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Anti-Aging Status of Skin Characterized by the Skin Antioxidative Protection SAP – Efficacy of Topically Applied Antioxidants

Keywords: skin, free radicals, antioxidants, antiaging

Abstract

Aging of skin is strongly correlated to the existence of free radicals/ROS, which are generated by external and internal stress factors. The skin, as a link between the exterior and interior milieu of human body is exposed eminently to the oxidative stress of both sides. Topical and systemic applied antioxidants increase or balance the Skin Antioxidative Protection SAP. The SAP parameter characterizes the dynamic performance of the antioxidative activity of skin against the generation of free radicals/ROS. Some antioxidant agents (coenzyme Q 10, green tea polyphenols, vitamin A, C and E) encapsulated in a liposome (ROVISOME™) formulation were topically applied and tested for their increasing effect on the SAP. The antioxidant power (AP) of the antioxidant substances was determined and the effect of the application time on the SAP was measured and represented.

lipoperoxides leading to a considerable membrane alteration (membrane permeability increases, fluidity of the lipids and proteins mosaic changes, all the active transport processes and enzymatic activities are modified). Structural proteins and enzymes undergo the alteration of lateral chains, that turns into aggregation phenomena, fragmentation, cross-linking. Sugars react immediately with ROS giving result to the formation of toxic and unstable products. Nucleic acids are not excluded from the ROS action, even though they are a quite sheltered target. Whatever is the substrate of ROS action, the outcome is the oxidation of intracellular and extracellular components, resulting into phenomena such as cytotoxicity, macromolecular depolymerisation, alteration of the structure and function of cell proteins and proteins of the extracellular matrix (collagen, elastin).

The balance between ROS production and antioxidant defenses (Fig. 1) determines the degree of oxidative stress. Unfortunately, the activity of the antioxidant systems declines during aging, so the consequences of this stress include modification to cellular components.

■ Skin Aging

The skin ages because of damage to proteins and DNA, induced by both chemical and physical means and because of the formation of toxic products, such as Co-proteins and cross-linked macromolecules. Modified proteins and cross-linking are irreversible phenomena responsible for the permanent, deep wrinkling

■ Introduction

In the anti-aging wars, the enemy combatant is the free radical. In the body, a free radical is an oxygen molecule that is lacking an electron and steals one from a nearby cell or molecule. Although free radicals serve a beneficial purpose – such as killing bacteria – their sneaky way of robbing electrons can result in cell damage, which manifests as the signs of aging.

Numerous studies have been carried out in the last decades to elucidate the biochemical and molecular mechanism of aging. The general consensus appears to be that the aging process is multifactorial and that reactive oxygen species

(ROS) are a contributing factor, even though the extent of their contribution remains uncertain. Substantially, the more reliable theory of aging remains the »free radical theory«, articulated in the mid-1950s by *Denham Harman* (1-3), which speculates that endogenous oxygen radicals were generated in cells and resulted in a pattern of cumulative damage.

All the biological structures undergo the detrimental action of ROS. Cytoplasmic and the other membranes are very easily attacked: the unsaturated fatty acids of the phospholipidic and proteic bilayer suffer a hydrogen abstraction from their molecules because of the action of ROS and this results in the formation of

in the dermis. Those structural and functional changes turn into a senescent appearance. Skin is mottled and gray to yellow with red spots and dilated veins, and it becomes thin. So any stress could cause permanent damage. The human body has mechanisms to reduce and repair damages produced by free radicals. Avoiding the environmental causes or free radical production and taking measures to increase the intake of antioxidants are the best defense against free radicals. Both of these anti-aging strategies will keep you looking younger for longer.

Exposure to the ultraviolet (UV) radiation in sunlight is the biggest culprit in the premature aging of the skin; everything from wrinkling to dryness to easy bruising can be largely attributed to exposure to UVA and UVB radiation. Free radicals break down the skin's collagen, which is a key to keeping the skin soft and supple. Other external stresses like high temperatures, mechanical or chemical treatments generate also free radicals which can damage the skin and reduce its SAP (Skin Antioxidative Protection), the comprehensive ability to reduce free radicals. There are two options to increase the SAP of skin: an internal by systemic application and/or an external by topical application of antioxidants. The efficacy of both is determined by the Antioxidative Power AP (4) of the substances used. Cosmeceuticals containing antioxidants are among the most popular anti-aging remedies. Topically applied antioxidants exert their benefits by offering protection from damaging free radicals. Cutaneous benefits include promoting of collagen synthesis, photoprotection from UVA and UVB, lightening hyperpigmentation, and improvement of variety of inflammatory dermatoses.

The selection of antioxidants is determined by their individual lack in the skin and their capacity and reactivity to remove free radicals/ROS. Capacity and reactivity of antioxidants are evaluated by the AP method.

Three classes of antioxidants: vitamins, polyphenols and coenzymes were investigated for their beneficial effect onto the SAP.

In order to compare the effect of the different antioxidants on the SAP their

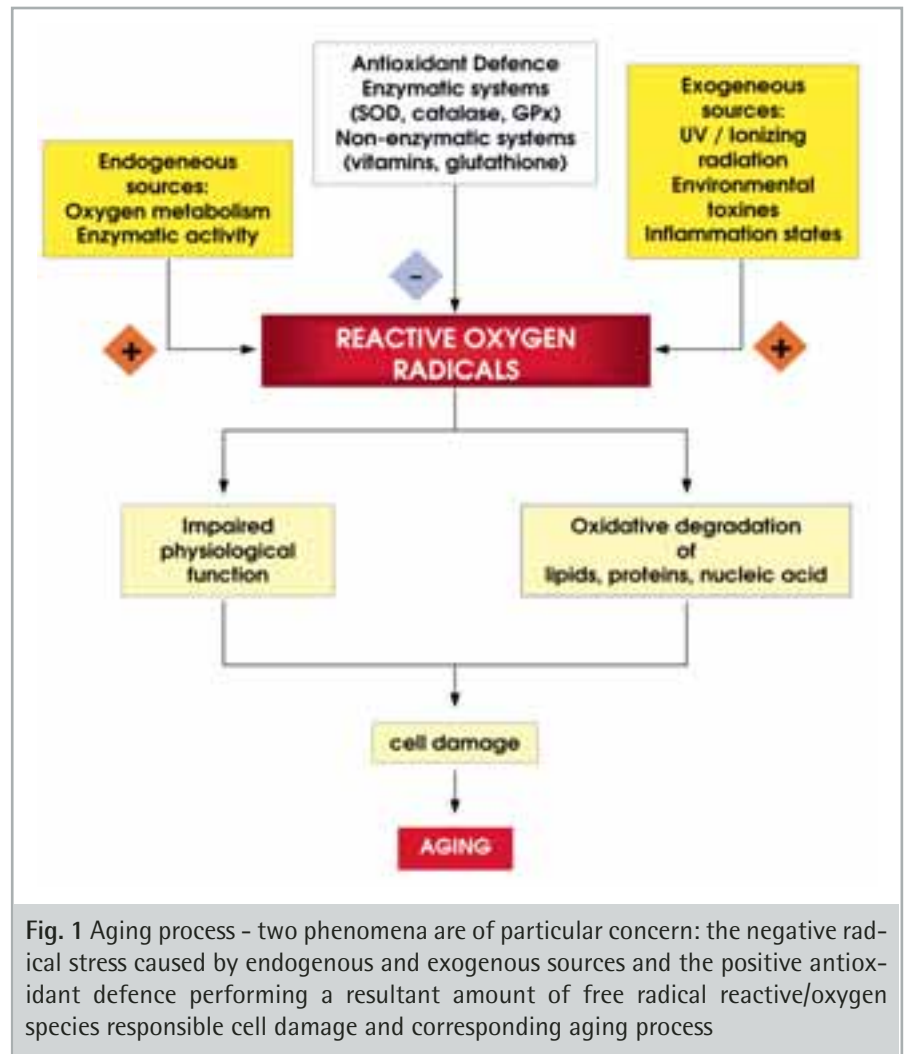


Fig. 1 Aging process - two phenomena are of particular concern: the negative radical stress caused by endogenous and exogenous sources and the positive antioxidant defence performing a resultant amount of free radical reactive/oxygen species responsible cell damage and corresponding aging process

penetration procedure should be optimized and standardized by using the same penetration enhancer for all ingredients. The SAP method offers the possibility to quantify the protection effect of antioxidants in cosmetic formulations and to define the optimum concentration of the active ingredient. Furthermore the influence of the application time, of the cosmetic formulation and of encapsulation in carrier systems can be assessed.

■ Experimentals

Skin samples

For testing the effect of antioxidants on the SAP the pig skin model was used (5). Numerous reports suggest anatomical, physiological and biochemical similarities between man and pig skin. Pig

skin, a waste product of the meat industry, can cheaply be obtained in large amounts and give reproducible results when studying photochemical and photo-toxicological processes. Pig skin was obtained from the local butcher. Skin strips (1x1cm) were placed in Petri dishes (epidermal side up, in immediate contact with air) on filter paper soaked with phosphate buffer (PBS) solution containing a nitroxyl probe as the free radical trap. Cosmetic formulations containing the liposomally encapsulated antioxidants in ROVISOMES™ were applied with an amount of 2 mg/cm² onto the epidermal surface of a pig skin flap, incubated for different periods of time and stored in the dark before pursuing the experiment.

A punch biopsy (Ø = 4 mm) was then taken and investigated with an ESR (electron spin resonance) spectrometer.

Test probes

Three different substances known in the anti-agent therapy and representative for different classes of antioxidants were encapsulated into liposomes. ROVISOME™ was used as a carrier system for the actives and should ensure nearly the same penetration properties for all active ingredients. But the active ingredients are contained in different concentrations in the ROVISOMES™ resulting in different diffusion gradients and distinct optimum penetration times. All formulations are manufactured and supplied by ROVI Cosmetics International:

- (1) ROVISOME DEFENCE containing 2% green tea extract
- (2) ROVISOME Q10 containing 0.5% coenzyme Q10
- (3) ROVISOME ACE Plus containing 1% vitamin A (retinol), 2% vitamin C-palmitate (ascorbyl palmitate) and 1% vitamin E (α-tocopherol)

Instrumentation

The measurements on skin biopsies were performed with a commercial high sensitive X-band bench top ESR spectrometer MiniScope MS200. Skin biopsies were supported in a special tissue cell. Both equipments were supplied from Magnettech GmbH (Berlin, Germany).

SAP-Determination

The SAP describes the ability of the skin to remove free radicals /reactive oxygen species ROS. Skin has its intrinsic antioxidative protection against oxidative stress due to the enzymatic and non-enzymatic antioxidants present in the epidermis and dermis. The measurement of this antioxidative activity is performed by the SAP method, where the reduction of a test radical (spin) probe inside the skin biopsy is measured by ESR spectroscopy. The SAP values of untreated skin were set to 100% in order to represent the full scope of intrinsic skin protection. The application of antioxidants on the skin enhances the antioxidant activity and therefore enhances the SAP values. High-

er SAP means lower number of existing free radicals in skin. Radical trapping experiments have the potential to allow a characterization of the SAP. Nitroxyl probes were used as traps for scavenging antioxidant molecules. The nitroxyl probe is a suitable probe to monitor the biological redox reaction between free radicals and antioxidants, particularly when a nitroxyl probe is localized at an area of interest. The hydrophilic nitroxyl probes widely used for ESR measurements should have no capability to pass through the stratum corneum (SC) barrier, but should be retained and accumulated in the skin (epidermis). With the application of nitroxyl probes we measured the SAP of the skin. The nitroxyl probes were purchased from Sigma (Munic, Germany). Other reagents were of the highest grade of purity commercially available.

Results

Before treating the skin with the antioxidant ROVISOME™ formulations the determination of their Antioxidant Power AP should be performed (4).

The measured AP-values of the three liposome (100%) formulations are listed in Table 1 and are represented graphically in Fig. 2. It is clearly seen that formulation ROVISOME Defence has the greatest AP of 104390 AU caused by its high concentration of 2% green tea extract. ROVISOME ACE Plus has an AP = 7691 AU followed by ROVISOME Q10 with a lower AP of 420 AU resulting from the low concentration of 0.5% coenzyme Q 10. The higher reactivity of ROVISOME Q10 characterized by a reaction time $t_r = 0.17$ min can not balance the AP of ROVISOME Defence with low-

	AP (AU)	t_r (min)
ROVISOME ACE Plus	7691	0.18
ROVISOME Defence	104 390	0.21
ROVISOME Q10	420	0.17

Table 1

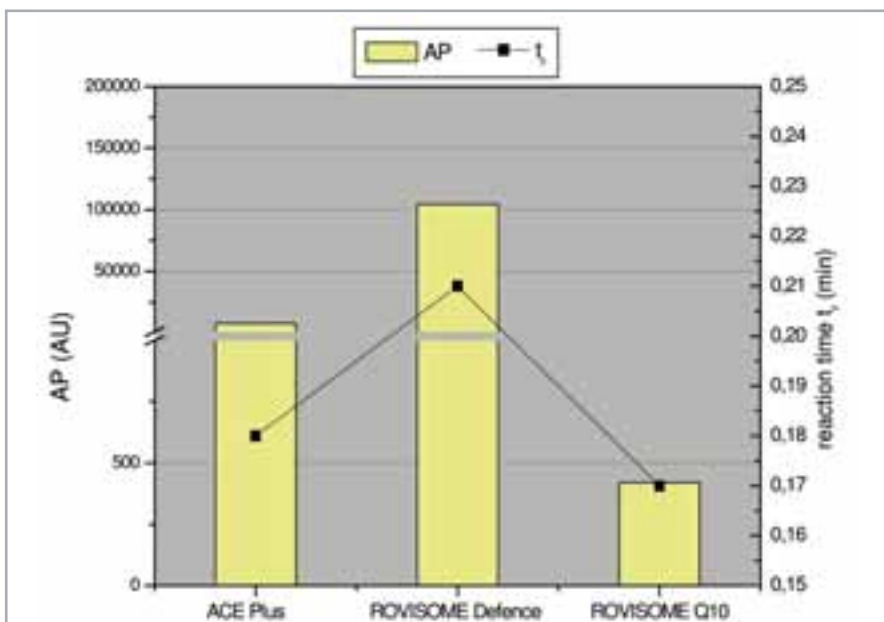


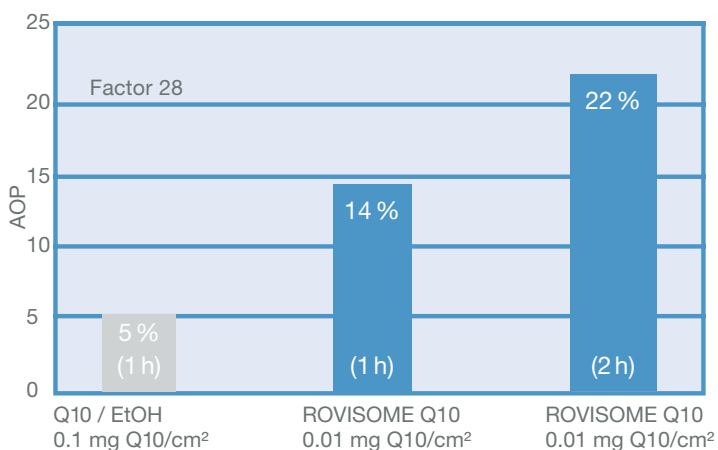
Fig. 2 Antioxidative Power AP and reaction time t_r of different ROVISOME™ formulations containing antioxidants coenzyme Q10 (0.5%), green tea extract (2%), vitamin A, C and E (1%, 2% and 1%)



ROVISOME Q10

The right combination makes the difference. ROVISOME Q10 are specially developed liposomes which transport Coenzyme Q10 as highly active Ubiquinol into the deeper skin layers. **The liposomal encapsulation is cell-identical and leads to an increase in the skin's natural anti-oxidative potential by 22 %.**

The test-results clearly show: ROVISOME Q10 is 28 times more efficient than the pure, non-encapsulated Coenzyme Q10. These significant radical-scavenging effects have also been shown when UV-irradiated. Cosmetic effect and high cost-value ration guaranteed!



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er reactivity and longer reaction time $t_r = 0.21$ because of its low concentration. With regard to the AP-values of the formulations different SAP of the treated skin should be expected provided that the active ingredients have nearly the same penetration properties.

An essential parameter for influencing the SAP is the residence time of the active ingredients on the skin – the incubation time. The SAP of the skin was determined for different residence times of 5; 10; 20 and 30 minutes. The increase of the SAP as a function of the residence time is represented for the different formulations in Fig. 3. Corresponding data are given in Table 2. The SAP of untreated skin (control) was set to 100%. The SAP curves of the tested formulations ROVISOME Defence and ROVISOME ACE Plus show distinctly a saturation effect after a residence times of 10 minutes. Residence times over 10 minutes do not show a further SAP increase for both ROVISOME™ formulations – a saturation plateau is reached.

Considering this saturation effect a maximum ingredient concentration contained in a pure ROVISOME™ formulation (100% liposomes) seems not to be necessary to reach the maximum possible SAP increase typical for the corresponding antioxidant. A final cosmetic O/W formulation containing 5% active ROVISOME™ was tested for its effect on the increase of the SAP. Prior to the SAP investigation the physical stability of the liposomes in the O/W cream formulation was investigated using a special ESR assay. This test reveals 99% of the ROVISOMES™ to be stable and integer over long period of time (data not shown). The difference between the application of pure ROVISOME™ (100%) and 5% ROVISOME™ containing O/W formulation is shown graphically in Fig. 4 and the SAP-values are listed in Table 3, including the standard deviation. It is seen that there is no linear correlation between the SAP-decrease and the concentration decrease of the active ROVISOME™ in the O/W formulation which is caused by the saturation effect of the SAP in the skin. That means that small amounts of the antioxidative active substance almost immediately penetrate through the SC and reach the epidermis layer, where the re-

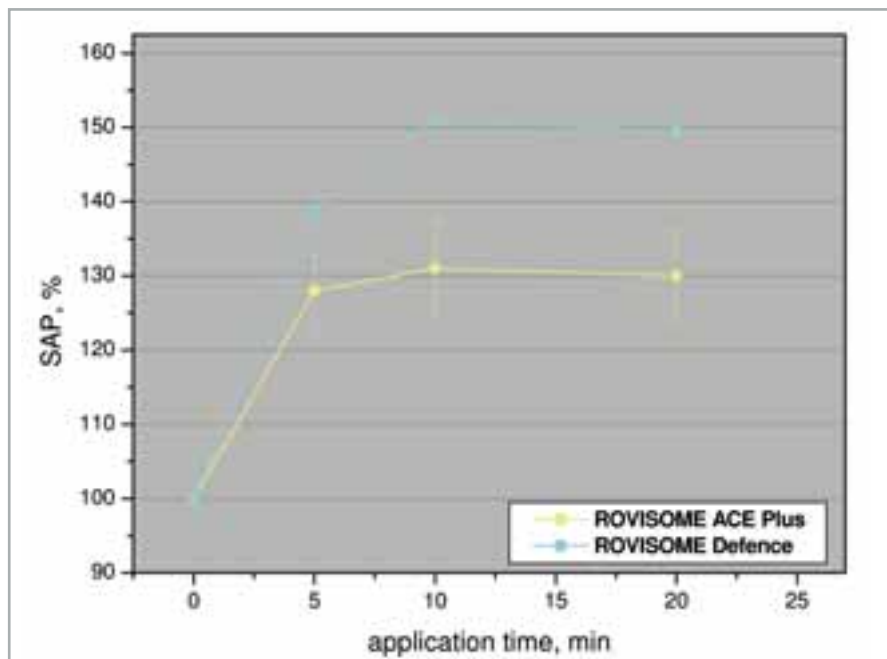


Fig. 3 Measured SAP of the formulations ROVISOME Defence and ROVISOME ACE Plus as a function of the application time using an amount of 2mg/cm²

	ROVISOME ACE Plus		ROVISOME Defence	
Application time	SAP %	± sd	SAP %	± sd
0	100	± 5	100	± 5
5	128.3	± 5	138.9	± 4.8
10	131.2	± 6.2	151	± 6.3
20	130.4	± 6.2	149.5	± 6.4

Table 2

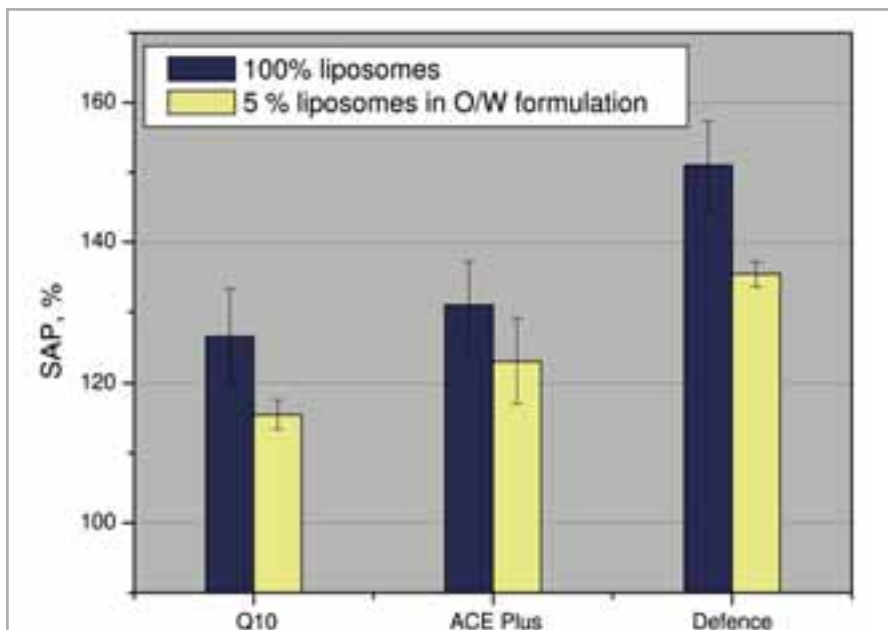
action between the antioxidant and the free radicals takes place. The SC acts as a reservoir of topically applied actives, from where a slow release into the lower skin layers may proceed.

■ Conclusion

The measured results show that the beneficial effects of topical applied antioxidant formulations on the SAP depend on their radical scavenging activity and on the antioxidative power AP. To guarantee a good comparison all antioxidants were inserted in the same carrier system of liposomes/ROVISOMES™ ensuring the same penetration properties

into the skin. A higher SAP can better counteract the actions of free radicals and could avoid a premature aging process.

Considering the AP (Antioxidative Power) values of the antioxidant formulations it is expected that the product with the highest AP value (ROVISOME Defence) will cause the highest increase in the SAP value. As a first approximation this hypothesis is true, although the data demonstrate that there is no linear correlation between the AP value and the increase in the SAP. To act inside the skin, the antioxidant has to cross the horny layer and has to remain active for a longer time. Lipophilic antioxidants as coenzyme Q 10 are particularly active in



INCI of the O/W cream:

Water, Hydrogenated Jojoba Oil, Steareth-2, Glycerin, PPG-15 Stearylether, Hydrogenated Canola Oil, Dioctyl Adipate, Steareth-21, Dicaprylylether, Sheabutter, Cyclomethicone, Polyacrylamide, C13-14 Isoparaffin, Laureth-7, Xanthan Gum, Sodium Hyaluronate, Preservatives

Fig. 4 SAP measured for two different concentrations of the active ROVISOME™ formulation of 5% and 100% applied on skin with 2 mg/cm² applied for 10 minutes

Formulation	SAP %	
	100% Liposomes	5% Liposomes
ROVISOME ACE Plus	131.0	123.1
ROVISOME Q10	126.5	115.5
ROVISOME Defence	151.0	135.4

Table 3

SAP has to be tested by *in vivo* methods. New cosmeceuticals, combined products for simultaneous topical and systemic application to improve the anti-aging status of the skin are under discussion (6).

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the lipophilic environment of cell membranes and their SAP enhancement is stronger than expected from their AP values. The total amount of topically applied actives that reaches the epidermis layer is surely limited. From the data in Fig. 3 results that there is a saturation effect of SAP values that reflects the saturation of the active ingredient that passes into the epidermis. The SAP values in Fig. 4 show that there is no linear correlation between the amount of antioxidants applied onto the skin and the increase of the SAP values. Even formulations containing only 5% of ROVISOMES™ with encapsulated active ingredients show

significant increase of the SAP. That means, that a low concentration of antioxidants will cross the horny layer and that this low amounts are sufficient to increase skins antioxidative activity almost immediately. Appropriate carrier systems like liposomes/ROVISOMES™ protect the antioxidants from oxidation, enhance the penetration into skin and guarantee a standardized penetration profile. The selection of appropriate cosmetic formulations can enhance the penetration through the SC.

Additionally to topical applied antioxidants and radical scavengers the effect of supplemented antioxidants on the

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