ± 2.0 °C)	10.94 ^{c}	12.82	365 ^{c}	0.438 ^{d}	9.89 ^{c}	8.86	369 ^{b}	0.450 ^{c}	48.5	1.1	11.2
MS	**G** (5.0 ± 0.5 °C)	20.00 ^{a}	8.27	369 ^{a}	0.449 ^{a}	20.72 ^{a}	5.76	369 ^{a}	0.448 ^{a}	2.3	-	0.2
	E (45.0 ± 0.5 °C)	20.57 ^{a}	11.95	369 ^{a}	0.448 ^{a,b}	20.89 ^{a}	9.67	369 ^{a}	0.447 ^{b}	3.2	-	0.4
	RT (25.0 ± 2.0 °C)	20.65 ^{a}	13.60	369 ^{a}	0.449 ^{a}	20.57 ^{a}	13.45	369 ^{a}	0.448 ^{a}	1.6	-	0.2

Legend: % SPF, λc and UVA/UVB ratio: percentage changes of the values for Sun Protection Factor, zero critical wavelength and UVA/UVB ratio, respectively, from the initial time point (t0) to day 90; Mean ± standard deviation (n = 2); Addition of: chemical and physical filters (**M**), rutin succinate (**S**) or rutin succinate and chemical and physical filters (**MS**). **CV**: Coefficient of Variation. Different superscript letters in the same row represent statistically different data according to Fisher LSD test ($p < 0.05$). Probability values were calculated for SPF, λc and UVA/UVB ratio mean values by one-way ANOVA ($p < 0.05$).

Table 4. Analysis of photoprotective efficacy as assessed by Normal Stability Test of formulations developed with cream base **B** with addition of chemical and physical filters (**M**), rutin succinate (**S**) or rutin succinate + chemical and physical filters (**MS**).

B													
Time (Days)		**t0**				**t15**				**t30**			
Formulation	**Environmental Condition**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**
S	**G** (5.0 ± 0.5 °C)	1.00	0.90	393 ^{a}	0.880 ^{a}	1.00	0.60	394 ^{a,b}	0.997 ^{d}	1.00	0.50	394 ^{a,b}	0.913 ^{e}
	E (45.0 ± 0.5 °C)					1.00	0.70	393 ^{a}	1.183 ^{d}	1.00	0.65	395 ^{a}	1.238 ^{c}
	RT (25.0 ± 2.0 °C)					1.00	0.75	395 ^{b}	1.158 ^{c}	1.00	0.75	395 ^{b}	0.946 ^{e}
M	**G** (5.0 ± 0.5 °C)	10.31 ^{a}	14.25	366 ^{a}	0.454 ^{a}	9.56 ^{a}	4.80	367 ^{a}	0.466 ^{c}	10.37 ^{a}	14.65	367 ^{a}	0.447 ^{b}
	E (45.0 ± 0.5 °C)					9.00 ^{a}	7.10	366 ^{a}	0.444 ^{e}	11.00 ^{a}	10.85	366 ^{a}	0.466 ^{b}
	RT (25.0 ± 2.0 °C)					9.89 ^{a}	9.25	366 ^{a}	0.454 ^{a}	9.38 ^{a,b}	12.65	366 ^{a}	0.449 ^{b}
MS	**G** (5.0 ± 0.5 °C)	9.73 ^{a}	11.55	366 ^{a}	0.451 ^{a}	9.52 ^{a}	15.20	366 ^{a}	0.451 ^{a}	9.50 ^{a}	4.55	366 ^{a}	0.450 ^{a}
	E (45.0 ± 0.5 °C)					9.83 ^{a}	6.75	366 ^{a}	0.450 ^{a}	9.33 ^{a}	8.00	366 ^{a}	0.450 ^{a}
	RT (25.0 ± 2.0 °C)					9.89 ^{a}	10.55	366 ^{a}	0.451 ^{a}	9.22 ^{a}	13.20	366 ^{a}	0.451 ^{a}

Time (Days)		**t60**				**t90**				**t0 a t90**		
Formulation	**Environmental Condition**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**SPF**	**CV (%)**	**λ_c (nm)**	**UVA/UVB Ratio**	**% SPF**	**% λ_c**	**% UVA/UVB Ratio**
S	**G** (5.0 ± 0.5 °C)	1.00	0.55	392 ^{a}	1.131 ^{b}	1.00	0.45	395 ^{b}	1.039 ^{c}	-	0.5	18.0
	E (45.0 ± 0.5 °C)	1.00	0.45	393 ^{a}	1.360 ^{b}	1.00	0.85	393 ^{a}	0.982 ^{e}	-	-	11.6
	RT (25.0 ± 2.0 °C)	1.00	0.35	392 ^{a}	1.010 ^{d}	1.00	0.90	395 ^{b}	1.320 ^{b}	-	0.5	50.0
M	**G** (5.0 ± 0.5 °C)	9.89 ^{a}	10.35	367 ^{a}	0.471 ^{d}	9.11 ^{a}	10.35	366 ^{a}	0.454 ^{a}	11.6	-	-
	E (45.0 ± 0.5 °C)	8.78 ^{a}	6.30	366 ^{a}	0.463 ^{c}	9.55 ^{a}	12.95	366 ^{a}	0.448 ^{d}	7.4	-	1.3
	RT (25.0 ± 2.0 °C)	7.22 ^{b}	5.20	366 ^{a}	0.460 ^{c}	8.63 ^{a,b}	8.85	368 ^{b}	0.473 ^{d}	16.3	0.5	4.2
MS	**G** (5.0 ± 0.5 °C)	9.43 ^{a}	8.75	366 ^{a}	0.450 ^{a}	9.83 ^{a}	6.45	366 ^{a}	0.450 ^{a}	1.0	-	-
	E (45.0 ± 0.5 °C)	9.11 ^{a}	7.95	366 ^{a}	0.451 ^{a}	9.32 ^{a}	10.55	366 ^{a}	0.451 ^{a}	4.2	-	-
	RT (25.0 ± 2.0 °C)	9.32 ^{a}	10.35	366 ^{a}	0.451 ^{a}	9.53 ^{a}	12.10	366 ^{a}	0.451 ^{a}	2.1	-	-

Legend: % SPF, λc and UVA/UVB ratio: percentage changes of the values for Sun Protection Factor, zero critical wavelength and UVA/UVB ratio, respectively, from the initial time point (t0) to day 90; Mean ± standard deviation (n = 2); Addition of: chemical and physical filters (**M**), rutin succinate (**S**) or rutin succinate and chemical and physical filters (**MS**). **CV**: Coefficient of Variation. Different superscript letters in the same row represent statistically different data according to Fisher LSD test ($p < 0.05$). Probability values were calculated for SPF, λc and UVA/UVB ratio mean values by one-way ANOVA ($p < 0.05$).

Formulations containing rutin succinate when incorporated into cream base (**A**), associated or not with chemical and physical filters, were found to be stable regarding their apparent viscosity throughout the 90 days of study (Table 5). However, there were increases of apparent viscosity in **M** formulations of 43.4% (0.972 to 1.394 Pa.s) and 90.0% (0.972 to 1.852 Pa.s), when under storage conditions of 5.0 ± 0.5 °C and 45.0 ± 0.5 °C, respectively. Variations of apparent viscosity observed for **S** formulations were not greater than 20%, but significant variations when the formulations were under storage conditions of 45.0 ± 0.5 °C and 25.0 ± 2.0 °C started to occur as soon as after day 15 of study.

Analysis of hysteresis areas from **MS** formulations prepared in cream base **A** resulted in no significant variations throughout the 90-day study period for all storage conditions tested: 5.0 ± 0.5 °C, 45.0 ± 0.5 °C and 25.0 ± 2.0 °C (Table 5). However, **M** formulations showed an increase of hysteresis area of 32.6% (from 34,693 to 45,817 mPa/s) when stored at 5.0 ± 0.5 °C, considering the whole t0 to t90 period. **S** formulations showed an increase of hysteresis area of 50% at the end of the 90-days study period when kept under all three study conditions: 5.0 ± 0.5 °C, 45.0 ± 0.5 °C and 25.0 ± 2.0 °C, and significant changes could be seen as soon as after 15 days of storage. Both apparent viscosity and hysteresis area suffered significant variations when assessed on **M** and **S** formulations after 15 days of storage, which can be taken as indicative signs of instability of their structures.

Table 5. Results for apparent viscosity at 200 s^{-1} and hysteresis area of photoprotective formulations developed with cream base **A** with the addition of chemical and physical filters (**M**), rutin succinate (**S**) and rutin succinate + chemical filters and physical (**MS**) during Normal Stability Test.

				A			
Time (Days)		**t0**	**t15**	**t30**	**t60**	**t90**	
Formulation	**Condition**			**Apparent Viscosity (Pa.s ± DP)**			**% η**
S	**G**	0.773 ± 0.002 ^{a}	0.826 ± 0.011 ^{a}	0.815 ± 0.020 ^{a}	0.828 ± 0.054 ^{a}	0.789 ± 0.001 ^{a}	2.06
	E		0.975 ± 0.078 ^{b}	0.892 ± 0.015 ^{a,b}	0.867 ± 0.054 ^{a,b}	0.834 ± 0.015 ^{a,b}	7.89
	RT		0.893 ± 0.026 ^{a,b}	0.913 ± 0.045 ^{b}	0.765 ± 0.034 ^{a}	0.866 ± 0.060 ^{a,b}	12.03
	Condition			**Hysteresis Área (mPa/s ± DP) % AH**			**% AH**
	G	13,423 ± 345 ^{a}	17,663 ± 435 ^{b}	17,607 ± 379 ^{b}	18,833 ± 691 ^{c}	20,087 ± 990 ^{d}	49.64
	E		23,283 ± 647 ^{c}	21,616 ± 950 ^{b,c}	20,504 ± 246 ^{b,c}	18,567 ± 1983 ^{b}	38.32
	RT		20,232 ± 206 ^{d}	19,963 ± 228 ^{d}	16,555 ± 1024 ^{d}	18,917 ± 46 ^{c}	40.92
	Condition			**Apparent Viscosity (Pa.s ± DP)**			**% η**
M	**G**	0.972 ± 0.088 ^{a}	1.254 ± 0.089 ^{a,b}	1.287 ± 0.033 ^{a,b}	1.295 ± 0.165 ^{a,b}	1.394 ± 0.094 ^{b}	43.41
	E		1.599 ± 0.431 ^{a,b}	1.773 ± 0.092 ^{b}	1.988 ± 0.077 ^{b}	1.852 ± 0.182 ^{b}	90.01
	RT		1.067 ± 0.049 ^{a,b}	1.387 ± 0.0107 ^{b}	1.088 ± 0.132 ^{a,b}	1.032 ± 0.085 ^{a}	6.17
	Condition			**Hysteresis Área (mPa/s ± DP)**			**% AH**
	G	34,693 ± 944 ^{a}	36,657 ± 1772 ^{a}	42,965 ± 313 ^{b}	47,661 ± 398 ^{c}	45,817 ± 1186 ^{b,c}	32.6
	E		32,072 ± 745 ^{c}	32,923 ± 522 ^{b,c}	31,927 ± 302 ^{c}	31,742 ± 354 ^{c}	8.5
	RT		38,127 ± 850 ^{b}	46,007 ± 500 ^{c}	37,063 ± 696 ^{a,b}	43,297 ± 1019 ^{c}	24.08
	Condition			**Apparent Viscosity (Pa.s ± DP)**			**% η**
MS	**G**	1.023 ± 49 ^{a}	1.284 ± 0.012 ^{b}	1.235 ± 0.045 ^{b}	1.237 ± 0.033 ^{b}	1.246 ± 0.088 ^{b}	21.79
	E		1.327 ± 0.092 ^{b}	1.433 ± 0.001 ^{b}	1.342 ± 0.084 ^{b}	1.330 ± 0.024 ^{b}	23.02
	RT		1.154 ± 0.094 ^{a}	1.054 ± 0.004 ^{a}	1.073 ± 0.086 ^{a}	1.049 ± 0.103 ^{a}	2.54
	Condition			**Hysteresis Área (mPA/s ± DP)**			**% AH**
	G	36,581 ± 943 ^{a}	37,779 ± 632 ^{a}	38,967 ± 898 ^{a}	37,412 ± 272 ^{a}	37,938 ± 1800 ^{a}	3.70
	E		37,028 ± 674 ^{a}	36,996 ± 896 ^{a}	38,355 ± 544 ^{a}	37,767 ± 555 ^{a}	3.24
	RT		37,913 ± 39 ^{a}	38,112 ± 472 ^{a}	37,833 ± 470 ^{a}	38,055 ± 932 ^{a}	4.02
P (ANOVA) *		<0.05	<0.05	<0.05	<0.05	<0.05	

Legend: % **η**: percentage changes of the values for apparent viscosity from the initial time point (t0) to the last measurement after 90 days (t90). % **AH**: percentage changes of the values for hysteresis area from the initial time point (t0) to the last measurement after 90 days (t90). **G**: 5.0 ± 0.5 °C; **E**: 45.0 ± 0.5 °C and 75% relative humidity; **RT**: 25.0 ± 2.0 °C under indirect lighting. Mean ± standard deviation (n = 2). Different superscript letters in the same row represent statistically different data according to Fisher LSD test ($p < 0.05$). * Probability values were calculated by one-way ANOVA ($p < 0.05$).

Given the results observed in the rheograms (Figure 1A), we chose to evaluate the different rheological profiles of formulations **S**, **M** and **MS** by adjusting the flow curves according to the power-law mathematical model, also called Ostwald-Waele model ($t = K \cdot \gamma^{n}$). After proper adjustments,

analysis of the new values in correlation with the non-adjusted values resulted in correlation coefficients (R^2) between 0.9900 and 0.9970. Yield stress was considered as being nearly zero, and therefore negligible for further calculations ($\tau_0 = 0$). This model was used in order to determine flow behavior index (n) and consistency index (K), making the above mentioned equation linear ($\log t = \log K + n \log \gamma$), where **n** represents the angular coefficient (slope) and **log K** represents the linear coefficient. All calculations were carried out with the aid of Excel 2003® software (Table 6).

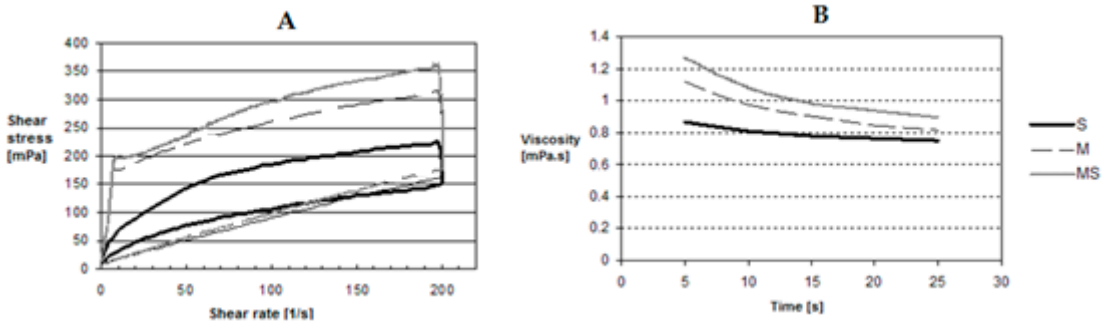

Figure 1. (**A**) Upward and downward flow curves representing hysteresis areas for formulations **S** (rutin succinate), **M** (chemical and physical filters) and **MS** (rutin succinate + chemical and physical filters) obtained 24 h after formulation samples were prepared (t_0). (**B**) Apparent viscosity curves versus time for formulations **S** (rutin succinate), **M** (chemical and physical filters) and **MS** (rutin succinate + chemical and physical filters). Variations of apparent viscosity with time refer to data obtained at a shear rate of 200 s^{-1}.

Table 6. Results for flow behavior index value (*n*) and consistency index (K) of photoprotective formulations developed with cream base **A** with the addition of chemicals and physical filters (**M**), rutin succinate (**S**) and rutin succinate + chemical and physical filters (**MS**) during Normal Stability Test.

A						
Time (Days)		**t0**	**t15**	**t30**	**t60**	**t90**
Formulation	**Condition**	**Flow Behavior Index (Dimension Less ± DP)**				
S	**G**	0.40 ± 0.01 [a]	0.27 ± 0.01 [a]	0.27 ± 0.03 [a]	0.32 ± 0.04 [a]	0.27 ± 0.14 [a]
	E		0.26 ± 0.01 [a]	0.21 ± 0 [a]	0.28 ± 0.02 [a]	0.30 ± 0.03 [a]
	RT		0.29 ± 0.02 [a]	0.28 ± 0.02 [a]	0.28 ± 0.02 [a]	0.27 ± 0 [a]
	Condition	**Consistency Index (mPa.s ± DP)**				
	G	1441 ± 3 [a]	1767 ± 45 [a]	1767 ± 59 [a]	1654 ± 75 [a]	1769 ± 321 [a]
	E		1866 ± 15 [a]	1942 ± 2 [a]	1783 ± 37 [a]	1704 ± 87 [a]
	RT		1846 ± 99 [a]	1800 ± 36 [a]	1696 ± 70 [a]	1801 ± 10 [a]
M	**Condition**	**Flow Behavior Index (Dimension Less ± DP)**				
	G	0.25 ± 0.03 [a]	0.22 ± 0.05 [a]	0.25 ± 0.03 [a]	0.27 ± 0 [a]	0.23 ± 0 [a]
	E		0.26 ± 0.10 [a]	0.25 ± 0.02 [a]	0.17 ± 0.10 [a]	0.15 ± 0.10 [a]
	RT		0.25 ± 0.02 [a]	0.23 ± 0.01 [a]	0.27 ± 0 [a]	0.27 ± 0 [a]
	Condition	**Consistency Index (mPa.s ± DP)**				
	G	1956 ± 11 [a]	2117 ± 0 [a]	2028 ± 9 [a]	1998 ± 0 [a]	2076 ± 55 [a]
	E		2138 ± 47 [a]	2140 ± 61 [a]	2393 ± 55 [a]	2419 ± 19 [a]
	RT		2033 ± 32 [a]	2183 ± 60 [a]	1981 ± 15 [a]	1958 ± 69 [a]
MS	**Condition**	**Flow Behavior Index (Dimension Less ± DP)**				
	G	0.21 ± 0.1 [a]	0.28 ± 0.08 [a]	0.29 ± 0.02 [a]	0.26 ± 0.01 [a]	0.26 ± 0.02 [a]
	E		0.25 ± 0.05 [a]	0.27 ± 0.01 [a]	0.30 ± 0.02 [a]	0.27 ± 0.01 [a]
	RT		0.27 ± 0.03 [a]	0.23 ± 0.01 [a]	0.28 ± 0.02 [a]	0.26 ± 0.01 [a]
	Condition	**Consistency Index (mPa.s ± DP)**				
	G	2032 ± 18 [a]	1987 ± 0 [a]	2183 ± 60 [a]	2019 ± 19 [a]	1776 ± 15 [a]
	E		1974 ± 74 [a]	2072 ± 26 [a]	2021 ± 7 [a]	2085 ± 6 [a]
	RT		2009 ± 82 [a]	2079 ± 16 [a]	1858 ± 19 [a]	1825 ± 6 [a]
P (ANOVA) *		<0.05	<0.05	<0.05	<0.05	<0.05

Legend: G: 5.0 ± 0.5 °C; E: 45.0 ± 0.5 °C and 75% relative humidity; **RT**: 25.0 ± 2.0 °C under indirect lighting. Mean ± standard deviation ($n = 2$). Different superscript letters in the same row represent statistically different data according to Fisher LSD test ($p < 0.05$). * Probability values obtained by one-way ANOVA ($p < 0.05$).

Pseudoplastic materials are characterized by flow curves in which shear stress decreases while shear rate increases [16], a behavior shared by a large number of (bio) technologically relevant systems. Such behavior is a consequence of orientational rearrangements of the internal structure within the flow area, which diminish the material resistance to the applied shear stress [22]. This is found to be the case for the systems studied in this work.

The intensity of this property is reflected on factor **n** of the Ostwald de Waele equation (the lower **n** is from 1.0, the more intense is pseudoplasticity). Table 6 depicts the significant pseudoplasticity of the studied formulations. This is a desirable aspect in formulations meant to be spread on skin, as is the case in this study. Table 6 also displays the consistency indexes K of the assessed formulation samples. The higher the K index, the more consistent, or "viscous", is the formulation. Results show that K values are situated around 2000 mPa.s.

Since viscosity is defined as the ratio between shear stress and shear rate, $\eta = d\tau/(d\gamma/dt)$, in the case of pseudoplastic fluids, it necessarily diminishes as flow curves are drawn. That is the why viscosity of non-ideal fluids (pseudoplastic included) is called "apparent viscosity"; for such systems, viscosity does not vary independently, as it essentially depends on either shear rate or shear stress applied [16,22].

Another characteristic observed in the studied emulsions was that apparent viscosities varied with time. In the formulation samples assessed, apparent viscosities at a constant shear rate (200 s^{-1}) diminished with time (Figure 1B), which characterizes the so-called thixotropic behavior (this shear rate was chosen due to the fact that it closely resembles the shear rate of a formulation being spread on skin). Such behavior is desirable, considering that a decrease of viscosity with time might speed up the absorption process at the skin when rubbing is required during application. Thixotropy is a property of a number of different systems, such as dispersions and emulsions.

Viscosity variations during storage reflect internal microstructural changes in the formulations. In some cases, decrease of viscosity may lead to unavoidable effects such as flocculation, coalescence or sedimentation, which, however, have not been observed for the formulations studied in this work.

Some authors, such as Brummer (2006) [23] and Tadros (2004) [16], discuss the relevance of thixotropy (phenomenon where apparent viscosity decreases with time) (Figure 1B) and thinning (pseudoplastic behavior, phenomenon where apparent viscosity decreases while shear rate increases). They correlate both phenomena to the gradual breakdown of the structure of formulations and flattening of emulsion droplets leading to possible breakage of aggregates, thus aligning molecules and droplets within a flow. For consumers, this would mean the formulation becomes more fluid, spreading more easily and without sagging when applied on skin, forming a homogeneous film that comes in intimate contact with the skin microrelief. As a result, an increase in product effectiveness would occur due to both the release of filters during the process where structural changes of the emulsions take place and to the longer time available for a better accommodation of the product on the skin, as indicated by the higher hysteresis area observed (anionic phosphate cosmetic base employed) [16,22].

Larger hysteresis areas indicate that the return to original structural conditions while shear application decreases takes longer for some samples (e.g., greater hysteresis area for **MS** formulations = 36,581 mPa/s compared to **S** formulations = 13,423 mPa/s). The differences of behavior between these formulations could be associated with the presence of a dispersed physical filter in the **MS** formulation that impairs recovery of the structural system.

Structural recovery of the **MS** formulation was constant throughout the whole study period of 90 days. The variations on hysteresis area values determined for the **M** formulation, however, were greater for most conditions tested and the variations in its hysteresis areas were also found to be greater than those observed for formulations **MS** and **S**. This suggests that rutin succinate, which is present in formulations **MS** and **S**, might have improved the alignment of the droplet stream. This could have been partly due to the nature of rutin succinate hydrophilic ($\log P = -1.13 \pm 0.02$) [17], and

to its role in the inhibition of peroxyl radicals formation, which could react with components of the oil phase, destabilizing the system and ultimately affecting its rheological behavior.

4. Conclusions

Development of formulations of improved photoprotective properties was possible due to the addition of rutin succinate to traditional sunscreen filters. Physicochemical and functional parameters did not suffer variations due to different storage conditions as assessed by the Normal Stability Test. It could be observed that the formulations containing rutin succinate associated with sunscreen filters that suffered the least overall variations (less than 10%) were the ones prepared with cosmetic base A (auto-emulsifying base with phosphated anionic properties, which emulate the properties of epithelial tissue).

Results also show that addition of rutin succinate to photoprotective formulations containing 2-ethylhexyl 4-methoxycinnamate and 2-hydroxy-4-methoxybenzophenone stabilized the process of photodegradation of these filters; this preserved the functionality of the filters by inhibiting formation of peroxyl radicals that might cause photoinstability. This can be concluded by assessing parameters of in vitro photoprotective efficacy, as the formulation with the highest SPF was the one containing rutin succinate (MS), which also suffered no significant variations of hysteresis area throughout the whole study period.

Besides, the pseudoplastic and thixotropic properties of this formulation remained constant during all of the storage time, indicating it possesses desirable rheological properties. When applied on skin, a homogeneous film would be formed and properly spread, allowing the filters to be dispersed and delivered as the formulation reversibly organizes/disorganizes its internal structure.

Finally, such properties here described are vital for sunscreen formulations to remain stable and to maintain photoprotective functionality, while also ensuring desirable rheological properties that are well received by consumers.

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Conflicts of Interest: The authors declare no conflict of interest.

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