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UV - Generated Free Radicals (FR) in Skin and Hair – Their Formation, Action, Elimination and Prevention A General View

Keywords: UV, skin, hair, free radicals, sun protection

■ Introduction

Human skin and hair are very susceptible target organs to UV radiation.

The acute reactions of human skin to solar ultraviolet radiation (280–400 nm) are recognised as a form of inflammation reactions that are mediated by several possible mechanisms (1) including (a) direct action of photons on DNA; (b) generation of reactive free radicals and reactive oxygen species (ROS) involving the formation of O_2^- , O_2^1 , H_2O_2 , $\cdot OH$, $ROO\cdot$, ecc.; (c) generation of prostaglandins, histamine, leucotrienes, and other inflammatory mediators. It is conceivable that UV-induced reactions represent oxidative stress mediated by the formation of free radicals, reactive oxygen species (ROS), lipid peroxidation, liberation of membrane phospholipids, and subsequent formation of prostaglandins by cyclooxygenase pathway.

While the energy of photons of the UVB range seems to be sufficient to damage directly the DNA, the photon energy of UVA radiation generates more free radicals. Although the effects of UVA radiation have been described, the molecular mechanisms by which the effects are produced have not been clarified. It has been suggested that skin exposure to UVA involves the production of reactive oxygen species which may be the first step of the multiple damages induced by UVA (2). It has also been reported that irradiation with UVA produces a decrease in the levels of antioxidants (3), inactivation of antioxidant enzymes (4) and an increase in the markers of lipid peroxidation in skin (5). Recent studies have

shown that UVA can induce epidermal tumours (6), and contributes to erythema caused by solar exposure (7).

Solar UV exposure is one of the most important factors that leads also to photo ageing and photo damage of the hair.

Abstract

Skin and hair are the direct target organs of sun radiation, of electromagnetic waves with wavelengths between 1000nm and 280 nm. The wavelength determines the penetration depth into the target object and the energy of the photon which determines the effect on the molecular structure of the object. The superoxide anion is the primary free radical generated by UV from molecular oxygen. It sets off a chain reaction resulting in further free radical compounds like hydroxyl radicals and further on to free radicals with intermediate activity which are the most likely species to lead to direct biological damages and numerous pathological changes of the skin. The reduction of the sun incidence is the only way to prevent these damages of skin. A sunscreen is the first defence line to prevent the generation of dangerous free radicals. An endogenous protection is realized by the topical application of antioxidants which forms a second defence line. Modern sunscreen products should provide broad spectrum UV protection. The UV filter should be heat- and photostable, water resistant, nontoxic, and easy to formulate.

But the quality characteristic of all these properties is determined last not least by the UV protection of the final product. An *ex vivo* test method applied on skin which can give in a short time exact, reliable and reproducible results is necessary during the development and production phase of a sunscreen product. The RSF and RHF method should be such a method. The AP method allows in an early stage to choose the right antioxidant ingredients concerning the second defence line of a sunscreen. All these methods belong to a set of quality assessment during development and production of a modern universal cosmetic product.

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The melanin pigment in the hair protects from UV-induced free radicals, since melanin is an efficient radical scavenger. The amount of UV induced free radicals in hair can be quantified by measuring the melanin free radicals.

Solar exposure and oxidative coloring are the most common and important factors that can lead to color fading and loss in hair manageability. In fact, ultraviolet (UV) radiation is considered to be the most damaging of all environmental factors. UV rays also effect the color and brilliance of hair. The UV induced damage involves deep changes in the structure of keratin caused by the photo-oxidation of amino acids, sterols and fatty acids, resulting in rupture of sulfur bridges, decomposition of lipids, decrease in melanin as well as numerous micro-molecular lesions (8,9).

Oxidative damage is the main reason for hair condition changes and the most important contributor to oxidation is UV radiation. Oxidative reactions in biological systems lead to the generation of free radicals. An efficient approach to alleviate the damage is the use of UV filters and antioxidants able to reduce the amount of free radicals.

Melanin, a biological polymer which is responsible for the pigmentation of skin and hair, acts both as a sunscreen and a radical scavenger. In human hair its concentration is responsible for hair color ranging from very black to blond.

The role of reactive oxygen species (ROS) and of their antagonists, the antioxidants and UV filters is examined in *ex vivo* measurements on pig skin biopsies and excised human hair. Whereas antioxidants reduce free radicals or repair radical damages the application of UV filters prevent the generation of free radicals. The measurements of these free radicals and their prevention or elimination can directly carried out by electron spin resonance (ESR) spectroscopy.

■ The Physics of Sun Exposure

The electromagnetic spectrum

The electromagnetic radiation is characterized by its dualism, the wave nature and the particle nature.

Wave Nature

- Relationship between wavelength (λ) and frequency (f): $c = \lambda f$
- Energy carried in fields

Particle Nature

- Photon Energy (ϵ): $\epsilon = h f$
- Energy carried in photons

These two physical parameters of solar radiation are decisively concerning their effect on skin. The wavelength λ (Fig. 1) is responsible for the penetration depth of the electromagnetic radiation into the skin and the energy of photons (Table 1) determines their effect on the molecular structure of the target.

The relevant part of the sun radiation reaching the earth and influencing the human skin and hair expands from infrared to UV enclosing wavelength from 1000 nm (IR) to 280 nm (UVB). The most

painful effects are generated by UVB and UVA. Corresponding to their penetration depth UVB and UVA radiation generate primary free radicals/ROS followed by secondary daughter radicals like Lipid radicals.

Photon energy and the primary effects corresponding to the UV spectrum

Ultraviolet Interactions

The near ultraviolet (UVB – UVA) from 280 nm to 400 nm is absorbed very strongly in the surface layer of the skin by electron transitions. As we go to higher energies (UVC – UVB) from 100nm to 280nm the ionization energies for many molecules are reached and the more dangerous photo ionization processes take place. Sunburn is primarily an effect of near UV. Ionisation produces the direct risk of skin cancer.

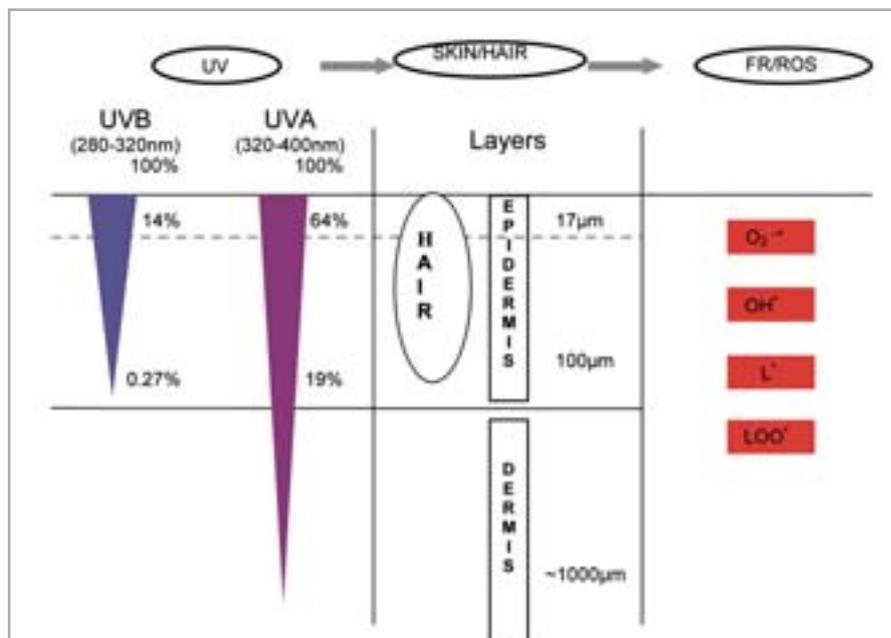


Fig. 1 Penetration depth of UVA and UVB wavelength in skin/hair and the FR/ROS consequently generated

Spectrum	Wave length λ	Energy ϵ
C	100 nm – 280 nm	12.3 – 9.8 eV
UV B	280 nm – 320 nm	9.8 – 9.2 eV
A	320 nm – 400 nm	9.2 – 8.1 eV
IR	760 nm – 1 mm	1.6 – 10 ⁻³ eV

Table 1 Spectrum of UV radiation and its photon energy

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Infrared Interactions

The quantum energy of infrared photons is in the range 0.001 to 1.6 eV which is in the range of energies separating the quantum states of molecular vibrations. Infrared is absorbed more strongly than microwave radiation, but less strongly than visible light. The result of infrared absorption is heating of the tissue since it increases the molecular vibration activity.

■ **The Formation and Action of Free Radicals (FR) and Reactive Oxygen Species (ROS) in Biological Material**

Primary reactive free radical species and their secondary daughter radicals
The presence of molecular oxygen (O_2) within skin cells in the mid – lower levels of the epidermis is a primary target for UV light waves that penetrate the skin. The incoming UV light donates an electron to molecular oxygen within skin cells. Molecular oxygen becomes to an unstable, aggressive free radical known as the superoxide anion ($O_2^{\cdot-}$). To stabilize itself electrically, the superoxide anion sets off a chain reaction resulting in further free radical compounds like OH \cdot . Reactive oxygen products and other biologically important free radical species are usually very unstable in biological material due to their high reactivity. High chemical reactivity results in low specificity of the reaction in biological material; biological oxidants with low reactivity have a high chemical specificity. Free radicals have a characteristic half – life due to their chemical reactivity and are distinguished from radicals bound to, e.g. enzymes (ribonucleotide reductase), which are not considered »free« radicals. Some radicals are stable enough to diffuse across biological membranes; others are so reactive that they react in the chemical microenvironment at their site of formation. Average half – life and half – life ways of biologically relevant free radicals and reactive oxygen species are listed in Table 2. For comparison, the thickness of the phospholipid bilayer is 4 nm.

Very reactive free radicals (hydroxyl radical) cause biological damage only if gen-

erated in close proximity to a potential target molecule (e.g. DNA), because they are immediately scavenged by the high concentration of organic molecules in the cell. If they are to cause cell damage directly, they need to be generated directly at the critical cellular target site.

Free radicals of intermediate reactivity are able to diffuse over significant distances and may then react with some specificity and selectivity with target molecules. They are the most likely species to lead to direct biological damage. Other types are physiological reaction intermediates which do not primarily cause cell damage. Examples of such endogenous radicals are tocopheroxyl, ascorbyl, and certain semiquinone – type radicals. Only under special circumstances may these species lead to pathophysiological reactions.

Persistent free radicals are rather biologically unimportant with respect to direct cell damage. However, they may be damaging by indirect means. Nitroxide – type radicals are one of the best characterized group of persistent radicals. Organic nitroxides are used as contrast enhancing agents in magnetic resonance imaging (MRI). Under special conditions nitrox-

ides may cause cell mutations. Otherwise, organic nitroxide radicals do not have physiological or pathophysiological relevance, with exception of the inorganic $NO\cdot$ radical (endothelial derived relaxing factor, EDRF). $NO\cdot$ is involved in the regulation of vasotonus and is a product of arginine metabolism. Other persistent free radicals of physiological relevance are melanin radicals, which can be detected directly in skin and hair.

Biological Oxidants

Radicals of interest in biological systems can have their unpaired electron on different atoms. Oxygen – centred radicals are to be distinguished from carbon –, sulphur –, and nitrogen – centred radicals. The latter can be products of drug metabolism. Carbon – centred radicals are also formed during lipid peroxidation, and sulphur – centred radicals result from the oxidation of endogenous thiol compounds. Free radicals can be formed by homolysis, photolysis, or radiolysis of molecules or be redox reactions, and they may be uncharged (OH \cdot ; hydroxyl radical) or charged ($O_2^{\cdot-}$; superoxide anion radical).

Reactive Species	Half-life	Half-life way
Hydroxyl radical ($\cdot OH$)	0.3 ns	1.8 nm
Lipid alkyl radical ($\cdot L$)	10 ns	60 nm
Lipid alkoxy radical ($\cdot LO$)	1 μs	6 μm
Lipid-peroxy radical ($\cdot LOO$)	1 – 10 s	
Superoxide anion radical ($O_2^{\cdot-}$)	0,4 μs – 1ms	55nm – 3 μm
Singlet oxygen (1O_2)	μs – ms	
Nitric oxide (NO)	seconds	
Ascorbyl radical	seconds	
Tocopheroxyl radical	seconds	
Melanin	persistent	
Metal ions	persistent	

Table 2 FR/ROS generated in skin by UV

Reactive nonradical oxygen compounds (reactive oxygen species ROS) in oxidative injury are ozone, hydroperoxides, hypobromic and hypochloric acid, hydrogen peroxide, singlet oxygen, and excited carbonyls.

Pathological changes of skin caused by UV exposure

Free radical damage to the skin from UV light is known to produce accelerated aging of the skin. In fact, all changes due to normal aging of the skin can be distinguished from those caused by UV – light – induced skin damage, as this causes dermatoheliosis: distinctive changes to five parts of the skin

epidermis:	actinic keratosis;
dermis:	solar elastosis;
blood vessels:	telangiectasia;
sebaceous glands:	solar comedones;
melanocytes:	diffuse or mottled brown patches

Solar elastosis accounts for much of the skin wrinkling, the result of exposure to UV light. In solar elastosis, the free radicals generated in the skin from UV light cause »up-regulation« of the elastin promoter gene, which increases synthesis and accumulation of elastin and fibrin in the upper dermis. This produces the characteristic »deep-wrinkle« appearance of the skin. On the face, solar elastosis is visible as yellowish skin, crisscrossed by deep wrinkles, and on the neck and extended surfaces of the limbs, as atrophied and dyschromic skin. Free radicals reaching the dermis also cause translocation of glycosaminoglycans and alter their main disaccharide units. As a result, dermal glycosaminoglycans are repositioned from between collagen fibers (where they belong) to be deposited on the elastic material of the superficial dermis. As the correct structure and location of glycosaminoglycans within the skin is responsible for retaining water molecules and moisture, the repositioning and re-structuring of glycosaminoglycans result in less hydration and suppleness of the skin, further contributing to acceleration of skin aging.

Detection of reactive oxidants

Evidence for the existence of reactive oxidants in skin and hair is difficult to establish because of the high reactivity of these species, their low steady state concentration, and the heterogeneity of the organs especially the skin. Skin is composed of three morphologically distinct layers (epidermis, dermis, subcutis). The epidermis has a complicated substructure, and all three skin layers consist of a variety of distinct cell populations.

1. *Direct evidence* of the formation of free radicals can be obtained by ESR spectroscopy
 - Low temperature (-70 °C) ESR for highly reactive (unstable) radicals
 - ambient temperature ESR for persistent radicals
2. *Indirect experimental evidence* for the formation of reactive oxidants can be obtained by the detection of reaction products, spin trapping, or measurements of chemiluminescence
 - Detection of free radical reaction products: lipid peroxidation products (TBRS)
 - Spin trapping highly reactive radical (DMPO, PBN) by ESR
 - Detection of light emission (chemiluminescence; luminol marker)

■ Prevention and Elimination of Free Radicals generated by UV

There are four primary ways to reduce free radical damage generated from UV light:

- avoid excess exposure to sunlight
- wear protective clothing
- antioxidant-containing sun block creams and lotions
- ingest antioxidant supplements

Classical approach: sunscreen (UV filter) as first defence line

It is beyond any doubt that sunscreens are very valuable and should be part of the first defence line against UV radiation. Thus, sunscreens are commonly

used as protection against sunburn, in the form of topical preparations that reduce the penetration of damaging solar UV wavelengths in skin by reflecting or absorbing them. Sunscreens are generally qualified with a sun protection factor (SPF), defined as the minimal erythema dose (MED) of protected skin divided by the MED of unprotected skin. Therefore, sunscreens are only calculated for a reduction of erythema solare or sunburn, one of the acute effects of UVB. Protection against sunburn does not necessarily mean protection against skin cancer. Broad spectrum sunscreens, effective against UVA and UVB, were introduced to overcome this disproportion. A classification of these new sunscreens with the SPF method seems to be impossible.

Endogenous protection by antioxidants

DNA is not only damaged in a direct way, but also indirectly via free radicals/ROS induction. Moreover these free radicals/ROS also damage proteins and membranes within the cell. Cells are well-equipped with antioxidant enzymes (super oxide dismutase, catalase and peroxidase) and ROS scavenging molecules, including L-ascorbic acid (vitamin C) and α -tocopherol (vitamin E). The scavenging capacity of this system is not endless and thus it might become saturated after oxidative stress insults, e.g. UV light. As abundance of free radicals/ROS is one of the causes of inflammation and erythema formation after UV, topical administration of antioxidants can obviously reduce these immediate UV effects. The predominant antioxidants in skin are vitamin C and E, as they neutralize reactive oxygen species before these can produce oxidative stress in respectively fluids and lipid phase. Although the amount of vitamins –originating from nutrition– delivered to skin is limited, it appears to be possible to achieve a higher level of photoprotection by using topical vitamins. It is notable that topical application of these vitamins did not effect UV-induced reactions (s.a. erythema formation) when applied only immediately after irradiation.

Beside application of indigenous skin antioxidants, also other –plant-derived or synthetic–antioxidants, e.g. flavonoids

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can give additional photo protection. For instance, ferulic acid, and an ubiquitous plant antioxidant, not only increased stability to a solution of vitamins C and E, but also adds a substantial synergistic photo protection against erythema and sun burn cell formation. Unfortunately, also this approach has its limits; several antioxidants can have – mainly when administered at high doses – also a paradoxical, prooxidant effect. Thus by determining the application dose one should find a balance between optimisation of positive protective effects and a minimal risk on toxic side effects.

■ Classification of Antioxidants and UV Filters by ESR

The protective effect of UV-filters (sunscreens) and antioxidants in skin and hair can be clearly assessed and classified by methods using the detection of UV gen-

erated free radicals. The application of ESR spectroscopy to study free radical processes in skin and hair appears to be an attractive and effective approach. An exact quantification of the radical protection of sunscreens and antioxidants is possible by using the RSF (Radical Skin protection Factor) and RHF (Radical Hair protection Factor) methods (10,11). The effectivity of antioxidants can be tested *in vitro* by the AP (Antioxidative Power) method.

The Radical Skin/Sun Protection Factor (RSF)

Radical trapping experiments have the potential to allow identification of the generated free radical species and were employed successfully in the detection of oxygen and carbon centered free radicals generated in skin exposed to UV radiation. A nitroxyl probe was used as trap for the detection of free radicals. The ni-

troxyl probe is a suitable probe to monitor the biological redox reaction, particularly when a nitroxyl probe is localized at an area of interest. Nitroxyl probes are susceptible to oxygen concentration, reactive oxygen species (ROS), and biological redox systems and are widely used as probes for in ESR measurements. Sunscreens are the first defence line which should prevent the generation of free radicals ($\cdot\text{OH}$, $\text{O}_2^{\cdot-}$) caused by UVA and UVB. Different sunscreen formulations were tested which contained different concentrations of UVB, UVA and broadband UVAB filters. Their protection against UVAB radiation is represented in Fig. 2 which shows the measured RSF of the tested UV filter formulations.

$$\text{RSF} = \frac{\text{N}(\text{free radicals})_{\text{unprotected}}}{\text{N}(\text{free radicals})_{\text{protected}}}$$

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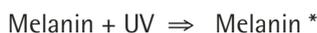
The RSF is a factor characterizing the protection of a sunscreen against the generation of free radicals and presents the ratio between the number N of generated free radicals in the unprotected and protected skin assuming the same applied UV dose (constant irradiance, variable irradiation time) for both or the increase of the time staying in the sun by using UV filter protection assuming the generation of the same amount N of free radical/ROS like for the unprotected skin.

The Radical Hair/Sun Protection Factor (RHF)

Oxidative damage is the main reason for hair condition changes and the most important contributor to oxidation is UV radiation. Oxidative reactions in a biological system like hair leads to the generation of free radicals. An efficient approach to alleviate the damage is the use of UV filters and antioxidants able to reduce these free radicals. Therewith a method is necessary which can quantify the protection factor of UV filters and antioxidants contained in hair care products.

As indicator for the detection of UV generated free radicals in hair melanin is used. Melanin is a biological polymer which is responsible for the pigmentation of many animals and plants. Melanin is present in human hair with different concentrations ranging from very black to blond hair.

One remarkable characteristic of the melanin is the presence of free radicals in its structure. Electron Spin Resonance (ESR) is therefore a suitable technique to study melanin. The intrinsic free radical is sensitive to light, leading to an increased ESR signal after UV radiation.



Using this effect it is possible to measure not only the UV protection of hair care products but also the efficacy of antioxidants present in a hair care products. The RHF method quantifies the amount of free radicals that are generated in the hair according to:

$$\text{RHF} = \frac{\text{N(free radicals)unprotected}}{\text{N(free radicals)protected}}$$

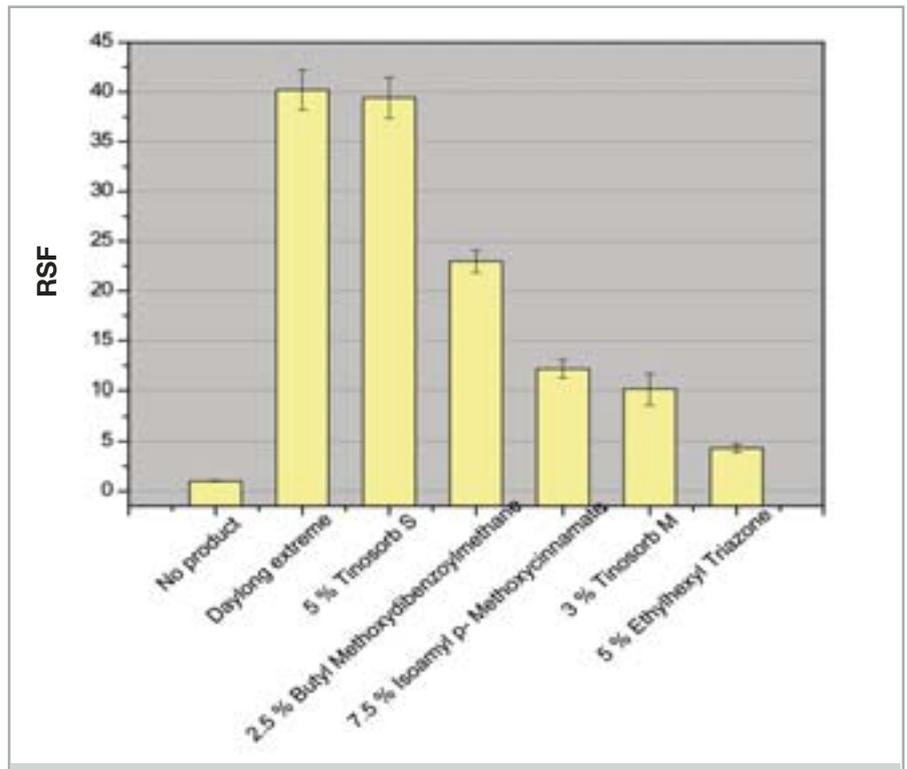


Fig. 2 RSF values of the tested sunscreen and UV-filters

The RHF is a factor characterizing the protection of a sunscreen against the generation of free radicals and represents the ratio between the number N of generated free radicals in the unprotected and protected skin assuming the same applied UV dose (constant irradiance, variable irradiation time) for both. The RSF is also a measure for the increase of the time staying in the sun by using UV filter protection assuming the generation of the same amount N of free radical/ROS like for the unprotected hair.

The UV filters constitute the first defense line that aims to reduce the amount of free radicals by absorbing or scattering the UV radiation. The second defense line is constituted by antioxidants that can neutralize the free radicals generated during UV radiation.

Both strategies are successful in reducing the free radical injury.

Determination of the Antioxidative Power (AP)

The reaction between antioxidants and free radicals is characterized mainly by the reaction capacity, thus the amount

of free radicals neutralized, and the reactivity, thus the velocity of the reaction process. Both parameters can be evaluated simultaneously by the Antioxidative Power (AP) method (12), able to analyse all the different classes of antioxidants. One common effect all antioxidants should have is the reduction of free radical reactions. Free radicals are the first and common cause of skin aging, in particular photoaging, and the reduction of the free radical injury in the epidermis is the most important strategy for a modern skin and also sun care product. The Antioxidative Power (AP) quantifies both the reaction capacity AP (AU) and velocity t_r (min), that can be applied to all kind of samples, independent on their physicochemical properties, and that is able to compare different antioxidants by using the benchmark vitamin C with the measuring unit AU. One AU corresponds to the radical scavenging activity of a solution of 1 ppm vitamin C. The AP method, based on ESR spectroscopy, provides the opportunity to obtain a meaningful parameter that characterizes the antioxidant system or mixture inside a cosmetic formulation. It can be used for the



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screening of different antioxidant classes and to compare the activity of raw material during the development process. Moreover, it is an excellent tool for the quality control of both raw materials and final products.

The measurements of the antioxidant capacity and reactivity of the different antioxidants were performed by using Electron Spin Resonance (ESR) spectroscopy. This technique is able to quantitatively detect free radicals and to analyse opaque, colored and viscous samples as well. The test radical 2,2-diphenyl-1-picryl-hydrazil (DPPH) was used in this assay. DPPH reacts with antioxidants and its signal intensity decay is recorded during the whole reaction.

In conclusion, from the above results appear that it is important to analyse carefully the antioxidant activity of bioactive ingredients (Table 3) in order to produce an efficient cosmetic product. Many influences can have adverse effects on the antioxidant activity, such as fermenta-

tion, oxidation, and reactions of the active with the components of the cosmetic formulation. The AP method can be an excellent tool for the creation of a meaningful parameter of the antioxidant capacity and reactivity. First the raw materials and their long time stability should be tested in order to select a powerful antioxidant with the desired characteristics. Then, the final formulation containing the selected antioxidant should be tested again in order to exclude oxidation reactions. The active ingredients can be protected by adequate procedures, for instance by encapsulation, to protect the antioxidant. To be effective, an antioxidant should be able to penetrate into the outer skin or hair which could be achieved by encapsulation strategies as well and should finally be able to neutralize free radicals. These efficacy tests can be performed using the SAP (Skin Antioxidative Potential) method (13) on skin biopsies and/or the RHF (Radical Hair Protection Factor) on human hair.

Both techniques use ESR spectroscopy and are suitable to demonstrate the antioxidant activities in skin and hair.

■ Closing Remarks

In conclusion, intensive investigation into this area of free radical research indicates that free radicals produced in the skin (and his appendix the hair) from the interaction between UV-light and molecular oxygen (within skin cells), and from the interaction of UV-light and melanin, are primary factors in accelerated skin aging and hair damage. The biological mechanisms through which free radicals are created within the skin and hair are largely understood, as are their deleterious effects on skin and hair cells and related structures, and the ensuing depletion of antioxidants that result from their presence.

Modern sunscreen products should provide broad spectrum UV protection and often contain several UV filters. A modern UV filter should be heat- and photostable, water resistant, nontoxic, and easy to formulate.

But the quality characteristic of all these properties is determined last not least by the UV protection of the final product. An *ex vivo* test method applied on skin which can give in a short time exact, reliable and reproducible results is necessary during the development and production phase of a sunscreen product. The RSF and RHF method should be such a method. The AP method allows in an early stage to choose the right antioxidant ingredients concerning the second defence line of a sunscreen. Using ESR methods it is also possible to investigate the penetration time profile of an antioxidant in a cosmetic formulation and to obtain information about the penetration of these active substances into the skin. All these methods belong to a set of quality assessment during the development and production of a modern universal cosmetic product.

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	Substance	AP (AU)	t _r (min)	Class	Form
1.	Vitamin C	1.000.000	0,24	Ascorbic acid	powder
2.	Vitamin E	404.000	0,33	tocopherol	Oil
3.	Vitamin E-acetate	0		Tocopherol-acetate	powder
4.	Wheat bran extract	120	2,87	Tocopherols, carotenes	Oil
5.	Amaranth seed oil	730	0,31	Tocopherols, tocotrienols	Oil
6.	Liquid tocopherol	165.300	0,57	Tocopherols, tocotrienols	Oil
7.	Ellagic acid	1.352.000	0,60	polyphenol	powder
8.	Rosmarinic Acid	971.200	0,51	polyphenol	powder
9.	Rosemary extract	243.500	0,79	polyphenol	powder
10.	Rooibos 1 Rooibos 2	90.000 – 715.000	0,331 – 0,79	polyphenol	powder powder
11.	Honeybush Extract	102.900	0,97	polyphenol	powder
12.	Aspalathin	1.531.000	0,22	polyphenol	powder
13.	Grape seed extract 1 Grape seed extract 2	357.000 930.000	0,95 0,81	polyphenol	powder powder
14.	Dihydroquercitin	1.030.000	0,23	polyphenol	powder
15.	Ginger Extract	98.700	0,73	terpene, polyphenol	Oil

Table 3 AP and tr values of the tested bioactive ingredients/antioxidants

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