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Photostability and efficacy studies of topical formulations containing UV-filters combination and vitamins A, C and E

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Abstract

It is already known that the photostability of a sunscreen is important for its performance on human skin. On the other hand, there are many formulations besides sunscreens containing combinations of UV-filters and daily use active substances with other claims like hydration and anti-aging effects. Vitamins A, C and E are frequently added in these kinds of products and it is not known if the UV-filters have some influence on the hydration and anti-aging effects of these vitamins on the skin as well as on their stability mainly when photounstable UV-filters like avobenzone and octyl methoxycinnamate are present in the formulation. Thus, the aim of this study was to evaluate the influence of two different UV-filters combinations, a photostable and a photounstable one, on the photostability as well as on the efficacy of a formulation containing vitamin A, C and E derivatives. The formulations that were investigated contained or not (vehicle: formulation 1) a combination of 0.6 % (w/w) vitamin A palmitate (1,700,000 UI/g), 2 % (w/w) vitamin E acetate and 2% (w/w) ascorbyl tetraisopalmitate (formulation 2) supplemented with a photounstable UV filter combination octyl methoxycinnamate (OMC), avobenzone (AVB) and 4-methylbenzilidene camphor (MBC) (formulation 3) or with a photostable UV filter combination OMC, benzophenone-3 (BP-3) and octocrylene (OC) (formulation 4). In the photostability studies, all formulations were spread onto a glass plate and exposed to UVA/UVB irradiation. The filter components and vitamins were quantified by HPLC analysis with detection at 325 and 235 nm and by spectrophotometry. To simulate the effects of these formulations daily use, all of them (formulations 1–4) were applied on the dorsum of hairless mice, which were submitted to a controlled light–dark cycle (and were not irradiated), once a day for 5 days. Transepidermal water loss (TEWL), water content of the stratum corneum and viscoelastic properties of the skin were analyzed by using different non-invasive Biophysics Techniques in order to evaluate hydration and anti-aging effects of these formulations as well as erythema to assess skin irritation. Histopathology, viable epidermal thickness as well as the number of epidermal cell layers were also evaluated. It was observed that both UV filters combinations (photounstable one containing OMC, AVB and MBC and photostable one containing OMC, BP-3 and OC) enhanced vitamin A photostability and F4 was more photostable than F3, in terms of vitamin A. *In vivo* efficacy studies showed that F2, F3 and F4 enhanced the viable epidermal thickness, the number of epidermal cell layers, TEWL and Uv/Ue parameter, when compared to the vehicle, which can suggest that they enhanced viable epidermis hydration and acted in cell renewal. However formulation 2 (containing only vitamins), which was the most photounstable formulation, provoked an irritation on hairless mouse skin, and consequently it cannot be considered as safe as the other formulations. It can be concluded that both UV filters combinations did not influence the hydration and anti-aging effects of the formulations containing the vitamins under study and reduced the skin irritation observed when the vitamins were present in the formulation. In addition, the photostable UV-filters combination had the highest recovery of vitamin A in the photostability studies. Finally, it could be suggested that the presence of UV-filters can be considered interesting for the reduction of skin irritation and the most suitable formulation was the one containing the combinations of vitamins A, C and E with photostable UV-filters.

Keywords: Sunscreens; Photostability; Vitamins A, E and C derivatives; TEWL; Skin hydration; Skin viscoelasticity

1. Introduction

Besides UV-induced skin cancers, chronic sun exposure is also responsible for the photoaging process characterized by the formation of deep wrinkles, abnormal pigmentation and a leathery aspect of the skin (Kligman, 1986), consequently, there are

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many daily use products containing not only active substances for anti-aging treatment but also UV-filters. On the other hand, sunscreens containing only organic UV filters are useful to protect the skin from UV-induced damage, but their protection is not ideal because of inadequate use, incomplete spectral protection and toxicity. Since skin is naturally protected from photodamage by its antioxidants (F'guyer et al., 2003), when there is excessive sun exposure, the body may not be able to completely neutralize the free radicals generated by UV exposure, which consequently can lead to many damages. This way, topical application and systemic administration of antioxidants acting as photoprotectives could maintain or restore a healthy skin barrier (Pinnell, 2003). Among the frequently used antioxidants in anti-aging products we can point out vitamin A, C and E derivatives. Vitamin A palmitate acts on epithelization in dry and rough skin, as well as on keratinization considered being abnormal (Maia Campos et al., 1999) and it also absorbs UV radiation between 300 and 350, with a maximum at 325 nm (Antille et al., 2003), which can suggest that it may have a biologically relevant filter activity as well. Vitamin E acetate is a free radical scavenger and can reduce DNA damage and keratinocytes death (sunburn cell formation) (Mcvean and Liebler, 1997; Gaspar and Maia Campos, 2003). In addition, it can enhance stratum corneum hydration (Gehring et al., 1998) and reduce skin roughness (Mayer, 1993). Ascorbic acid is known for its antioxidant potential (Colven and Pinnell, 1996), its regulation in collagen production (Chung et al., 1997) and for its action on the improvement of UV-induced modifications in skin relief and ultrastructure (Humbert et al., 2003), however it is extremely unstable. To overcome this problem, ascorbic acid is chemically modified by esterification of the hydroxyl group leading to some derivatives such as ascorbyl palmitate, ascorbyl tetraisopalmitate and magnesium ascorbyl phosphate, which are widely used in topical formulations.

Direct application of antioxidant vitamins on the skin has the added advantage of targeting the antioxidants to the area of skin needing the protection. However, for topical application of antioxidants to be useful, several obstacles must be overcome. Firstly, they are inherently unstable compounds, which make them difficult to formulate in an acceptable, stable composition for cosmetic use (Pinnell, 2003). In addition, it is already known that the more photounstable a filter, the more rapidly it is consumed after exposure to UV radiation. The photostability depends not only on the filter but also on the presence of other UV filters, other active substances (i.e., antioxidants) and raw materials in the product, as well. However, although there are many combinations of UV-filters and vitamins in anti-aging products, it is not known if the UV-filters have some influence on the effect of these vitamins on the skin as well as on their stability. Consequently, it is very important to evaluate the performance of whole formulation in the development of a new product.

Thus, the aim of this study was to evaluate the photostability and the efficacy of a formulation containing vitamin A, C and E derivatives and two different UV-filters combinations, a photostable and a photounstable one.

Table 1
Components of the formulations under study

Components	Percentage of components in each formulation (w/w)			
	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)	Formulation 4 (F4)
Phase A				
Hydroxyethyl cellulose	0.50	0.50	0.50	0.50
Glycerin 86%	3.00	3.00	3.00	3.00
Distilled water	74.15	69.56	55.56	53.56
Phase B				
Octyl octanoate	3.00	3.00	3.00	3.00
BHT	0.05	0.05	0.05	0.05
Phosphate-based self-emulsifying wax (cetearyl alcohol, dicetyl phosphate, ceteth-10 phosphate)	3.50	3.50	3.50	3.5
C12-c15 alkyl benzoate	6.00	6.00	6.00	6.00
Octyl methoxycinnamate	–	–	10.00	7.00
Benzophenone-3	–	–	–	4.00
Avobenzone	–	–	2.00	–
4-Methylbenzilidene camphor	–	–	2.00	–
Octocrylene	–		–	5.00
Phase C				
Methylphenyl Polysiloxane	4.00	4.00	4.00	4.00
Cyclopentasiloxane	2.00	2.00	2.00	2.00
Vitamin A palmitate 1,700,000 UI	–	0.59	0.59	0.59
Ascorbyl tetraisopalmitate (vitamin C)	–	2.00	2.00	2.00
Vitamin E acetate	–	2.00	2.00	2.00
Propyleneglycol	3.00	3.00	3.00	3.00
Phenoxyethanol and methylparaben, ethylparaben, propylparaben and buthylparaben	0.80	0.80	0.80	0.80

F1 (vehicle), F2 (formulation containing vitamins A, C and E), F3 (formulation containing vitamins A, C and E and a photounstable UV-filter combination), F4 (formulation containing vitamins A, C and E and a photostable UV-filter combination).

Finally, considering that there are many formulations besides sunscreens containing combinations of UV-filters and vitamins with other claims such as hydration and anti-aging effects this research should contribute to a better understanding of UV-filters influence on the effects of these vitamins on the skin as well as on their stability mainly when photounstable UV-filters are present in the formulation.

2. Materials and methods

2.1. Formulations studied

The formulations that were investigated were based on a phosphate-based self-emulsifying wax (cetearyl alcohol, dicetyl phosphate, ceteth-10 phosphate) and on hydroxyethyl cellulose, as described in Table 1, and contained or not (vehicle: formulation 1) a combination of 0.6% (w/w) vitamin A palmitate (1,700,000 UI/g) (DSM, Swiss), 2% (w/w) vitamin E acetate (DSM, Swiss) and 2% (w/w) ascorbyl tetraisopalmitate (Nikko Chemicals, Japan) (formulation 2) supplemented with a photounstable UV filter combination octyl methoxycinnamate (OMC), avobenzone (AVB) and 4-methylbenzilidene camphor (MBC) (formulation 3) or with a photostable UV filter combination OMC, benzophenone-3 (BP-3) and octocrylene (OC) (formulation 4). The procedure of obtention of the four formulations is described here briefly: phases A and B were heated separately to ∼75 °C. When both phase A and phase B reached the temperature, A was added to B with moderate mixing (using a Heidolph mixer, RPZ 2021, at 650 rpm for 20 min) until ambient temperature was reached. After that, phase C was added to AB and the emulsion was homogenized again under stirring.

Both photostable and photounstable UV-filters combination had their photostability and *in vivo* photoprotection previously determined (SPF 15) according to COLIPA methodology (Gaspar and Maia Campos, 2006; Gaspar et al., 2006).

2.2. Photostability studies

In these studies, 40 mg of each formulation containing UV filter and/or vitamins (formulations 2, 3 and 4) were spread onto an area of 10 cm^2 (approximately 4 mg/cm^2) of a glass plate and left to dry for 15 min before exposure to 30 min UVA/UVB irradiation (280–400 nm) from a 96000 Oriel 150 W Xenon arc solar simulator (Oriel Corporation, Stratford, CT). Irradiance, which was approximately 0.186 mW/cm^2, was measured at 290 nm with a 70260 Oriel Radiant Power Meter equipped with a silicon probe and U-340 filter, which resulted in a cumulative UVB dose of approximately 334.8 mJ/cm^2 or 15.9 MED/cm^2 (Gaspar and Maia Campos, 2006). For each exposed plate, a duplicate plate serving as a negative (non-irradiated) control was kept in a dark place at 30 °C. Three replicate pairs of samples were prepared. Exposed samples (formulations 2, 3 and 4 and the vehicle without UV filters: formulation 1) were immersed in 25 mL of isopropanol and the dried film was dissolved ultrasonically. This solution was quantified by HPLC analysis (Shimadzu) on a C-18 column (LiChrospher® 100 RP-18, 5 μm, Merck) at 325 and 235 nm. Gradient elution was employed using 74% of methanol:isopropanol (45:55, v/v) as solvent A and 26% water as solvent B for 3 min, with a linear gradient decreasing from 26 to 0% B over 6 min, followed by an isocratic elution (0% B).

For spectrophotometric evaluation, the samples were diluted (1:4, v/v) and the ratio of the mean UVA (320–400 nm) to the mean UVB (280–320 nm) absorbances was calculated as proposed by Diffey (1994).

$$\frac{\int_{320}^{400} A(\lambda)d\lambda / \int_{320}^{400} d\lambda}{\int_{280}^{320} A(\lambda)d\lambda / \int_{280}^{320} d\lambda}$$

Results obtained were statistically analyzed using Kruskal–Wallis, a non-parametric test.

2.3. Safety and efficacy studies

2.3.1. Study protocol

Adult male hairless mice (HRS/J-hairless, Jackson, Bar Harbor, ME) weighing on average 30 g were used. The animals were kept in individual cages with a controlled light–dark cycle and received commercial ration (Nuvilab CR-1), as well as water *ad libitum*. This study was carried out in accordance with the "Principles of Laboratory Animal Care" (NIH).

The formulations (5 mg/cm^2), which were not irradiated, were applied on the dorsum of the animals once a day for 5 days to simulate a daily use condition, as follows: (a) no treatment (control); (b) application of the vehicle only (formulation 1); (c) application of the formulation containing vitamins A, C and E (formulation 2); (d) application of the formulation containing vitamins A, C and E and a photounstable UV-filter combination (formulation 3); (e) application of the formulation containing vitamins A, C and E and a photostable UV-filter combination (formulation 4). Different techniques, which are described in the next paragraphs, were used to assess different aspects of cosmetic safety and efficacy (superficial and deeper layers effects).

2.3.2. Biophysical techniques

The water content of stratum corneum was determined with a skin capacitance meter (Corneometer® CM 825, Courage & Khazaka, Electronic GmbH, Germany) that expresses the values in arbitrary units (Leonardi et al., 2002).

TEWL was determined by an evaporimeter (Tewameter® TM 210, Courage & Khazaka, Electronic GmbH) and values were expressed as g/m^2 h (Gaspar and Maia Campos, 2003).

Skin viscoelastic properties of the skin were determined using a non-invasive, *in vivo* suction, skin-elasticity meter (Cutometer® SEM 575, Courage & Khazaka, Electronic GmbH). The following group of mechanical parameters was analyzed: Ue (immediate distension), Uv (delayed distension), Uf (skin distensibility), Ur (immediate retraction), Ua/Uf (the ratio of total retraction to total distension, called gross elasticity), Ur/Ue (neto-elasticity of the skin without viscous deformation), Ur/Uf (the ratio of immediate retraction to total distension, called biological elasticity) and Uv/Ue (the ratio of viscoelastic to elastic distension) (Dobrev, 2000; Nishimori et al., 2001).

The erythema index was measured by reflectance spectrophotometry using a Mexameter® MX16 (Courage & Khazaka, Electronic GmbH). The degree of erythema was quantified as an erythema index and was related to the hemoglobin content (Gaspar and Maia Campos, 2003). This index is often used to assess erythema or skin irritation in safety tests (Fluhr et al., 2001).

2.3.3. Histology and histometry

Mice were euthanized by CO_2 inhalation and skin fragments were obtained and immediately immersed in a fixing solution consisting of 85 mL of 80% (v/v) alcohol, 10 mL formaldehyde and 5 mL acetic acid. After 24 h the fixed fragments were dehydrated, cleared and embedded in paraffin. Semi serial 6 mm-thick sections were then obtained and each section corresponded to an interval of fifty sections, i.e., 10 sections were obtained from the 2 mm biopsy. The sections were stained with hematoxylin and eosin for general histopathological, and histometric analysis (Silva and Maia Campos, 2000; Lu et al., 1999).

Viable epidermis thickness as well as the number of epidermal cell layers were analyzed by using a light microscope Leica DMLB, coupled with a digital camera DC 300, using 100-fold magnification. The number of nucleated cell layers was counted at 10 randomly selected locations per slide and averaged, as described previously (Lu et al., 1999). Viable epidermis thickness was also measured in a similar manner, and the means ± S.E.M. were calculated.

2.3.4. Statistical analysis

Results obtained for efficacy studies were statistically analyzed using Kruskal–Wallis, a non-parametric test.

3. Results and discussion

3.1. Photostability studies

The chromatographic separation of the UV filters and vitamins on C18 columns was optimized with regard to the mobile phase, which gave good results in separating the 5 UV filters and the three vitamins under study (Fig. 1A).

The chromatographic profiles of formulations 2, 3 and 4 exposed and not exposed to 30 min. UVA/UVB irradiations are shown in Fig. 2.

Our results were validated in terms of linearity, precision and accuracy. The correlation coefficients were all above 0.999. The precision (CV) was between 0.6 and 4.52. The accuracy values were low (−11.75 to 2.9) because a small amount of the formulation was lost when it was spread onto the glass plate, but the procedure was kept because it is currently used for the photostability evaluation of sunscreens.

To analyze the alterations that occurred in the formulations under study after UVA/UVB irradiation, and to observe the influence of the photostability on vitamins stability, the recovery of the five studied UV filters and three vitamins contained in the formulations was plotted on a graph as shown in Fig. 3. It was observed that the photostability of the formulations under study only influenced the stability of vitamin A palmitate. In addition, both UV filters combinations (F3 and F4) enhanced vitamin A photostability and F4 was more photostable than F3 (in terms of vitamin A and OMC recovery) (Fig. 3). Carlotti et al. (2002) suggested that the degradation process of vitamin A palmitate has an oxidative mechanism, thus the use of an antioxidant such as BHT (which was used in all formulations under study) is necessary for their proper storage over time. These authors observed that MBC could protect vitamin A from photo degradation, and the same protection was observed when AVB was employed. Both UV filters (MBC and AVB) are present in formulation 3, which enhanced vitamin A stability, however a sunscreen (formulation 4: composed by OMC, OC and BP-3), which was previously considered photostable in other studies (Gaspar and Maia Campos, 2006; Gaspar et al., 2006) promoted a statistically better protection of vitamin A palmitate (Fig. 3). Once formulation 3 (photounstable) presented lower vitamin A recovery than formulation 4 (photostable) (Fig. 3), it can be suggested that when AVB and MBC were used in combination with OMC (formulation 3), they produced an unstable UV-filter combination (Chatelain and Gabard, 2001) that enhanced vitamin A photo degradation.

The UVA/UVB-absorption ratio, obtained by spectrophotometric analysis, has been often used for the *in vitro* UVA protection (Diffey, 1994) as well as for the determination of the

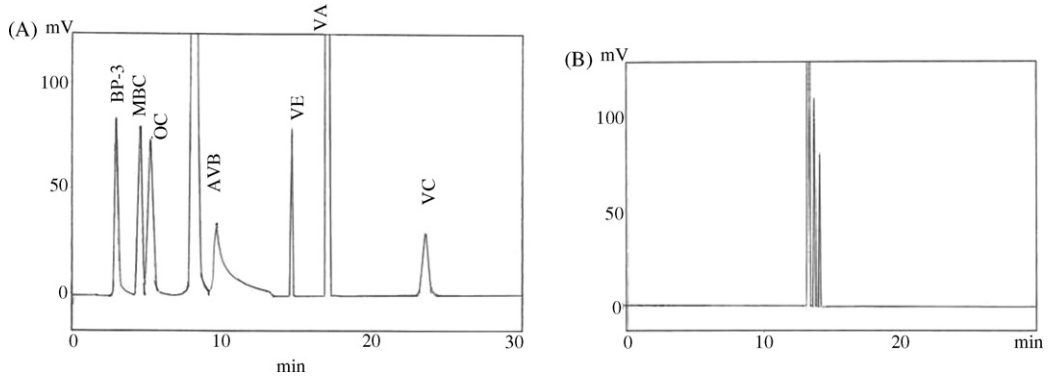

Fig. 1. HPLC chromatographic analysis of (A) an isopropanol solution of the five sunscreen agents and three vitamins studied. Peaks: benzophenone-3,4-methylbenzilidene camphor, octocrylene, octyl methoxycinnamate, avobenzone, vitamin E acetate, vitamin A palmitate and ascorbyl tetraisopalmitate (VC) and (B) placebo. Detection at 325 and 235 nm; C18 column; gradient elution, flow rate 0.8 mL min.

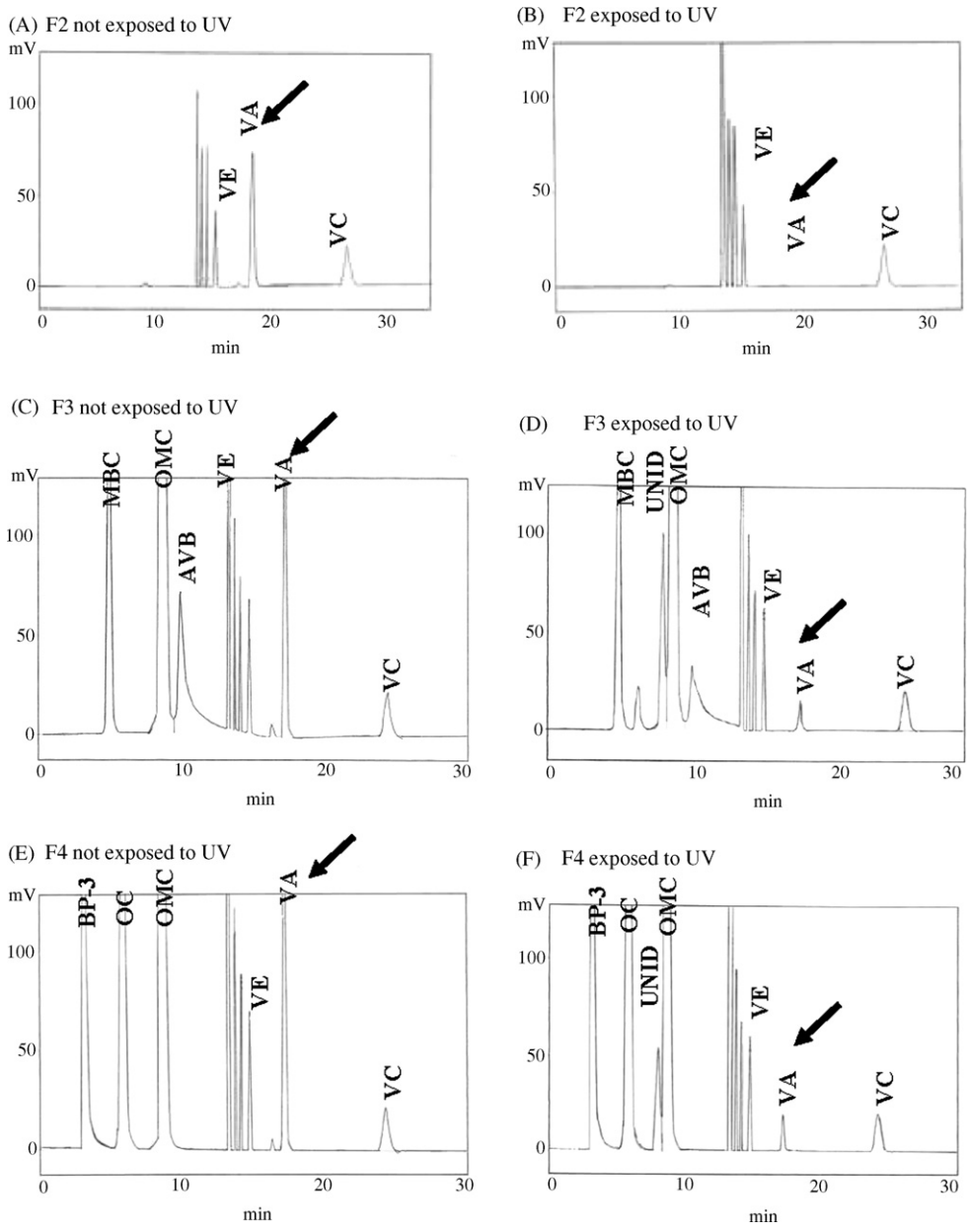

Fig. 2. Chromatographic profiles (HPLC) of formulation 2 (vitamins A, C and E) (A) not exposed and (B) exposed to 30 min UVA/UVB irradiation; formulation 3 (4-methylbenzilidene camphor, octyl methoxycinnamate, avobenzone, vitamins A (✓), C and E) (C) not exposed and (D) exposed to 30 min UVA/UVB irradiation and formulation 4 (benzophenone-3, octocrylene and octyl methoxycinnamate) (E) not exposed and (F) exposed to 30 min UVA/UVB irradiation. Conditions, similar to Fig. 1. Observe unidentified peaks (UNIND) after irradiation of all formulations (D and F).

photostability of sunscreens (Osterwalder and Herzog, 2002) because it is a simple method that does not involve human tests.

These results showed that both formulations (formulation 3 and 4) had their UVA/UVB-absorption ratio reduced after irradiation (Fig. 4). However, formulation 4 had lower reduction of UVA/UVB-absorption ratio (3.5% for 30 min of irradiation) than formulation 3 (8.26% for 30 min of irradiation), which indicates that F4 had a lower reduction in UVA absorption capacity than F3. This lower reduction in UVA absorption observed in F4 is related to a higher protection in the UVA region and may be due to improved vitamin A stabilization (seen in HPLC results), since vitamin A absorbs UVA radiation: between 300 and 350 nm) (Antille et al., 2003).

3.2. Safety and efficacy studies

3.2.1. Biophysical techniques

The statistically analyzed results for all parameters obtained from Biophysical Techniques are shown in the Figs. 5 and 6.

Formulations 2, 3 and 4 enhanced the TEWL when compared with the vehicle (F1) and with the control site ($p < 0.01$) but they were not statistically different among themselves. This enhancement in the TEWL could suggest that the formulations under study enhanced cell renewal since vitamins A and C present these properties (Maia Campos et al., 1999; Silva and Maia Campos, 2000). Besides this, previous studies conducted by our group showed that ascorbic acid, which acts in cell

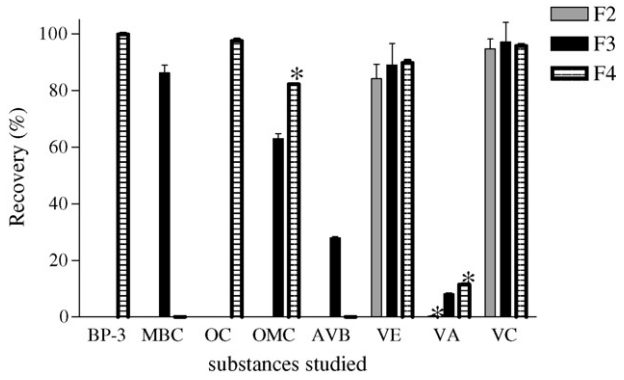

Fig. 3. Recovery of the UV filters: BP-3, MBC, OC, OMC and AVB and vitamins E, A and C contained in the formulations 2, 3 and 4 under study, after 30 min. UVA/UVB irradiation, which were expressed as percentage of the initial filter amount (negative control). *Statistically different from formulation 3 ($p < 0.05$).

renewal, enhanced TEWL in clinical studies (Maia Campos et al., 2005). During the process of terminal differentiation, several biochemical changes and loss of water occurs in the skin surface (Rieger, 2000) and since the vitamins (F2) and the combination of vitamins with photounstable or photostable UV-filters (F3 and F4, respectively) provoked an enhancement in TEWL, we have another indication that epidermal cells are in intense renewal process.

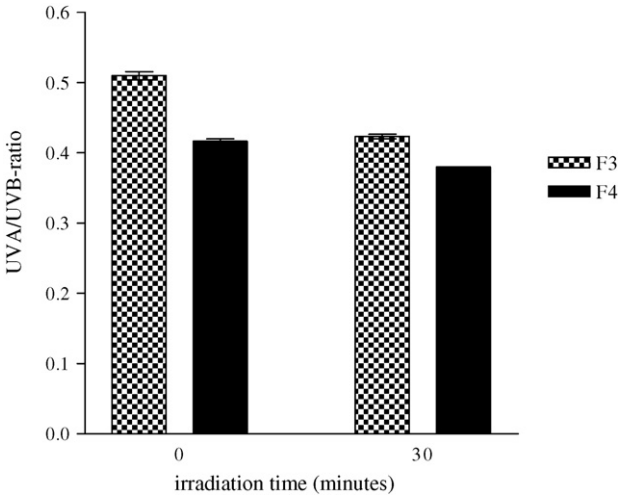

Fig. 4. UVA/UVB absorption ratio for formulations 3 and 4 after 0 (negative control) and 30 min. UVA/UVB irradiation.

When the erythema index, which has a good correlation to skin irritation and is often used in safety tests (Fluhr et al., 2001), was analyzed, formulations 3 and 4 did not provoke any irritation that could be measured by erythema index, since the values obtained for the vehicle, F3, F4 and control site were not statistically different (Fig. 5B). However formulation 2 (containing only vitamins) provoked an irritation along with an enhancement in erythema index. This irritation have been reported by many authors and Dermatologists since despite many beneficial effects on dermatological applications, retinol and its derivatives cause severe local irritation manifested as mild erythema and stratum corneum peeling of the skin (Kim et al., 2003), which can be considered adverse effects. Consequently, the enhancement of erythema index seen in the present study could be provoked by the presence of vitamin A palmitate that hydrolyses into retinal and oxidizes into tretinoin in the skin (Duell et al., 1996) which in turn presents cell receptors inducing keratinocytes proliferation and consequently acts on cell renewal (Orfanos et al., 1997) reducing stratum corneum thickness and epidermis protection (Maia Campos et al., 1999) and causing local irritation manifested as erythema (Kim et al., 2003). Once the formulation 2 contains 10,000 UI/g of vitamin A palmitate combined with two other vitamins derivatives (vitamin E acetate and ascorbyl tetraisopalmitate) a potentiation of their effects such as cell renewal, enhancement of epidermis and reduction of stratum corneum thickness and also skin irritation might have occurred. The difference observed with formulations 2, 3 and 4 effects might have occurred due to the presence of organic UV filters (F3 and F4), which are lipid soluble substances that can form a protective film in the stratum corneum and consequently reduce skin irritation (Loden and Andersson, 1996). In addition, these UV filters (photostable and photounstable combinations) may act as stabilizers protecting vitamin A palmitate from hydrolysis and oxidation, reducing its conversion into tretinoin and consequently the irritation caused by tretinoin. Our results can be supported by Carlotti et al. (2002) that reported that avobenzone and BHT protected vitamin A palmitate and vitamin A from photodegradation.

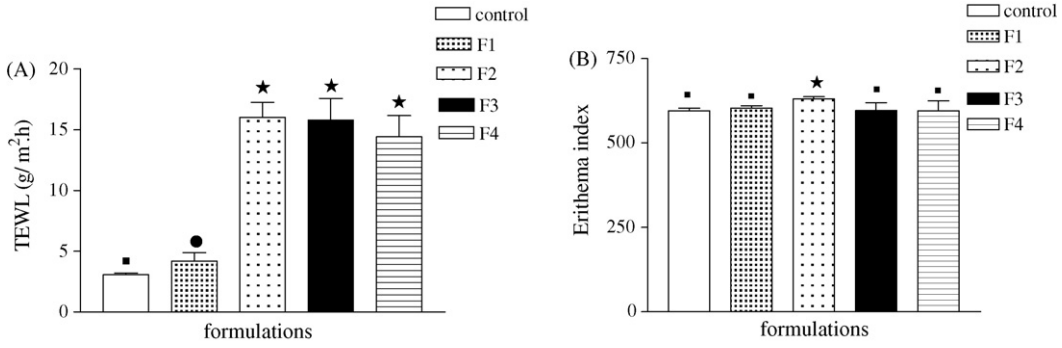

Fig. 5. Transepidermal water loss (TEWL) (A) and erythema index (related to skin irritation) (B) obtained for the three treated areas: F1 (vehicle), F2 (formulation containing vitamins A, C and E), F3 (formulation containing vitamins A, C and E and a photounstable UV-filter combination), F4 (formulation containing vitamins A, C and E and a photostable UV-filter combination) and the values obtained for the control area. Different symbols indicate statistically significant differences between treatments (Kruskal–Wallis test, $n = 8$ hairless mice, mean ± S.E.M., $p < 0.05$).

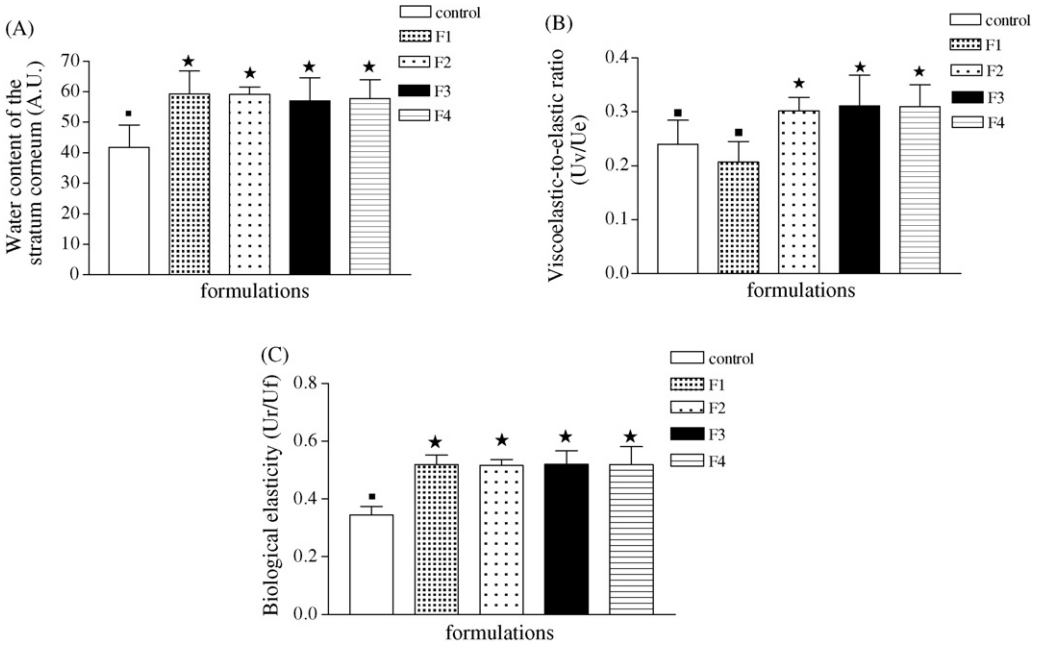

Fig. 6. Water content of the stratum corneum (A), viscoelastic-to-elastic ratio (Uv/Ue) (B) and biologial elasticity (Ur/Uf) (C) obtained for the three treated areas: F1 (vehicle), F2 (formulation containing vitamins A, C and E), F3 (formulation containing vitamins A, C and E and a photounstable UV-filter combination), F4 (formulation containing vitamins A, C and E and a photostable UV-filter combination) and the values obtained for the control area. Different symbols indicate statistically significant differences between treatments (Kruskal–Wallis test, $n = 8$ hairless mice, mean ± S.E.M., $p < 0.05$).

Formulations 2, 3, 4 and the vehicle (F1) enhanced the water content of the stratum corneum when compared to the control site ($p < 0.01$) but they were not statistically different among themselves.

When the biological elasticity was analyzed it was observed that formulations 2, 3, 4 and the vehicle enhanced Ur/Uf values in the same intensity ($p < 0.05$) when compared to the control (Fig. 6C). This enhancement could be due to epidermal hydration, since Dobrev (2000) also observed an enhancement of Ur/Uf after the use of glycerin based moisturizing creams.

When the viscoelastic-to-elastic ratio was analyzed it was observed that formulations 2, 3 and 4 enhanced the Uv/Ue parameter when compared with the vehicle and with the control site ($p < 0.01$) showing that they enhanced epidermis hydration. Our results are similar to the ones obtained by Dobrev (2000), who observed that, when using small-diameter measuring probes (as used in this study), Uv/Ue is an indicative of epidermis and superficial dermis hydration. He also suggests that Cutometer parameters Uv and Uv/Ue are the most indicative of epidermal and dermal water content, and that their enhancement indicates a decrease in the viscosity of the interstitial fluid as a result of the increased water content which leads to the softening of outer layers of the epidermis (stratum corneum mainly) and to the reduction of the friction between the fibers, which facilitates the movement of the interstitial fluid.

3.2.2. Histology and histometry

The statistically analyzed results for viable epidermal thickness as well as the number of epidermal cell layers obtained in the histometric evaluation are shown in Fig. 7, where it can be seen that formulations 2, 3 and 4 enhanced the viable epidermal thickness (Fig. 7A) and the number of epidermal cell layers (Fig. 7B) when compared with the vehicle and with the control site ($p < 0.001$) but they were not statistically different.

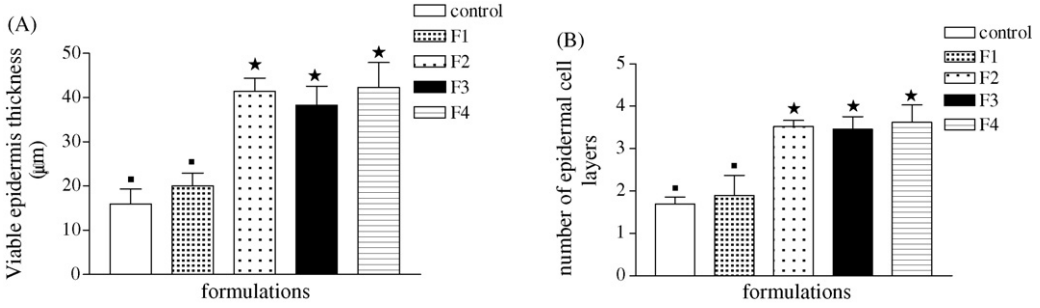

Fig. 7. Viable epidermis thickness (A) and number of epidermal cell layers (B) obtained for the three treated areas: F1 (vehicle), F2 (formulation containing vitamins A, C and E), F3 (formulation containing vitamins A, C and E and a photounstable UV-filter combination), F4 (formulation containing vitamins A, C and E and a photostable UV-filter combination) and the values obtained for the control area. Different symbols indicate statistically significant differences between treatments (Kruskal–Wallis test, $n = 8$ hairless mice, mean ± S.E.M., $p < 0.001$).

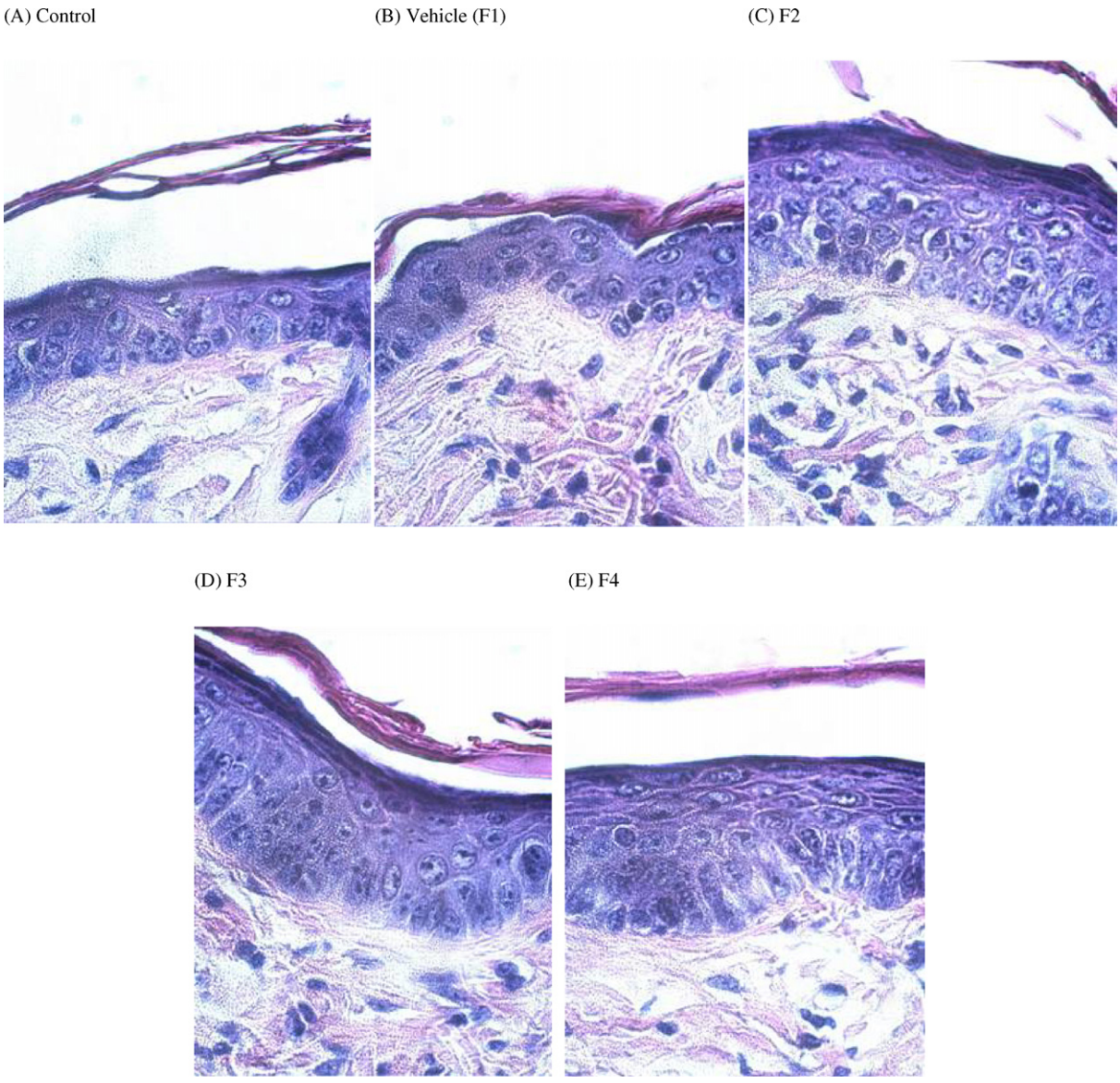

Fig. 8. Photomicrographs of hairless mice skin. Initial magnification ×1000, HE. Control (no treatment) (A), F1: vehicle (B), F2 (formulation containing vitamins A, C and E) (C), F3 (formulation containing vitamins A, C and E and a photounstable UV-filter combination) (D), F4 (formulation containing vitamins A, C and E and a photostable UV-filter combination) (E).

Our results are similar to the ones obtained by Maia Campos et al. (1999) when analyzing vitamin A effects on the skin. Fig. 8 shows the photomicrographs of all treated groups comparing to the control group.

In summary, the photostability of the formulations under study only influenced the stability of vitamin A palmitate (both UV filters combinations, F3 and F4, enhanced vitamin A photostability). In the efficacy studies, which simulated a daily use of these formulations, once formulations 2, 3 and 4 enhanced the viable epidermal thickness, the number of epidermal cell layers, TEWL and Uv/Ue parameter, it can be suggested that they improved viable epidermis hydration and acted in cell renewal. These effects are important for the improvement of aged skin conditions, which presents a reduction in epidermis thickness and in the cell renewal process, however formulation 2 (containing only vitamins) provoked an irritation on hairless mouse skin.

Consequently, it can be concluded that both UV filters combinations did not influence the superficial and in deeper layers hydration and anti-aging effects of the formulations containing the vitamins under study and reduced the skin irritation observed when the vitamins were present in the formulation. This reduction in skin irritation occurred probably due to the protective film formed by these lipid soluble UV filters in the stratum corneum and also for the protective effects of the UV filters, which can reduce vitamin A conversion into tretinoin and consequently skin irritation. In addition, the photostable UV-filters combination had the highest recovery of vitamin A in the photostability studies. Finally, it could be suggested that the presence of UV-filters can be considered interesting for the reduction of skin irritation and the most suitable formulation was the one containing the combinations of vitamins A, C and E with photostable UV-filters.

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