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Antioxidative Power of Formulations Over Life Time: Unique Active Superior than Vitamins

Keywords: antioxidants, antioxidative power, ESR spectroscopy, free radicals, anti-ageing

Introduction

The antiradical and antioxidant properties of cosmetic skin and hair care products are gaining more and more importance in modern anti-ageing strategies. There are several hundreds of active anti-ageing actives that have been selected for their beneficial effects on skin. The main claims used for anti-ageing products are »protection«, »regeneration«, »revitalizing«. These actives may influence the most diversificate functions, beginning with collagen stimulation, protein synthesis, DNA protection, regeneration of membrane functionality, and many others (1). But one common effect of about 90% of all anti-ageing active ingredients is their anti-oxidant and anti-radical properties. The capacity to reduce the number of intrinsic or extrinsic free radical injury in skin and hair is the first and most important feature that an active must be able to do in order to confer efficient protection. The existence of free radicals, as the first and main cause of skin ageing, photo-ageing, pigmentation, and wrinkling is the precondition for an effective performance of all other actives that enhances the biological functions of the skin (2).

Classical antioxidants used for cosmetic formulations are the vitamins C and E and their stabilized derivatives. Furthermore, secondary plant derivates, such as polyphenols, chinones, flavonoides are gaining an increasing importance in modern skin care products. Nevertheless, the high activity and reactivity of vitamins lead to a decrease in shelf-life inside the formulations and cause disadvantages such as product discoloration and loss of activity. The derivative forms of vitamin C and E, i.e. tocopherol acetate or ascorbyl palmitate, lack in antioxidant activity and are often not able to overcome the disadvantages of the pure vitamins. Also the plant extracts are difficult to formulate without loosing their antioxidant activity due to possible interactions with the formulation matrices (e.g.fermentation processes) (3, 4).

It is therefore of main importance to create new antioxidant actives that remain stable inside cosmetic formulations while maintaining the best possible antioxi-

Abstract

The human skin is situated at the interface of the organism and its environment and therefore exposed to a variety of physical and chemical assaults. Exposure to ionizing and UV radiation or xenobiotics generates free radicals in excessive quantities that quickly overwhelm tissue antioxidants and stress-degrading pathways. Apparently there is a need for providing the skin with potent antioxidants to prevent accelerated ageing processes. Commonly used antioxidants in cosmetics like vitamin C, its derivatives or vitamin E are effective, but highly instable during storage. Ideal anti-(photo) ageing concepts should provide maximum efficacy paired with excellent stability and galenic elegance. RonaCare® AP as candidate of a newly developed class of antioxidants fulfils all these requirements. In the present study cosmetic formulations containing different antioxidants are tested with respect to their Antioxidative Power (AP) directly after processing and after different periods of time at different storage conditions. Vitamin-containing formulations show a dramatic decrease in the Antioxidative Power and some of them show undesirable discolorations. RonaCare® AP did not show galenic disadvantages such as yellowing of the formulation and remains stable without loss of its antioxidative activity. These results indicate RonaCare® AP as superb antioxidant not only for anti-ageing skin care but also for sunscreen formulations.

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dant and antiradical performance. This performance should be maintained in the cosmetic formulation for longer storage and also on the skin of the consumer for a certain period under realistic conditions.

In the present work the antioxidant properties of cosmetic products containing the classical antioxidants vitamin C or E on one side and the new skin antioxidant RonaCare® AP on the other side are compared. The emphasis is placed on the influence of environmental stress factors such as storage time, storage temperature and UV radiation on the antioxidant performance.

RonaCare® AP (Bis-Ethylhexyl Hydroxydimethoxy Benzylmalonate, HDBM) is a novel key active for skin care products, and especially for modern sun care products. It is a pure, stable, and transparent cosmetic oil without solubility problems. It can be directly added to the oil phase of an emulsion and remains stable over a broad pH range of 4-7,5. HDBM has a phenolic structure **(Fig. 1)** that is able to neutralize two free radicals through a two-electron-step reduction. The oxidation product of RonaCare® AP itself has antioxidant properties and therefore its

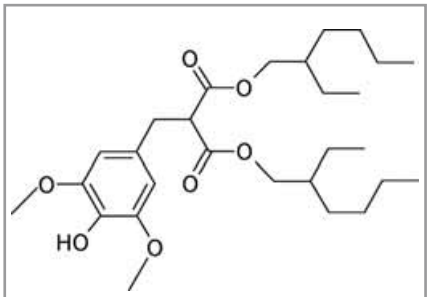

Fig. 1 Chemical structure of Bis-Ethylhexyl Hydroxydimethoxy Benzylmalonate (RonaCare® AP)

activity maintains. As a result of oxidation, the single-bond (HDBM) can be converted into a double-bond (HDBMox) which then possesses the ability to quench singlet oxygen. Singlet oxygen itself is not a radical but also known as very Reactive Oxygen Species. Therefore we consider the HDBM-HDBMox redox-pair as an ideal and complete antioxidant system (5, 6).

This antioxidative activity can be determined for raw materials and final products. In cosmetic formulations antioxidants are expected i) to be highly reactive against free radicals; ii) to penetrate into the living layers of the skin; iii) to be stable inside the cosmetic formulation and easy to handle from a formulator's point of view. Here, we used an *in vitro* method based on ESR spectroscopy to determine the antioxidative capacity and reactivity of cosmetic formulations (Antioxidative Power, AP). This 2 D parameter allows the quantitative comparison of different antioxidants in cosmetic formulations.

■ Materials and Methods

The test radical DPPH (2,2-di-phenyl-1-picryl-hydrazyl) was purchased from Sigma-Aldrich (Munich, Germany) and dissolved in ethanol at a stock concentration of 0.2 mM.

The measurements of the antioxidant capacity and reactivity were performed by using Electron Spin Resonance (ESR) spectroscopy. Since this spectroscopic technique is able to quantify free radicals and since it is applicable to opaque, viscous, and coloured samples, it is particular suitable for the analysis of antioxidants in cosmetic products. The measurements discussed in this article were performed with the X-band ESR spectrometer Miniscope MS 300 (Magnettech, Germany) and the following technical parameters: 60 G sweep width, 100 Gain, 1 G modulation amplitude, 7 mW attenuation, 3365 G central field, 0.14 sec time constant. In the present work we used the Antioxidative Power (AP) method to determine the activity of different cosmetic formulations containing RonaCare® AP, vitamin E, vitamin C palmitate, and vitamin C, all at 1% concentration within an O/W formulation **(Table 1)**. The Antioxidative Power (AP) is a parameter able to quantify both the reaction capacity and velocity of antioxidants (7). The test radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) is used as a detector molecule. At least 3 concentrations of the test sample were prepared and added to DPPH to obtain a initial radical concentration of 0.1 mM. The signal intensity decay of each concentration is recorded at different time intervals during the reaction until saturation is reached and all antioxidant active molecules had react with the test radical. From these intensities a first order kinetic is obtained for each concentration set. The kinetic parameters are used to calculate the reaction time tr and the static parameters

	Ingredient	%
Phase A	Oil Soluble Antioxidants	0.00/1.00
	Glyceryl Stearate, Steareth-25, Ceteth-20, Stearyl Alcohol	8.00
	Cetearyl Alcohol	1.50
	Cetearyl Ethylhexanoate	6.0
	Caprylic/Capric Triglyceride	6.50
	Stearoxy Diemethicone	1.20
	Dimethicone	0.50
	Propylparaben	0.05
Phase B	Propylene Glycol	3.00
	Methylparaben	0.15
	Aqua (Water)	72.10
Phase C	Water Soluble Antioxidants	0.00/1.00

Table 1 Oil in water (O/W) formulation for detection of the antioxidative power

are used to calculate the characteristic weight wc. Both parameters are used to calculate the Antioxidative Power by means of the following equation:

Antioxidative Power = RA • N (DPPH) / tr • wc

where N is the amount of free radical spins and RA is the reduction amplitude. For a direct comparison of different antioxidants, the AP method is standardized to the activity of vitamin C (ascorbic acid, supplied by Sigma-Aldrich, Munich, Germany at the highest grade of purity). The antioxidative activity of a solution of 1 ppm vitamin C is defined as an antioxidative unit (AU).
Each Antioxidative Power value is a result of three independent kinetic measurements. The errors of the measured DPPH intensities and the calculated parameters are below 3%. The standard deviation of each Antioxidative Power value is given to 5%.
The Antioxidative Power values and the reaction times of the formulations were determined after different storage conditions: a) storage at room temperature; b) storage at 40 °C; c) after UV radiation.

Results and Discussion

a) The influence of storage at room temperature on the Antioxidative Power of emulsions
In **Table 2** the Antioxidative Power values of the cosmetic emulsions containing the antioxidants RonaCare® AP, vit. E, vit. C palmitate and vit. C in the final concentration of 1% each are represented. The stabilized form of vitamin E, tocopheryl acetate, has no measurable antioxidative capacity at all. The oil-soluble form of vitamin C, ascorbyl palmitate, has a measurable antioxidative activity of 32% of the activity of ascorbic acid. The Antioxidative Power for each formulation were determined after different storage times. The emulsions were stored at room temperature protected from light in closed polyethylene tubes. **Table 2** reports the results of the antioxidative capacity and reactivity after 4, 10, 16, and 20 weeks storage. In **Fig. 2a** and **2b** the variation of the Antioxidative Power values are reported expressed in percentages from the initial (t = 0) value. It is clearly seen that all formulations containing the classical vitamins C and E, even in the stabilized form of ascorbyl plamitate, are affected by a dramatic loss in activity. After 20 weeks storage the activity of the vitamin C and vitamin C palmitate formulations is reduced to 1-

	t = 0 AP (AU)	t = 4 weeks RT AP (AU)	t = 10 weeks RT AP (AU)	t =16 weeks RT AP (AU)	t = 20 weeks RT AP (AU)	t = 52 weeks RT AP (AU)
Placebo	0	0	0	0	0	0*)
RonaCare AP	1312	1297	1487	1328	1416	1368*)
Vit. E	3400	3018	1985	1893	1396	0*)
Vit. C palmitate	4334	2039	1557	198	75	0*)
Vit. C	13712	10670	4070	1377	304	0*)

The margin of error for all AP values is ± 5%.
*) Calculated values representing a maximum theoretical shelflife of 1 year.

Table 2 Antioxidative Power (AP AU) values of O/W formulation containing 1% antioxidants each, stored at room temperature (RT) protected from light

2% of the initial values. In these two samples after 20 weeks an increase of the reaction time is observed (0.57 minutes), indicating that the antioxidants had lost their molecular functionality. Even the more stable vitamin E formulation decreases in activity up to 40% of the initial value after 20 weeks storage. The RonaCare® AP containing formulation maintained its activity over the whole storage period; even a small increase in its Antioxidative Power is observable.
After 10 weeks storage at room temperature, a clearly visible discoloration of the emulsions containing vitamin C and vitamin C palmitate occurred **(Fig. 4a)**. This colour change became more intense with longer storage times and the color changed from yellow to an orange-brown. The formulations containing vitamin E and RonaCare® AP remained white.

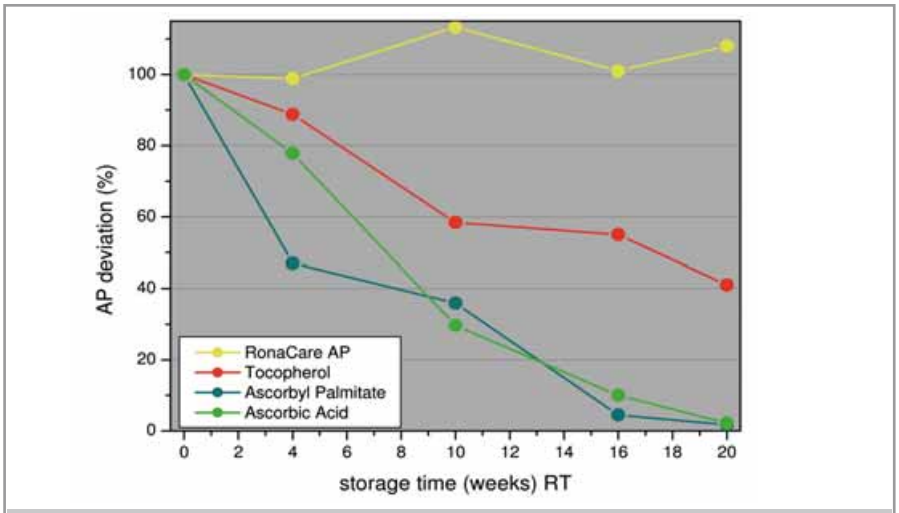

Fig. 2a Variation of the Antioxidative Power values, expressed in percentages from the initial value (t = 0)

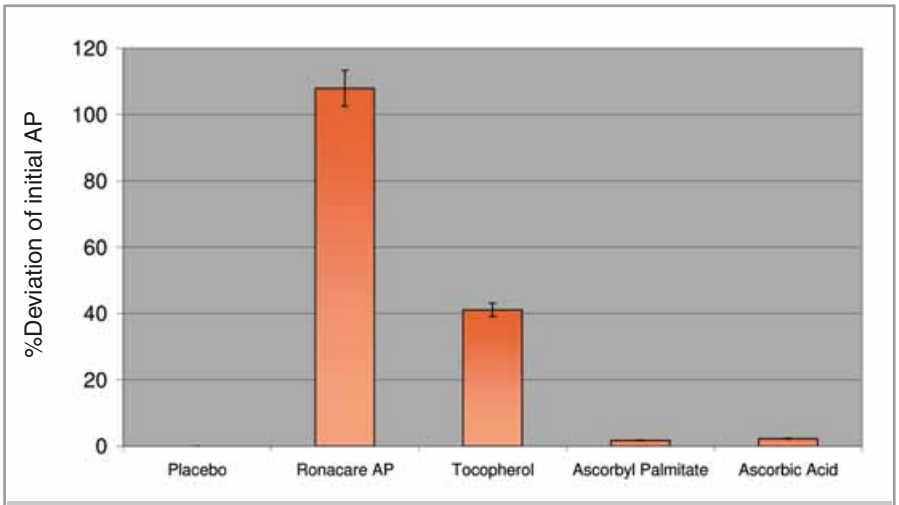

Fig. 2b Variation of the Antioxidative Power values after 20 weeks storage at room temperature, expressed in percentages from the initial value (t = 0), 1% active ingredient

b) The influence of storage at 40 °C on the Antioxidative Power of emulsions
The Antioxidative Power of the cosmetic emulsions stored at 40 °C were analysed after 1 day, 2 days, and 10 days (data not shown). The Antioxidative Power of the formulations containing vitamin E, vitamin C, and vitamin C palmitate decreased with increasing storage time; although this decrease is not as accentuated as the decrease observed at room temperature for longer storage. For the vitamin E formulation a decrease up to 75% of the initial value was determined. The vitamin C and vitamin C palmitate containing emulsions showed

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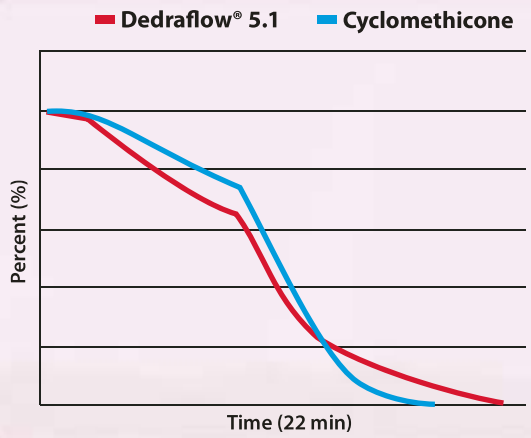

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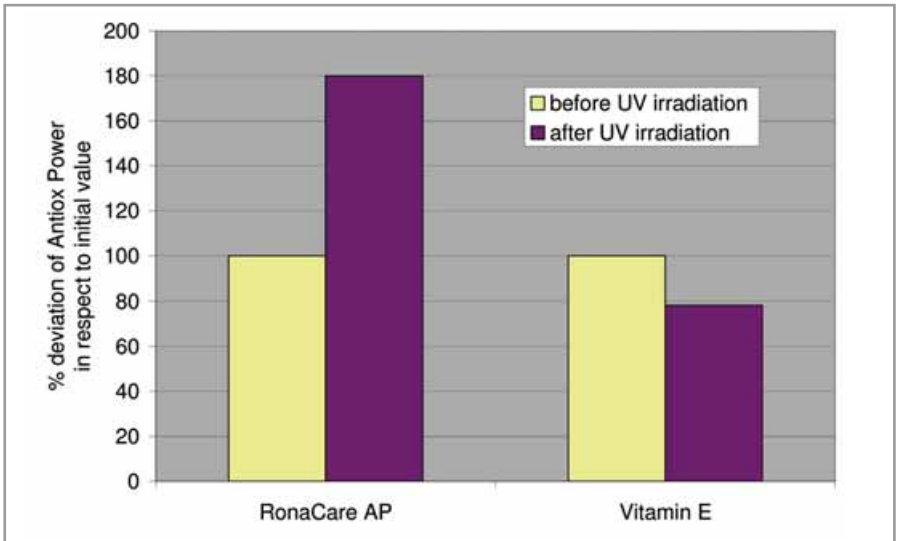

Fig. 3 Variation of the Antioxidative Power values, expressed in percentages from the initial value (t = 0) before and after UV radiation

	before UV radiation	after UV radiation
	Antioxidative Power (AU)	Antioxidative Power (AU)
RonaCare® AP (07-0-2-1)	1312	2362
Vitamin E (07-0-2-3)	3400	2664

Table 3 Antioxidative Power values of O/W formulation containing 1% antioxidants each, irradiated with UV light at 23,2 mW/cm²

a decrease in the Antioxidative Power values after 10 days up to 39% and 51% of their respective initial values. Only the RonaCare® AP containing formulations maintained its antioxidative potential and showed even an increase in the Antioxidative Power values due to shorter reaction times.

c) The influence of UV radiation on the Antioxidative Power of emulsions

UV radiation of antioxidant containing cosmetic products may cause oxidation processes that lead to a decrease in antioxidant activity. Formulations containing RonaCare® AP or vit. E, were UV radiated using a SOL 2 Hönle sun simulator. The irradiation intensity was 23,2 mW/cm². 4 mg cm^{-2} of the test emulsions were spread with a finger cot over a glass plate, the samples were irradiated for 10 minutes, corresponding to 13.92 kJ cm^{-2} or 2.4 MED. The emulsion containing vit. E showed a colour shift to yellow immediately after the irradiation **(Fig. 4b)**. Immediately after the irradiation, samples were recollected and analysed regarding their Antioxidative Power. The results are reported in **Table 3** and **Fig. 3**. The Antioxidative Power of all samples containing vitamins decreased significantly. Only for RonaCare® AP a strong increase in the Antioxidative Power was observed. In **Fig. 3** the variation in Antioxidative Power values, expressed in % of the initial value at t = 0 is depicted. The Antioxidative Power of RonaCare® AP increased up to 180% after UV irradiation.

RonaCare® AP has been shown to provide longlasting antioxidant activity in cosmetic formulations. Its Antioxidative Power was constant even at 40 °C. RonaCare® AP did not show galenic disadvantages such as yellowing of the formulation.

After 20 weeks of storage time at room temperature all other antioxidants have

Fig. 4a Colour change of the cosmetic emulsions after 16 weeks storage at room temperature

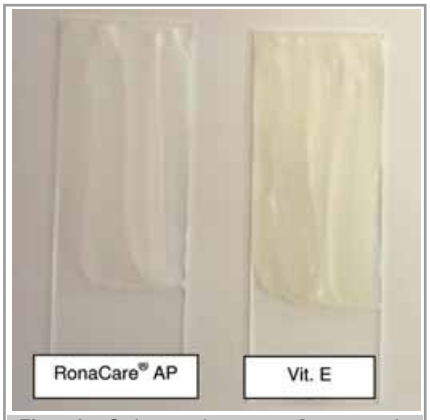

Fig. 4b Colour change of cosmetic emulsions containing 1% of each RonaCare® AP and Vitamin E after UV radiation

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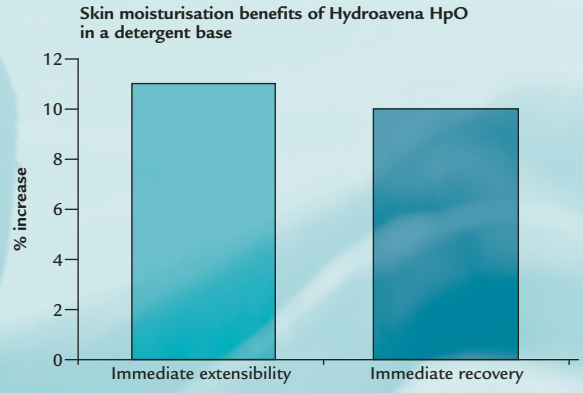

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fallen below the constant protection level of RonaCare® AP. For an extended storage period from week 20 to e.g. week 52 (~ 1 year shelf life) we can therefore expect a drastically improved integral performance versus the compared antioxidants. This perspective appears even more likely when taking into account that RonaCare® AP is known for its ability to even increase its protection level under UV light.
It is known that antioxidants stability might be increased at higher usage levels. The here applied concentration was 1 % of all antioxidants in a cosmetic cream. For lower usage levels instabilities are expected to be even higher unless an antioxidant would show perfect performance stability, such as in RonaCare® AP.

Conclusion

The ESR (Electron Spin Resonance Spectroscopy) based technique to determine the Antioxidative Power is particularly suitable for the analysis of cosmetic formulations and all materials with viscous and opaque characteristics. Not only the capacity of antioxidants but also their reactivity and long term stability can be determined with high precision and minimal efforts.

In comparison to commonly used antioxidants like Vitamin C and Vitamin E RonaCare® AP remained stable in cosmetic formulations over a long period of time with constant Antioxidative Power. After 20 weeks storage at room temperature all vitamins used in this study showed a tremendous reduction in the antioxidative activity accompanied by discoloration of the formulation. None of these undesirable side-effects were observed for RonaCare® AP-containing cremes indicating this newly developed substance as outstanding antioxidant for a multitude of skin care applications.

References

(1) *Fuchs J, Packer L.* Ultraviolet irradiation and the skin antioxidant system. Photodermatol Photoimmunol Photomed. 1990,7: 90-2

(2) *Halliwell B, Gutteridge BJC.* Free Radicals in Biology and Medicine, 3rd edition, Oxford University Press, 1999

(3) *Jung K, Herrling T.* Insights in the role of antioxidants in modern skin and heir care products – methods to prove their activity. Cosmetic Guideline 2007: 197 215

(4) *Jung K, Seifert M, Herrling T.* The role of antioxidants in cosmetic products to prevent free radical damage in skin – an efficacy study. EuroCosmetics 11/12 2007: 12-13

(5) *Rudolph T, Bühle P, Beck J, Pflücker F, Reiffen K-A, and Buchholz H.* Hydroxy Dimethoxybenzyl Malonate: A Novel Anti-(Photo)aging Concept; IFSCC magazine; Vol 9, Number 3; July/September 2006

(6) *Chauduri R, Puccetti G, Marchio F,* and *Lascu Z.* Methods for stabilizing ingredients with cosmetics, personal care and household products; US Patent US 7,150,876, December 2006

(7) *Jung K, Richter J, Kabrodt K, Lücke IM, Schellenberg I, Herrling T.* The antioxidative power (AP) – A new quantitative time dependent (2D) parameter for the determination of the antioxidant capacity and reactivity of different plants. Spectrochim. Acta A 2006, 63:846-50

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