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## ORIGINAL ARTICLE

# Development and stability studies of sunscreen cream formulations containing three photo-protective filters



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## KEYWORDS

Sunscreen cream;  
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**Abstract** The present study aimed to formulate and subsequently evaluate sunscreen cream (W/O/W emulsion) containing three photo-protective filters: benzophenone-3, ethylhexyl methoxycinnamate and titanium dioxide at different percentages. Formulations were stored at 8, 25 and 40 °C for four weeks to investigate their stability. Color, centrifugation, liquefaction, phase separation, pH and Sun Protection Factor (SPF) of sunscreen cream formulations were determined. The microbiological stability of the creams was also evaluated and the organoleptic quality was carried out for 28 days. Interestingly, the combination of 7% Benzophenone-3, 7% Ethylhexyl methoxycinnamate and 6% Titanium dioxide preserved physicochemical properties of the product and was efficient against the development of different spoilage microorganisms as well as aerobic plate counts, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and yeast and mold counts. Furthermore, a good stability was observed for all formulations throughout the experimental period. The newly formulated sunscreen cream was proved to exhibit a number of promising properties and attributes that might open new opportunities for the development of more efficient, safe, and cost-effective skin-care, cosmetic, and pharmaceutical products.

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## 1. Introduction

Sunlight is composed of wavelengths ranging from ultraviolet light to visible light. Ultraviolet (UV) is divided into UVA (320–400 nm), UVB (290–320 nm) and UVC (100–290 nm) (Hanson et al., 2006). Exposure to solar radiation has negative effects on the human skin. Among all, UV is the most harmful to the skin and causes sunburns and skin cancer after long-term exposure (Francis et al., 1998).

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Organic substances containing chemical groups that can filter UVA and UVB radiations are used as active ingredients of sunscreen formulations (Hanson et al., 2006; Nohynek and Schaefer, 2001; Ibrahim and Brown, 2008). UV absorbers with an intramolecular hydrogen bridge are widely employed as additives against UV radiation (Fluegge et al., 2007; Paterson et al., 2005). Accordingly, to make skin protection highly effective and prevent skin cancer and other types of skin damage, the sunscreens must involve appropriate sun-blocking agents and/or preparations that contain combinations of these active substances (Hanson et al., 2006; Nohynek and Schaefer, 2001; Palm and O'Donoghue, 2007; Pescia et al., 2012). Some of the approved compounds for use in the manufacture formulation are benzophenone-3, ethylhexyl methoxycinnamate and titanium dioxide.

Benzophenone-3 utilizes an excited state intramolecular proton transfer from a hydroxyl group to dissipate light energy. It was supposed to dissipate the absorbed light energy in a harmless manner, indeed benzophenone-3 converts the absorbed photon energy into heat without chemical damage (Schnabel and Kiwi, 1978). Benzophenone-3 has strong absorption in 280–340 nm (UV-B) range.

The photochemical behavior of ethylhexyl-p-methoxycinnamate represents the most widely used sunscreen compound (Hayden et al., 1998). The photo-induced degradation of ethylhexyl-p-methoxycinnamate in emulsion formulations has been demonstrated by many researchers (De Flandre and Lang, 1988).

The increasing demand of inorganic UV filters as titanium dioxide, known to block UVB/UVA sunlight (Serpone et al., 2007), is related to their low potential for producing irritant reactions and to their sunscreen efficacy (Serpone et al., 2007).

The present work was undertaken to investigate the potential effects of using three photo-protective chemicals, benzophenone-3, ethylhexyl methoxycinnamate and titanium dioxide, with regard to the continuous search for an enhanced formulation of cosmetic and pharmaceutical emulsions and, if any, to submit it to a battery of well-established tests for consistency and potential industrial application.

Accordingly, the present study was carried out to investigate the physical and microbiological stability of W/O/W emulsions containing three photo-protective filters: benzophenone-3, ethylhexyl methoxycinnamate and titanium dioxide at different percentages in order to make a comparative assessment of these active principles.

## 2. Materials and methods

### 2.1. Preparation of emulsions

Table 1 shows the components and concentrations of (W/O/W) multiple emulsions. The formulations used in this study are named  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$ . Table 2 shows trade name, chemical name and functions of the raw materials which are used in the formulation preparation. The oil phase consisted of Croduret PEG-40, Viatenza® Argan PE8, Lipex® Shea WL and Ethyl Paraben USP24/NF19 heated up to  $75 \pm 0.5^\circ\text{C}$ . At the same time, two aqueous phases, i.e. Uvinul® M40, Uvinul® MC 80, Micro titanium dioxide JMT-150AO, Lanette®O, Crodamol ICS, Eumulgin B2, Crodamol IPP and Crodamol GTCC and distillate water (the second aqueous phase),

**Table 1** Components and concentrations of the formulations.

Ingredients	Formulations			
	$F_1$ (%)	$F_2$ (%)	$F_3$ (%)	$F_4$ (%)
Benzophenone-3	10	5	5	7
Ethylhexyl methoxycinnamate	5	10	5	7
Titanium dioxide	5	5	10	6
Cetearyl alcohol	3.5	3.5	3.5	3.5
Glycerol monostearate	3.5	3.5	3.5	3.5
Cetareth-25	1	1	1	1
Isopropyl palmitate	1.5	1.5	1.5	1.5
Caprylic/capric triglyceride	1.5	1.5	1.5	1.5
Polyethylene glycol	1	1	1	1
Ethyl paraben	0.5	0.5	0.5	0.5
Distillate water	67.5	67.5	67.5	67.5

were heated to the same temperature. After that, the first aqueous phase was added to the oil phase using a mechanical stirrer with constant stirring at 2000 rpm for 15 min until the aqueous phase was added completely. Then the (W/O) phase was added to the second phase drop by drop. Finally, for homogenization, the speed of the stirrer was decreased to 1000 rpm until the emulsion was cooled to room temperature.

### 2.2. Physical analysis

The obtained emulsions were submitted to a set of organoleptic (color, look, feel, thickness) and physical (phase separation and creaming) analyses (Akhtar et al., 2011).

### 2.3. Stability tests

Stability tests were achieved at different conditions for emulsions to explore the effect of these conditions on the storage of emulsions. These tests were performed on samples kept at  $8^\circ\text{C} \pm 2^\circ\text{C}$ ,  $25^\circ\text{C} \pm 2^\circ\text{C}$  and  $40^\circ\text{C} \pm 2^\circ\text{C}$ . Color, phase separation and liquefaction of emulsions were observed at various time intervals during 28 days.

#### 2.3.1. Centrifugation tests

Centrifugal tests were performed for emulsions directly after preparation. Those tests were repeated after 1 day, 7 days, 14 days, 21 days, and 28 days of storage. They were performed at 5000 rpm and  $25^\circ\text{C}$  for 10 min by placing 10 g of each sample in centrifugal tubes.

#### 2.3.2. pH determination

The pH value of various emulsions stored at different conditions was determined using a digital pH Meter. The pH tests were repeated for multiple emulsions after 1 day, 3 days, 7 days, 14 days, 21 days, and 28 days of storage.

### 2.4. Determination of *in vitro* SPF of sunscreen cream

SPF was determined in the samples in which the previous assay of organoleptic characteristic evaluation was performed, as well on days 1, 2, 5, 8, 12 and 15. To do so, samples were prepared according to the method proposed by Dutra et al. (2004). In this manner, 0.5 g of each sample was mixed with an appropriate amount of distilled water to obtain a final concentration of  $0.2 \times 10^{-4}$  g/ml. Briefly, samples were dispersed

**Table 2** Chemical name, trade name and functions of raw materials used in the preparation of the formulations.

Chemical name	Trade name	Properties
Benzophenone-3	Uvinul® M40	A broad-band UV filter and anti-aging
Ethylhexyl methoxycinnamate	Uvinul® MC 80	UVB filter
Titanium dioxide	Micro titanium dioxide JMT-150AO	Negative charging/highly hydrophobic, exhibits water repellency and surface preparation agent
Cetearyl alcohol	Lanette ®O	Viscosity regulation in cosmetic O/W emulsions
Isocetyl stearate	Crodamol ICS	Emollient with spreading capacity, lubricant and solvent.
Ceteareth-25	Eumulgin B <sub>2</sub>	Non-ionic emulsifier for O/W emulsions.
Isopropyl palmitate	Crodamol IPP	Non-occlusive emollient and excellent dispersing medium.
Caprylic/capric triglyceride	Crodamol GTCC	Emollient, lubricant and solvent
Polyethylene glycol	Croduret PEG-40	O/W emulsifier, effective solubilizer and wetting agent
Argan oil PEG-8 esters	Viatenza® Argan PE8	Emollient and emulsifying agent
Shea butter oleyl esters	Lipex® shea WL	Offers low viscosity, intermediate spreading and provides high moisturization
Ethyl p-hydroxybenzoate	Ethyl paraben USP24/NF19	Microbiological preservative

in 100 ml of distilled water and were homogenized by ultrasonication for 5 min. The obtained dispersion was filtered with a filter paper and the first 10 ml was rejected. Then 2 ml of filtered solution was adjusted to 50 ml using distilled water. The absorbance of each sample was determined by spectrophotometry in the range of 290–320 nm (UVB), with 5 nm intervals, using distilled water as blank. A fresh sunscreen sample (not submitted to temperature effect) was used as control, in order to establish initial SPF. Three replicates of each group were performed. The SPF of each sample was determined with the data obtained by spectrophotometric analysis, using the Mansur equation:

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

where: CF: correction factor (= 10); EE ( $\lambda$ ): erythral effect spectrum; I ( $\lambda$ ): solar intensity spectrum; and Abs ( $\lambda$ ): absorbance of sunscreen product (Mansur et al., 1986).

## 2.5. Microbiological stability

One gram of emulsion was dispersed in a 4 ml sterile Ringer's solution containing 0.25% tween 80. Six dilutions were made in the same dispersing vehicle, and 0.1 ml was plated out on the appropriate solid medium using the surface viable method. Colonies were counted after the incubation and all operations were carried out in duplicates (ISO NF- 21148, 2000).

### 2.5.1. Aerobic plate count

Aerobic plate counts were determined by inoculating 0.1 ml of the homogenate sample onto triplicate sterile plates of pre-poured and dried Standard Method Agar. Then, plates were incubated for 48 h at 35 °C (ISO NF- 21149, 2006). Duplicates of each dilution (1 ml) of neutralized and non neutralized samples were pour-plated using Standard Method Agar (Oxoid, Basingstoke, Hampshire, England) and incubated at 30 °C for 48 h. Plates containing 25–250 colonies were counted and the average number of CFU/g was calculated.

### 2.5.2. *P. aeruginosa* count

*Pseudomonas aeruginosa* were enumerated on *Pseudomonas* Agar Base (CM 559, Oxoid) supplemented with fucidin, cephaloridine and cetrinide, providing a selective medium for *P.*

*aeruginosa*. Colonies were counted after two days of incubation at 25 °C (ISO NF- 22717, 2006).

### 2.5.3. *S. aureus*

Population of *S. aureus* was determined by standard plating methods (ISO NF- 22718, 2008). Colonies of *Staphylococcus* were selected, gram-stained, and observed for oxidase and catalase reactions to confirm their presence. All microbial counts were transformed into logarithms of the number of colony-forming units (log<sub>10</sub> CFU/g).

### 2.5.4. Yeast and mold counts

The method involved enumeration of colonies on the Sabouraud dextrose chloramphenicol agar medium. Enumeration was carried out as a pour plate, surface spread, or membrane filtration method (ISO NF- 16212, 2008). Microbiological tests were repeated for formulations at 25 °C after 0, 7, 14, 21 and 28 days of preparation.

## 2.6. Statistical analysis

All measurements were repeated in triplicates and microbial counts were transformed into logarithms of the number of CFU (log<sub>10</sub> CFU/g). Data were subjected to analysis of variance (ANOVA) of the Statistical Analysis System software of SAS Institute using the General Linear Models procedure (SAS, 1990). Differences among the mean values of different treatments and storage times were achieved by the least significant difference (LSD) test. The significance was defined at  $P < 0.05$  and the differences which are equal to or more than the identified LSD values are considered statistically significant.

## 3. Results and discussion

### 3.1. Stability of formulated emulsions

(W/O/W) emulsions are of interest in a number of application and research areas. In cosmetic research, the emphasis has been placed on double emulsions as delivery for various activities (Shum et al., 2008).

In this study, formulations were placed in different storage conditions (8, 25 and 40 °C) for a period of four weeks in

**Table 3** Physical characteristics of  $F_1$  and  $F_2$ , formulations kept at  $8 \pm 2^\circ\text{C}$ ,  $25 \pm 2^\circ\text{C}$  and  $40 \pm 2^\circ\text{C}$ .

		Fresh		24 h		3 day		7 day		14 day		21 day		28 day	
		$F_1$	$F_2$	$F_1$	$F_2$	$F_1$	$F_2$	$F_1$	$F_2$	$F_1$	$F_2$	$F_1$	$F_2$	$F_1$	$F_2$
Liquefaction	8 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	40 °C	–	–	–	–	–	–	–	–	–	–	–	–	+	+
Color	8 °C	PY	SPW	PY	SPW	PY	SPW	PY	SPW	PY	SPW	PY	SPW	YW	SPW
	25 °C	PY	SPW	PY	SPW	PY	SPW	PY	SPW	PY	SPW	PY	SPW	YW	W
	40 °C	PY	SPW	PY	SPW	PY	SPW	PY	SPW	PY	SPW	PY	W	YW	W
Phase separation	8 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	40 °C	–	–	–	–	–	–	–	–	–	–	+	–	+	–
Centrifugation	8 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	40 °C	–	–	–	–	–	–	–	–	–	–	+	–	+	–

– = No change; + = Slight change; PY = Pale yellow; SYW = Soft yellowish white; YW = Yellowish white; Y = Yellow; W = White.

**Table 4** Physical characteristics of  $F_3$  and  $F_4$ , formulations kept at  $8 \pm 2^\circ\text{C}$ ,  $25 \pm 2^\circ\text{C}$  and  $40 \pm 2^\circ\text{C}$ .

		Fresh		24 h		3 day		7 day		14 day		21 day		28 day	
		$F_3$	$F_4$	$F_3$	$F_4$	$F_3$	$F_4$	$F_3$	$F_4$	$F_3$	$F_4$	$F_3$	$F_4$	$F_3$	$F_4$
Liquefaction	8 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	40 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Color	8 °C	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
	25 °C	W	YW	W	YW	W	YW	W	YW	SYW	YW	SYW	YW	SYW	YW
	40 °C	W	YW	W	YW	SYW	YW	SYW	YW	SYW	YW	SYW	YW	SYW	YW
Phase separation	8 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	40 °C	–	–	–	–	–	–	–	–	–	–	+	–	+	–
Centrifugation	8 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	25 °C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	40 °C	–	–	–	–	–	–	–	–	–	–	+	–	+	–

– = No change; + = Slight change; PY = Pale yellow; SYW = Soft yellowish white; YW = Yellowish white; Y = Yellow; W = White.

stability chambers. Color, liquefaction and phase separation changes were presented in Tables 3 and 4.

### 3.1.1. Color

The findings revealed that the freshly prepared emulsions were pale yellow, soft yellowish white, yellowish white and white in color for  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$ . Little changes in color were observed for emulsions  $F_1$ ,  $F_2$  and  $F_3$ , as well as the end of storage period is characterized by the following colors: soft yellowish white, white and yellowish white (Tables 3 and 4). For example, for  $F_1$ , the change in color was observed from the 21st day. This change was presumably due to the oily phase separation promoted at higher temperature. Interestingly, no change in color was observed for  $F_4$  at the different storage conditions: 8, 25 and  $40 \pm 2^\circ\text{C}$ , up to 28 days of observation.

### 3.1.2. Liquefaction

The viscosity of emulsion is often reported to play a vital role in its flow properties (Nasirideen et al., 1998). Starting from the emulsion preparation, the temperature and time processes

begin to contribute to its separation, leading to a decrease in viscosity which results in liquefaction increase (Herbert et al., 1988). As far as the findings of the present study, no liquefaction was observed for the emulsions in any of the storage conditions under investigation, i.e., 8, 25 and  $40 \pm 2^\circ\text{C}$  during the 28 days of observation. The absence of liquefaction provided strong evidence for the stability of the emulsions under investigation.

### 3.1.3. Phase separation test

Creaming leads to phase separation and is often attributed to density differences between the two phases under the influence of gravity (Derick, 2000). The findings of this present work revealed that all the formulation samples were stable in all storage conditions, i.e., 8, 25 and  $40 \pm 2^\circ\text{C}$  during the 28 days of the observation period.

### 3.1.4. Centrifugation test

No phase separation was observed after centrifugation in any of the samples stored at different conditions up to 21 days. A

weak phase separation was, however, recorded on the 21st day and up to the 28th day for preparation of  $F_1$  and  $F_3$  kept at 40 °C. No other phase separation was observed till the end of the experimental period. This was presumably due to the proper homogenization speed during emulsion formulation which might have prevented the breakage of the formulations during testing (Abdurahman and Rosli, 2006).

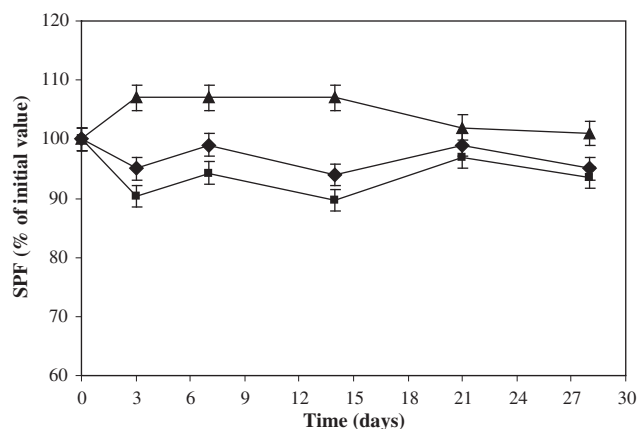
### 3.1.5. pH value determination

Monitoring the pH value is crucial for determining the emulsions' stability. In fact, pH changes indicate the occurrence of chemical reactions that can give an idea on the quality of the final product. Furthermore, the most important parts of chemical stability are performances on accelerated testing and kinetics of pH profiles (Issa et al., 2000).

The pH of human skin normally ranges from 4.5 to 6.0. Therefore, in order for a formulation to possibly gain admission for industrial application, it should have a pH that is included into this range (Matousek et al., 2003). Emulsions formulated in this work had a pH value of 6.1, which is close to the neutral pH. Moreover, the pH of the various emulsion samples stored at various storage conditions, i.e. 8, 25 and 40 °C, were noted to undergo a continuous decrease up to one month of observation (data not shown). The emulsions had stable pH values for almost all conditions tested (data not shown). In the end of storage, at 40 °C, a statistically significant decrease in the pH of the emulsion was observed. The high temperature contributes to the destabilization of the emulsion by hydrolysis, but it did not affect the overall quality of emulsions because the pH values remained around pH 6.0, which is an acceptable and non skin irritating pH value.

### 3.2. Evaluation of the sun protection factor

Since the formulation  $F_4$  seemed to have the best properties during stability tests, its sun protection factor SPF was calculated in predetermined days by applying Mansur equation (Mansur et al., 1986). Fig. 1 represents the variation of SPF of the sunscreen emulsion determined upon exposure to different temperatures 8 + 2 °C, 25 + 2 °C and 40 + 2 °C during the course of study (28 days). An initial SPF determination was performed in a fresh sample of sunscreen (prior to any



**Figure 1** Sun protection factor (SPF) variation of  $F_4$  during sunscreen incubation under different conditions: (■) 8 ± 2 °C, (◆) 25 ± 2 °C; and (▲) 40 ± 2 °C.

temperature exposure), which was considered to correspond to 100% SPF.

Generally, SPF values remained stable throughout the whole period of study. However, when the sunscreen was exposed to the temperature at 8 ± 2 °C, upon 3 days, a slight decrease of approximately 5% in SPF was identified ( $P < 0.05$ ) compared to the initial SPF value. A similar SPF reduction (6.5%) was perceived in the group of 25 + 2 °C, when comparing initial SPF with the one measured on day 28 ( $P < 0.05$ ). Nevertheless, in spite of the statistical significance of the values, these determinations do not compromise the general trend of results, which indicate the maintenance of the SPF.

The SPF variation of formulations  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$  at 8 + 2 °C, 25 + 2 °C and 40 °C + 2 °C, upon 28 days of exposition (data not shown), was obtained by comparison with the fresh sample not subjected to temperature effect, assumed as 100%. In fact, final SPF does not display accentuated alterations either when comparing the result of the experimental groups with the initial SPF or when comparing experimental groups themselves. An exception occurs for the maximum average temperature as compared to the initial SPF value, as previously referred, which is significant ( $P < 0.05$ ).

Although there are many studies concerning the determination of SPF in sunscreen of various semisolid dosage forms (lotion, milk and cream), most of them do not address the issue of their behavior when packages are exposed to the effect of high temperatures. Deccache, describes that a sunscreen in the form of gel did not exhibit significant SPF variations during a period of two weeks either at 25 °C or at 40 °C (Deccache et al., 2010).

### 3.3. Microbiological evaluation

#### 3.3.1. Aerobic plate count

The log mean count recorded for the Aerobic plate count of samples on day 0 was about 2.01 log<sub>10</sub> CFU/ g. On day 28 of storage, the log mean count of Aerobic plate count reached 4.33, 4.3, 3.7 and 3.33 for  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$ , respectively, which did not approximate the maximum limit of 6,9 log<sub>10</sub> CFU/g for Aerobic plate count recommended by ISO NF- 21149 (2006) in processed cosmetics (Table 5).

#### 3.3.2. *P. aeruginosa* and *S. aureus* counts

The results from the *Pseudomonas* and *S. aureus* detection tests were negative, thus confirming that all formulated emulsions met the conventional standards specified with regard to fitness for human consumption (ISO NF- 22717, 2006; ISO NF- 22718, 2008). (Table 5).

#### 3.3.3. Yeast and mold counts

Yeast and molds have been tested in cosmetic products to assess microbiological safety and product quality during processing and storage (ISO NF- 16212, 2008). The levels of these microorganisms were noted to remain under the standard limit. In fact, the initial yeast and mold counts recorded for all treatments were under the detection limit (ISO NF- 16212, 2008).

Moreover, the yeast and mold count values recorded for the formulated sample  $F_4$  were noted to show delayed proliferation when compared to  $F_1$ ,  $F_2$  and  $F_3$  (Table 5).

In conclusion, the combination of 7% benzophenone-3, 7% ethylhexyl methoxycinnamate and 6% titanium dioxide seems

**Table 5** Microbial load of aerobic plate count, *P. spp.*, *S. aureus* and Yeast and molds count of  $F_1$ ,  $F_2$ ,  $F_3$  and  $F_4$  during 28 days of storage at  $25 \pm 2^\circ\text{C}$ .

	Days of storage at $25 \pm 2^\circ\text{C}$				
	0	7	14	21	28
<i>Aerobic plate count</i>					
$F_1$	$2.0 \pm 0.30^a$	$2.43 \pm 0.34^c$	$2.85 \pm 0.26^b$	$3.19 \pm 0.19^b$	$3.43 \pm 0.30^c$
$F_2$	$2.02 \pm 0.31^a$	$2.15 \pm 0.37^a$	$2.36 \pm 0.18^a$	$2.98 \pm 0.22^a$	$3.03 \pm 0.29^b$
$F_3$	$2.04 \pm 0.25^a$	$2.28 \pm 0.29^b$	$2.88 \pm 0.17^b$	$3.15 \pm 0.18^b$	$3.40 \pm 0.18^c$
$F_4$	$2.01 \pm 0.27^a$	$2.11 \pm 0.19^a$	$2.34 \pm 0.15^a$	$2.96 \pm 0.15^a$	$3.33 \pm 0.11^a$
<i>P. spp.</i>					
$F_1$	<1	<1	<1	<1	<1
$F_2$	<1	<1	<1	<1	<1
$F_3$	<1	<1	<1	<1	<1
$F_4$	<1	<1	<1	<1	<1
<i>S. aureus</i>					
$F_1$	<1	<1	<1	<1	<1
$F_2$	<1	<1	<1	<1	<1
$F_3$	<1	<1	<1	<1	<1
$F_4$	<1	<1	<1	<1	<1
<i>Yeast and molds</i>					
$F_1$	$1.12 \pm 0.28^a$	$1.42 \pm 0.22^a$	$1.56 \pm 0.15^a$	$1.79 \pm 0.39^{a,b}$	$1.8 \pm 0.22^a$
$F_2$	$1.14 \pm 0.16^a$	$1.55 \pm 0.22^b$	$1.78 \pm 0.14^c$	$1.88 \pm 0.27^a$	$1.98 \pm 0.27^c$
$F_3$	$1.11 \pm 0.11^a$	$1.4 \pm 0.15^a$	$1.61 \pm 0.16^a$	$1.82 \pm 0.17^a$	$1.89 \pm 0.23^b$
$F_4$	$1.13 \pm 0.16^a$	$1.37 \pm 0.22^a$	$1.49 \pm 0.14^b$	$1.72 \pm 0.27^b$	$1.79 \pm 0.27^a$

±: Standard deviation of three replicates.

CFU: Colony-forming units.

<sup>a-c</sup> Averages for different microbial analyses with different letters in the same column are different ( $P < 0.05$ ).

to be very interesting since it preserved physicochemical properties of the product and was efficient against the growth of different spoilage microorganisms. It should be noted that the maximum authorized levels are 7.5% for -Ethylhexyl 4-methoxycinnamate are 7% for benzophenone-3 according to FDA (FDA, 1999) and 25% for titanium dioxide (Salvador and Chisvert, 2005) in the  $F_4$  formulation.

#### 4. Conclusion

The findings presented in the current study indicated that sunscreen cream (W/O/W emulsion) containing three photo-protective chemicals: benzophenone-3, ethylhexyl methoxycinnamate and titanium dioxide at different percentages yields good physical characteristics and microbiological stability, thus providing a safe and stable emulsion delivery system. Formulations and subsequent evaluation of the cosmetic emulsions from the photo-protective filters presented here, showed no phase separation in emulsions at different storage conditions during 28 days except for formulation  $F_1$  at  $40 \pm 2^\circ\text{C}$ . Emulsion liquefaction started in the emulsions at increased temperatures after the 28th day of storage for formulations  $F_1$  and  $F_2$ . On centrifugation, the phase separation was noted in both  $F_1$  and  $F_2$ , to start after the 21st day of storage at  $40^\circ\text{C}$ . Furthermore, the multiple emulsions prepared in this work had a pH value of 6.5, which is close to the neutral pH. On the other hand, SPF values of  $F_4$ , which seemed to be the more interesting formulation, remained stable throughout the whole period of study. Microbiological assays (Aerobic plate count, *P. aeruginosa*, *S. aureus*, and yeast and mold counts) on elaborated sunscreen cream revealed that the formulation  $F_4$  was stable during storage at  $25 \pm 2^\circ\text{C}$ .

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