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Formul'Ageing – Risks and countermeasures

Avoiding premature formulation and skin ageing - caused by the formulation itself

KEYWORDS: Formul'Ageing, Anti-ageing, Antioxidants, Photosensitivity, Protection

Abstract

Naming something is a way of bringing it into focus and an important step towards understanding any phenomena. Here we coin the term Formul'Ageing. Every coin has two sides: on one side, the term Formul'Ageing refers to the ageing of the formulation (i.e., it refers to premature ageing of the formulation due to excessive radical formation inside the formulation upon e.g., exposure to the sun). The other side of the term Formula'Ageing could be summed up as formulation-provoked skin ageing. It hints at a yet under-recognised and indirect phenomenon: premature ageing of the skin resulting from the unexpected induction of free radicals inside the skin when basic formulations are topically applied and exposed to sunlight. However, in both cases of Formul'Ageing, free radical formation is thought to be the main underlying cause.
Using electron spin resonance spectroscopy, we found that a cosmetic antioxidative active ingredient provided stable and efficient protection against free radicals for cosmetic (sun care) formulations and, more importantly, the skin. Radical protection will help to assure efficacy and stability for the formulations and to counterbalance the collateral radical-promoting activity of most conventional formulations for the skin.

INTRODUCTION

Reactive oxygen species (ROS) can be harmful to both skin and cosmetic formulations.
In the skin, excessive ROS can randomly damage lipids, proteins and DNA and are thought to be the main driver of premature (skin)ageing, photo-ageing, wrinkling, and pigmentation (1-4). Normally, the skin's antioxidant defence system can defend it against ROS damage with its antioxidant defence enzymes, such as catalases, and non-enzymatic small molecule antioxidants, such as ascorbic acid, tocopherols, and glutathione. However, that system can easily be overloaded, particularly by excessive UV(A) exposure. Older skin is even more susceptible to ROS overload because the activity of the antioxidant defence enzymes and the level of nonenzymic antioxidants decrease with age (5).
In cosmetic formulations, excessive ROS are probably not toxic to the consumer. Radicals formed in the product are unlikely to penetrate into the living layers of the skin and do harm (6). However, excessive ROS formation may accelerate the degradation of the functional ingredients and provoke physiochemical destabilisation and unpleasant olfactory sensations.

Formulation-provoked skin ageing
We and others already reported on the surprising and, in our eyes, yet under-recognized phenomenon that many cosmetic formulations, when applied to the skin and exposed to sunlight, provoke significant oxidative stress inside the skin (7-10). The application of basic cosmetic formulations, thus, can unintentionally increase the skin's photosensitivity.
This formulation-mediated pro-oxidative effect is thought to be due to a higher degree of hydration of the outer skin layers that allows more penetration by UV-radiation and, thereby, promotes radical formation. Thus, sunlight generates more free radicals in moisturized skin than in dry skin (8). Still more radicals might be produced in the presence of surface active agents (e.g., tensides) or harsh ingredients (e.g., DHA or erythrulose as found in self-tanners) (9).
In other words, conventional finished products, such as moisturisers or anti-ageing creams, may alleviate symptoms, such as dry skin or wrinkle formation, but simultaneously increase the skin's sensitivity to UV-induced ROS formation and, thereby, accentuate premature skin ageing. Fortunately, these unintended side effects can be eliminated by incorporating carefully selected cosmetic actives (10).

Ageing of the formulation
Before and especially as soon as it is applied to the skin, a product is exposed to external physical and chemical influences, including oxygen and UV-radiation. Molecular oxygen triggers autoxidation of basic ingredients, such

as fatty acids, and the formation of peroxide. Sunlight triggers disadvantageous radical chain reactions inside the formulation itself in a very unpredictable manner. Increased numbers of UV-induced free radicals increase the risk of photo-oxidative reactions that eventually may lead to formulation instability.
Antioxidants can effectively reduce product oxidation and thereby improve the formulation's shelf-live, quality and skin compatibility. However, not all achieve the desired results. Some plant extracts and certain UV filters can even exacerbate the problem if the exposure to sunlight transforms them into unwanted pro-oxidants (11).
For example, uncoated titanium dioxide and some organic UV-filters (e.g., BMDBM) generate free radicals during UV radiation. These free radicals, in turn, can degrade valuable compounds in the formulation and may compromise the photo-protective effect of the sunscreen (12).
Thus, for proper product function and stability, it is critical that formulations contain ingredients that prevent or neutralise UV-induced radical formation in both skin-care and sun-care formulations.

Countermeasures
As a consequence, antiradical and antioxidant efficacy is becoming more and more important for modern products. Classical antioxidants in cosmetic formulations include the vitamins C and E and their stabilized derivatives as well as secondary plant derivatives (e.g., polyphenols or flavonoids). These antioxidants, however, have major drawbacks, including product discoloration and loss of activity. The stabilised derivatives (e.g., tocopheryl acetate or ascorbyl palmitate) lack antioxidant activity and are thus not able to overcome the original's disadvantages (13).
Next-generation antioxidant actives must tackle both facets of Formul'Ageing. They must effectively reduce free radical injury in skin AND formulations and have several improved features. In particular, they should effectively neutralise the detrimental pro-oxidative effects of the basic raw materials and, thereby, decrease the skin's sensitivity to UV-induced ROS formation. Such actives, moreover, should remain stable in cosmetic formulations and maintain their antioxidant and antiradical performance over the lifetime of the formulation (i.e., have a longer life on the shelf and on the skin of the consumer) under realistic conditions. Sun-care products must also have adequate photostability.

Aim
Here we sought to build on our previous findings, showing that an antioxidative active that protects against free radicals in the skin and for skin care and sun care formulations (10). In the present work, we compared the antioxidant properties of cosmetic formulations containing classical antioxidants or the antioxidative active. We focused on the influence of environmental stress factors, such as storage time, storage temperature and UV radiation, on the antioxidant performance in the skin and in the formulation itself.

MATERIALS AND METHODS

Cosmetic active and formulations
CELLIGENT® (INCI: *Helianthus Annuus* Seed Oil, Ethyl Ferulate, Polyglyceryl-5 Trioleate, *Rosmarinus Officinalis* (Rosemary) Leaf Extract, Water, Disodium Uridine Phosphate, Tocopherol; hereafter referred to as CEL), developed by RAHN AG, is a cosmetic active ingredient designed to protect the skin and the formulation from radical formation and to regenerate UV-stressed skin. CEL displays remarkable antioxidative efficacy that is ascribed mainly to ethyl ferulate and rosemary extract. Ethyl ferulate is a natural antioxidant with UV-absorbing properties and, thereby, neutralises oxygen radicals that arise as a result of UV radiation (14). The rosemary extract developed for CEL contains an extremely high level of carnosolic acid: approximately 90% of rosemary's antioxidative efficacy is mediated by carnosolic acid (15).
O/W emulsions containing 0, 0.5, 1, 2, or 3% CEL were prepared as outlined in Table 1. Identical emulsions containing 0.3% tocopherol (i.e., Dermofeel® Toco 70 non-GMO, a solution of D–(α, β, γ, δ)-tocopherols in vegetable oil; Dr. Straetmans) or 3% tocopheryl acetate (i.e., Dermofeel® E74 A non-GMO, D-(α)-tocopheryl acetat; Dr. Straetmans) instead of CEL were used as benchmarks.

St	Substance	INCI	% w/w
1	Water demin.	Water	Add 100
	Glycerin 85%	Glycerin, Water	3.00
2	Carbopol Ultrez-10	Carbomer	0.15
3	Euxyl PE 9010	Phenoxyethanol, Ethylhexylglycerin	1.00
	Sisterna SP70-C	Sucrose Stearate	0.50
	Myritol 312	Caprylic/Capric Triglyceride	15.00
	Keltrol CG-SFT	Xanthan Gum	0.25
	Dermofeel GSC	Glyceryl Stearate Citrate	3.00
	Lanette O	Cetearyl Alcohol	2.00
4	NaOH solution 10%	Sodium Hydroxide, Water	0.40
5	CEL	*Helianthus Annuus* (Sunflower) Seed Oil, Ethyl Ferulate, Polyglyceryl-5 Trioleate, *Rosmarinus Officinalis* (Rosemary) Leaf Extract, Water, Disodium Uridine Phosphate, Tocopherol	0.00 0.50 1.00 2.00 3.00

Table 1. Test emulsions: O/W emulsions containing 0, 0.5, 1, 2, or 3% CEL were prepared. Identical emulsions containing 0.3% tocopherol or 3% tocopheryl acetate instead of CEL were used as benchmarks.

Measuring free radicals inside the skin
In a pigskin model (16), emulsions were applied on the epidermal side of a 1 x 1 cm skin biopsy (2 mg/cm^2) and allowed to penetrate for 15 min in the dark. The biopsy was then placed in a 1 mM solution of the radical indicator PCA (1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxylic acid) for 5 min. Punch biopsies (Ø 4 mm) were made and exposed to increasing doses of UV-light with a 300 W Oriel (Newport) UV-solar simulator. The irradiances as integrated values over the spectral ranges were E (UVB = 280-320) = 23.5 W/m^2 and E (UVA = 320-400 nm) = 180 W/m^2 and the cumulative dose for each test set was 1.2 Minimal Erythemal Dose (MED), corresponding to approximately 20 min of sun exposure (European summer noonday).
Signal intensity from electron spin resonance spectroscopy was measured before and after each irradiation and plotted against the respective UV dose. The resulting monoexponential decay curve allowed us to quantify the UV-induced free radicals within the skin (12). Four independent experiments were performed.

Antioxidative power
The inherent antioxidative activities of the emulsions were analysed by the antioxidative power (AP) method (17).
In brief, this method determines the overall AP of active

ingredients, such as plant extracts, vitamins, and other compounds, by measuring their reducing activity against a stable test radical (e.g., DPPH = 1,1-Diphenyl-2-picrylhydrazyl radical) by electron spin resonance spectroscopy. Unlike most other test systems, the AP method accounts for reduction potential and for reaction time. AP is expressed in antioxidative units (AU), in which 1 AU corresponds to the activity of a 1 ppm solution of pure ascorbic acid as a benchmark.
To access the oxidation- and photo-stability of the active ingredients in the formulation, AP values were determined in fresh emulsions and after 8 weeks of storage at room temperature (RT) while protected from light. Emulsions (500 mg) were then dispensed into one well of a 24-well plate, covered with a quartz glass and exposed to UV radiation with a 300 W Oriel (Newport) UV-solar simulator. After three exposures of 30 min each, 100 mg of each sample was used to determine the remaining AP value. The irradiances as integrated values over the spectral ranges were E (UVB = 280–320 nm) = 3,3 mW/cm^2 and E (UVA = 320–400 nm) = 13,4 mW/cm^2, and the cumulative dose for each test set was 5 MED, corresponding to approximately 75 min of sun exposure (European summer noonday).

RESULTS AND DISCUSSION

CEL protects the skin against formulation-mediated ROS formation

Basic formulations can increase the skin's photosensitivity. Skin samples that were supplied with a basic skin-care formulation showed a 48% higher cutaneous ROS-burden after UV exposure than skin samples that were not supplied with any skin care formulation at all (Figure 1). This finding is in agreement with previous reports where the application of a basic sun-care formulation (without filters) followed by UV -exposure even led to an increase in the skin's ROS burden by 75% as compared to non-supplied skin (10).
This finding has major implications. Finished products, such as conventional facial moisturizers and hand creams, when applied to the skin and exposed to sun light, can provoke significant oxidative stress and gradually accentuate premature skin ageing, even while they alleviate symptoms, such as dry skin or wrinkle formation.
However, the increased skin photosensitivity can be overcome by adding carefully tested cosmetic actives to the formulation. Indeed, adding 3% CEL to the basic formulation completely neutralised the pro-oxidative side-effects of the formulation. In fact, it decreased the quantity of UV-induced free radicals to a level lower than in untreated skin and conferred true radical protection. The calculated effectiveness of CEL against free radical formation was 158% (Figure 1; 144% in previous experiments (10)).
The dose-response curve follows a symmetrical sigmoidal rather than a linear shape. This shape implies a threshold effect that becomes apparent at concentrations above 0.5% (Figure 2).

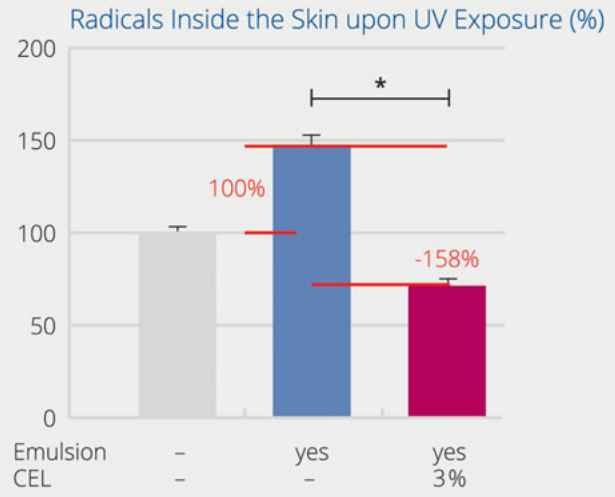

Figure 1. Formul'Ageing = Formulation-provoked skin ageing. Basic cosmetic formulations, when applied to the skin and exposed to sunlight, provoke significant oxidative stress in the skin (blue). Antioxidative cosmetic actives can reduce incidental pro-oxidative side effects (red). Percentage of free radical generation inside the skin, normalized to UV-exposed skin without application of formulation (grey). Means of four independent measurements + SEM. * indicates p < 0.05 (ANOVA).

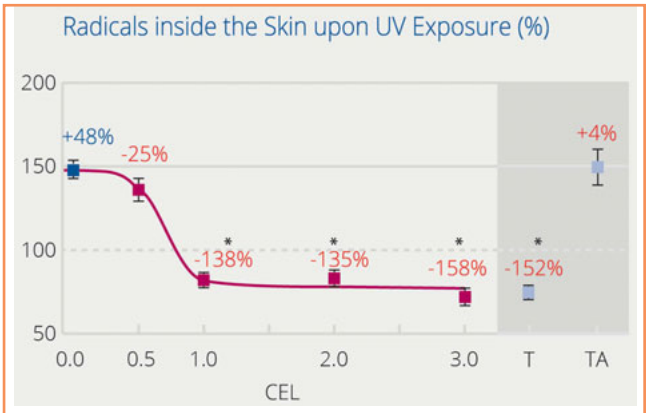

Figure 2. Dose-response curve. Skin samples supplied with a basic skin care formulation (blue) showed 48% higher cutaneous ROS-burden after UV exposure than skin samples not supplied with any skin care formulation (set to 100%). In contrast, incorporating > 0.5% CEL (red) completely counterbalanced the oxidative stress in the skin induced by the raw material. 0.3% tocopherol (T) also was very effective. However, tocopherol is unstable (see Figure 3). Tocopheryl acetate (TA, 3%) is a stable derivative but lacks protective efficacy. Means of four independent measurements ± SEM. * indicates p < 0.05 (ANOVA) significantly different from skin samples supplied with a basic skin-care formulation (blue).

From this observation, we can recommend that formulations include a minimal level of 0.5% CEL.
In a control formulation, 0.3% tocopherol also efficiently reduced the amount of UV-induced free radicals inside the skin (Figure 2). However, pure vitamins E and C are unlikely to overcome the formulation-mediated pro-oxidative effects in the skin, because they are highly unstable during storage (see next section). Tocopheryl acetate is often used as an alternative to tocopherol as it is considered more stable and is frequently claimed to be a powerful antioxidant. Unfortunately, the stability comes at the price of complete lose its antioxidant activity. Tocopheryl acetate is inactive, both biologically and as an antioxidant. It must be hydrolyzed to tocopheryl in the skin, but this rarely occurs (18). This observation agrees with our finding that the control formulation with

Description	Expected	AP (AU)	Recovery (%)
Pure cosmetic active CEL	n.a.	2603	n.a.
Formulation with 0.0 % CEL	0	0	n.a.
Formulation with 0.5 % CEL	13	8	62
Formulation with 1.0 % CEL	26	25	96
Formulation with 2.0 % CEL	52	43	83
Formulation with 3.0 % CEL	78	76	97
Formulation with 0.3% Tocopherol	n.a.	352	n.a.

Table 2. The antioxidative power (AP) of CEL. Functional recovery of CEL was almost 100% in all formulations. AU values are benchmarked against ascorbic acid. N=3; Mean ± 5%.

3% tocopheryl acetate offered absolutely no protection against UV-induced free radicals inside the skin (Figure 2). Thus, stabilised vitamin E and C derivatives, although fairly stable, are not suitable for preventing UV-induced radical formation inside skin: they simply lack antioxidant activity.

CEL maintains its antiradical performance over the lifetime of cosmetic formulations

The greater the capacity of a test substance to neutralize free radicals and the faster the reaction, the greater is the AP. As a raw material, CEL had an effective AP of 2,603 units (Table 2). We expect that a formulation with 3% CEL will have an AP of 78 units, based on full recovery and stability of the cosmetic active. In fact, the functional recovery of CEL in all formulations was almost 100%. Only the 0.5% concentration showed a lower reactivity than expected (Table 2).

The formulation with 0.3% tocopherol had higher AP values than formulations with CEL. However, the higher antioxidant activity did not translate to better protection for the skin (cf Figure 2). We explain this by differences in stability and hypothesize that tocopherol is quickly oxidised on skin when exposed to UV-light, whereas CEL is not. It is thus critical to know the AP of a cosmetic active and also to thoroughly understand its (photo)stability under realistic conditions.

We investigated the functional stability of CEL after long-term storage and UV radiation for 30, 60, and 90 min (Figure 3). After 8 weeks of storage at RT, the AP value of the formulation containing 3% CEL was unchanged. The APs remained constant even after 3 months of storage at various temperatures, such as 4, 20, or 40°C (data not shown). After UV radiation (three doses of 30 minutes each), the AP values were also unchanged (Figure 3). In stark contrast, the formulation with 0.3% tocopherol was not photostable and underwent rapid oxidation. After 8 weeks of storage at RT, even protected from light, its AP was reduced by 22%. The subsequent UV exposure resulted in a collapse of the AP; it decreased by 37%, 50%, and 67% after 30, 60, and 90 min of UV-exposure, respectively, indicating that tocopherol quickly loses its molecular functionality. Comparable instabilities were reported for other commonly used antioxidants, such as vitamin C (13).

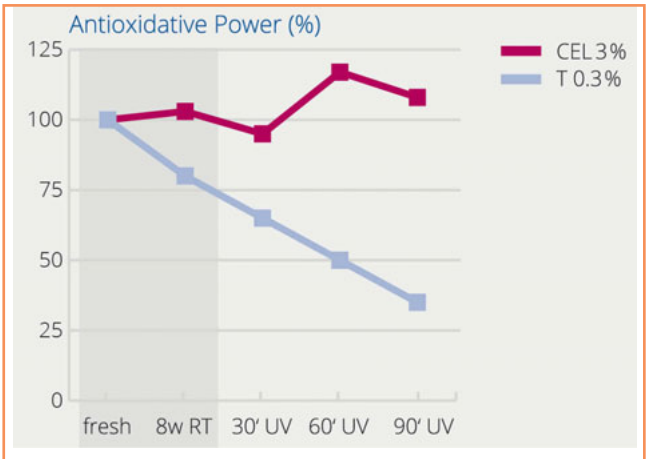

Figure 3. Formul'Ageing is the ageing of the formulation. Variation of the antioxidative power values of O/W formulations containing either 3% CEL (red) or 0.3% tocopherol (T, light blue), expressed in percentages of the initial value. The formulations were first stored at room temperature protected from light for 8 weeks (dark grey) and then exposed to UV light three times for 30 min each. The antioxidative power of CEL upon storage and irradiation did not statistically significantly differ from the initial value whereas the antioxidative power of T was significantly lower already after 8 weeks of storage ($p < 0.05$; ANOVA); N=3, means ± STD of 5%.

CONCLUSION

In conclusion, anti-photoageing ingredients should be both effective inside the skin and provide maximum stability inside the cream. CEL perfectly fulfils these requirements making it a promising antioxidant for both anti-ageing skin care and sunscreen formulations.

We coined the two-sided term Formul'Ageing. First, it is evident that many cosmetic formulations, when topically applied and exposed to sunlight, induce oxidative stress in the skin (formulation-induced skin ageing) (7-10). This is unfortunate. Even though they were developed to care the skin, many conventional finished products have an unpleasant side-effect: they may accelerate premature skin-ageing by aggravating ROS formation when the "cared" skin is subjected to sunlight. In particular, ROS formation inside the "cared" skin was 48 to 75% higher as compared to skin that was left "uncared".

We believe that this phenomenon is of great practical importance. The experimental protocol we used closely mimics real-life situations that could be found in morning or midday beauty routines. For example, a moisturiser could be applied to the face just before leaving home. Within 15 min, the facial skin could be exposed to the sun for 20 min.

For these reasons, we recommend that potential formulation-mediated pro-oxidative effects be considered during formulation development and that cosmetic actives be incorporated that can counteract this detrimental radical induction. UV filters would be ideal for protecting the skin against excessive ROS formation. Alternatively, supplementing the skin with antioxidative actives and thereby strengthening its antioxidative potential are an emerging approach to limit ROS-induced skin damage caused by UV radiation (8, 10). Indeed, we showed here that the incorporation of ≥ 1% of the cosmetic active CELLIGENT® completely neutralised the detrimental pro-oxidative effect of basic formulations; this will allow creating finished products that really do what they are supposed to do: to provide excellent skin care benefits without side-effects.

As a second consideration, not only the skin but also the cosmetic formulations for skin care and sun care are susceptible to oxidation of their ingredients (ageing of the formulation). The results could change the appearance

of the formulation or, more importantly, the effectiveness of its active ingredients. The new active has been proven to provide excellent radical neutralisation inside skin-care and sun-care formulations (10).

We thus believe that, in skin care formulations, the new active will lessen formulation destabilization and active ingredient degradation. In sun-care formulations, we believe that the new active may protect the formulation against photoinduced radical reactions, that take place in any sunscreen due to the activity of the inorganic and organic UV filters. Notably, the efficacy of sunscreens is one of the most important skin protection tools, and the stability of those products should be guaranteed and optimized (12). Antioxidants, however, that are promoted for the stabilisation of skin- and sun-care formulations should present exceptional long-term stability and, in particular for sun-care formulations, excellent photo-stability. Indeed, the new active developed is not only is highly effective but also very stable inside cosmetic formulations upon storage at different temperatures and upon heavy UV-exposure. This dual feature is in stark contrast to classical antioxidants, such as vitamins C/E, that either are effective but unstable or stable but ineffective.

For all these reasons, this new active may become the perfect innovative cosmetic ingredient to counteract Formul'Ageing and to protect the skin as well as the formulation from internal and environmental factors, which contribute to oxidative stress.

To finish, the selective addition of appropriate cosmetic actives may help to guarantee that cosmetic products that are meant to do a good deed will indeed do so.

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