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# The Free Radical Transformation from Cosmetic Product to Skin



## Introduction

Modern, multifunctional cosmetic products are increasingly complex and confront the cosmetic industry with new problems in product development. Active ingredients and particularly basic raw materials may increase the burden of free radicals in the formulations and/or inside the skin, even though they were designed to do just the opposite.
Applied on the skin, a cosmetic formulation is exposed to solar irradiation. UV radiation may lead to radical chain reactions inside the formulation. Especially unsaturated fatty acid, aromatic compounds, inorganic or organic UV-filters, among others, are responsible for the generation of different radical species inside a formulation (mainly lipid peroxides). These radical species may deteriorate the emulsion properties and may lead to the oxidative destruction of actives (i.e. UV-filters, antioxidants) and may therefore reduce the product's efficacy under *in vivo* conditions. Moreover, the degradation products of actives or components may have undesired effects and may be responsible for allergic reactions.
It is therefore important to create formulations with no or very low radical generation, in order to protect the chemical and physical properties of the product.
First, protecting the formulation from free radicals is pivotal for proper product function and stability.

## Abstract

**Free radicals are generated in cosmetic formulations especially in lipids(mostly poly unsaturated fatty acids – PUFA) and transform their radical properties to skin. It is described what characterizes the radical generation in the formulation and its transformation on to the skin.
The radicals in cosmetic formulations are generated by environmental impacts(chemical/physical stress or aging). The radical transformation is possible because it is in direct contact to the skin.
Free radicals in a cosmetic formulations are generated by UV exposition. With increasing time an ascending curve of free radicals is generated. The curve can be characterized by only one parameter the rate constant LRF(Lipid Radical Factor) which is typical for every formulation.
On the basis of the LRF two samples are selected which are applied on the skin to measure the Radical Status of Skin (RSF). It is seen that the formulation with a higher LRF generates a lower RSF. That means that samples with a high radical generation potential generates also more free radicals in skin, LRF↑ → RSF↓. The radical character of the cosmetic product is directly transformed to the skin.**

### In cosmetic product (*in vitro*)

In cosmetic formulations excessive ROS is toxic to the consumer as radicals from the product penetrate into the living layers of the skin and do harm. Excessive ROS formation may also accelerate the degradation of the functional ingredients and provoke olfactory and physiochemical destabilization.
Before and especially as soon as a product is applied to the skin, it is exposed to external physical and chemical influences, including oxygen and UV-radiation (1). Contact with molecular oxygen triggers autoxidation of basic ingredients such as fatty acids as well as peroxide

formation. Effective antioxidants can lessen product oxidation and thereby improve the formulation's shelf-live, quality and skin compatibility (2).
Contact with sunlight triggers disadvantageous radical chain reactions inside the formulation itself in a very unpredictable manner. The higher the amount of UV-induced free radicals, the higher is the risk of photo-oxidative reactions that eventually may lead to formulation instability. Antioxidants are used in formulations to counteract these oxidation processes. Therefore, compelling antiradical and antioxidant efficacy is becoming more and more important for modern products, but the selected raw materials not always achieve the desired results. Some plant extracts or certain UV-filters even exacerbate the problem if the exposure to sunlight transforms them into unwanted pro-oxidants (3). The generation of free radicals, in turn, will favor the degradation of other valuable compounds present inside the formulation.
Thus, for proper product function and stability, it is critical to add ingredients that truly prevent or neutralize UV-induced radical formation within both, skin-care and sun-care formulations.

**In skin (*ex vivo*)**

Second, free radical protection of the skin is important to prevent premature skin-aging.
Surprisingly, many cosmetic raw materials, when applied to the skin and exposed to sunlight, provoke massive oxidative stress in the skin. Incorporation of cosmetic actives completely counterbalanced the raw material-induced oxidative stress in the skin (4). Lipid peroxidation is devastating and rapid, because every lipid radical formed in the process can »burn« another lipid molecule. A chain reaction is the consequence.

## Materials and methods

**Skin**

Skin biopsies from pig were used in the *ex vivo* experiments (5). Pig skin has the greatest similarities to human skin and has the main advantage of a high structural and functional homogeneity. The ears of 6 month old pigs from local slaughter were washed, the cartilage and the sub-dermal fat was removed, the skin was cut into 1x1 $cm^2$ pieces and stored in phosphate buffered saline (PBS) with a pH = 7,4 at 77 °K until used. Skin biopsies (Ø 4 mm) were taken from the prepared skin flap. The samples were applied on the epidermal side of the pig ear.

**Chemicals**

**Cosmetic products**
Sample 1 and sample 2 were commercially available cosmetic market formulations. Both products were facial lotions. Sample 1 was a saturated system not containing natural unsaturated oils with very low perfume content. Sample 2 was a lotion containing natural oils and having high perfume content.

**Free radical indicator**
Carbon centred free radicals (lipid radicals) generated in the cosmetic formulation and the skin during UV irradiation were detected by using a radical trap on the basis of nitroxyl compounds 2,2,5,5 tetrametylpyrrolidine-N-oxyl – PCA from Sigma-Aldrich (Germany).
The spin probe PCA (2,2,5,5-tetrameth-yl-3-carboxylpyrrolidine-N-oxyl) was used as a radical indicator. It is photo stable and reacts with all oxygen- and carbon-centered radical species.
900 mg of the test formulations were mixed with 100 mL of a PCA solution to obtain a 0.01 mM PCA concentration. 50 µl of the sample preparation were inserted in capillary quartz tubes. The PCA signal intensity was measured using an ESR spectrometer. The PCA react with the radical species to give a ESR-silent hydroxylamine.
The capillary tubes were exposed to increasing UV radiation using a Newport sun simulator.

**UV irradiation**

The UV-irradiation was performed with xenon arc lamp Solar Simulator from Newport-ORIEL Product Line 81260 (US, Newport Solar Simulators – product specifications) equipped with a 300 W Xenon lamp supplying an irradiance in the plane of the sample of 16,5 mW/$cm^2$ for UVA (330-400 nm) and 5,0 mW/$cm^2$ for UVB (290-330 nm). The 81260 has a UVB/UVA dichroic mirror as a standard device. It passes 280 to 400 nm and greatly reduces the VIS and IR output of the lamp. The measurements were performed with an UV-Meter-BASIC (hönle UV technology, Germany). The UV solar simulator emits a continuous spectrum with no gaps or extreme peaks of emission in the UV region. The output from the solar simulator is stable, uniform across the whole output beam and suitable filtered to create a spectral quality that complies with the required acceptance limits. The RCEE % values are in the acceptance limit (6,7).

**ESR spectrometer**

A X-band ESR spectrometer Miniscope 300 Magnettech, Germany was used for *ex vivo* radical detection. A special tissue cell (GZ 170P) from Magnettech for skin measurements was also applied.

## Experimental process

Two experiments were performed: one aimed to detect the free radicals inside the formulation (Lipid Radical Factor LRF) and the other aimed to detect the free radicals inside the skin (Radical Skin Protection Factor, RSF).
The spin probe PCA (2,2,5,5-tetrameth-yl-3-carboxypyrrolidine-N-oxyl) was used as a radical indicator. It is photo stable and reacts with all oxygen – and carbon-centred radical species.
For the *in vitro* LRF experiment 900 mg of the test formulations were mixed with 100 mL of a PCA solution to obtain a 0.01 mM PCA concentration. 50 µl of the sample preparation were inserted in capillary quartz tubes. To generate lipid radicals in the formulation irradiations in the second range (10-180 s) is necessary. The irradiation and ESR measurements are performed in capillary tubes.
The spin probe PCA (2,2,5,5-tetrameth-yl-3-carboxylpyrrolidine-N-oxyl) was used as a radical indicator. It is photo stable and reacts with all oxygen- and carbon-centered radical species.

For the *ex vivo* measurements (RSF method) the test products (2 mg/cm², as defined by the COLIPA standard) were applied one time on a 1x1 cm² skin biopsy (epidermal side). The treated skin samples were allowed to remain for 15 minutes in the dark at room temperature in a normal humidity atmosphere. After that they were placed on a paper (filter discs grade 389, 84 g/m² from Munktell&Filtrak GmbH, Germany) imbued with a 1 mM solution of the test indicator (PCA) for 5 minutes. Then a skin biopsy of 4 mm diameter was taken with a biopsy punch, the skin was fixed on the tissue cell and the ESR spectrum was recorded. The skin in the tissue cell was UV irradiated with different UV doses (corresponding to irradiation times between 0,5 and 5 minutes) and different irradiance corresponding to the optical transmission given by the density filters. After that process the ESR spectra of the skin biopsy were measured and the data were analysed corresponding to a given numerical algorithm (8,9).

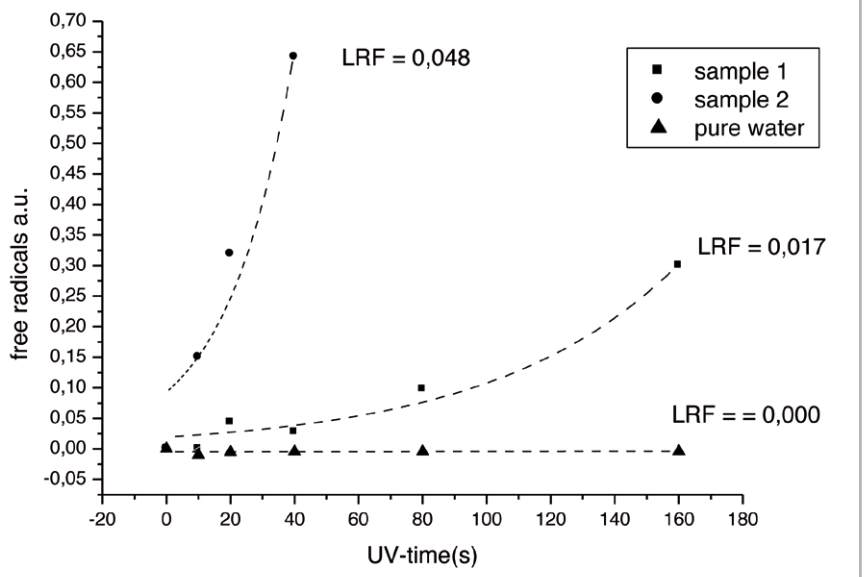


**Fig. 1** *In-vitro* measurements (normalized values) of lipid radicals in two cosmetic formulations (sample 1 and 2) and pure water.

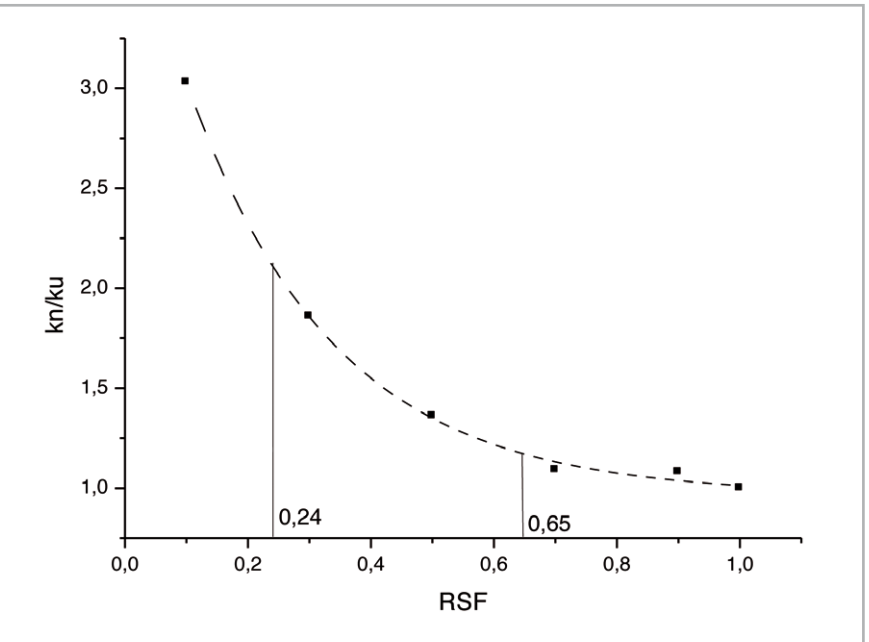


**Fig. 2** Calibration curve for the rate constant $k_n/k_u$ as a function of RSF (1<RSF >0) for the used skin.

## Results

Compelling antiradical and antioxidant efficacy is becoming more and more important for modern products. To test the radical status of a cosmetic we measure the LRF/10/. The LRF is a factor which detects the radical formation capacity of a formulation for lipid radicals. It is the rate constant for the generation of lipid radicals over a UVAB irradiation time (**Fig.1**). Mainly poly- unsaturated fatty acids and perfume oils are responsible for the formation of lipid peroxy radicals. For pure water the LRF=0. **Fig. 1** shows the LRF of two test samples and pure water. The LRF is measured by using the test probe PCA (10 µM) together with a UVAB irradiation over some seconds. Sample 2 which contains more unsaturated fatty acids has a higher LRF = 0,048. Sample 1 with LRF = 0,017 has a moderate LRF.

The question is now. Can the generated free radicals during UV in the applied formulation also generate free radicals in the skin? Is there a radical-transformation? In order to answer this question, the second analytical method the RSF measurement, was performed.

The samples were applied for 15 minutes on the skin and the RSF of skin were measured.

A quantitative expression of the RSF value is achieved by the construction of a calibration curve using optical density filters (UV-attenuation filters).

Corresponding to the used density filters of PF5 and PF10 we measured a normalized signal intensity of $k_u/k_5$ = 3,08 for PF5 and $k_u/k_{10}$ = 3,61 for PF10. With these

values the calibration curve in **Fig. 2** is calculated which is necessary for the determination of the RSF of the two formulations of sample 1 and sample 2.
Then the two formulations were applied on the skin for 15 minutes and the normalized PCA-reduction (**Fig. 3**) was measured.
The k-value of the sample 1 ($k_1$ = 0,22) is similar to that of untreated skin ($k_u$=0,188). On the contrary, we find for sample 2 a distinct increase of radical status of the skin ($k_2$=0,413). Corresponding to the calibration curve of the used skin in **Fig. 2** we find for sample 1 ($k_1/k_u$ = 1,17) a RSF = 0,65 which corresponds to a 53 % radical increase. For sample 2 a RSF = 0,24 ($k_2/k_u$=2,19) which corresponds to a radical increase of 316 %.

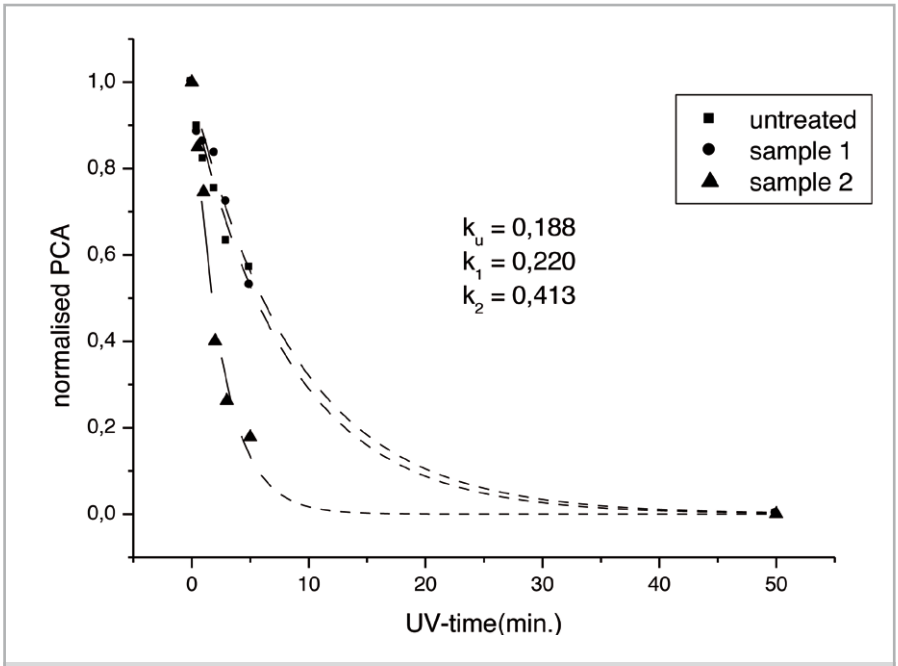


**Fig. 3** *Ex-vivo* measurements of the rate constants $k_n$ (n= p,1,2) for the application of different samples (sample 1 and 2 and untreated) on the excised pig skin.

## Discussion

It is clearly seen that skin products/ formulations have a great influence on the skin. The higher the LRF the higher the impact on the skin. More lipophilic substances have a better chance to penetrate in the stratum corneuml. Lipid peroxy radicals can so in a chain reaction transfer its radicals to the epidermis and so on. Lipid peroxidation is devastating and rapid, because every lipid radical formed in the process can burn another lipid molecule. Until something comes along to terminate the process by sopping up all those rad electrons, the fire will just keep burning. Only saturated fatty acids, highly purified peroxy-free perfume oils, or a sufficient concentration of antioxidants should be used to prevent the generation and chain reaction of free radicals. Skin care products with a high part of unsaturated lipids has a high LRF which generate many free radicals under UV-irradiation (10). Also other skin products like self tanning creams which containing DHA influence the radical status, thus, increase the susceptibility of the skin to external stressors like UV radiation (11) decreasing the RSF of skin. By adding unsaturated lipids (almond oil) and/or perfumes, the generation of free radicals is dramatically increased (to be published). Via the chain reaction radicals can transfer from the product to the skin. Lipid radicals generated in the product can transfer in to the skin. Especially products on the basis of polyunsaturated lipids can do so. So antioxidants have an indirect influence on to the skin. They can prevent the generation of free radicals in the formulation during UV-irradiation.

**Abbreveaitions**

| | |
|---|---|
| PCA: | 2,2,5,5-tetramethyl-3-carboxypyrrolidine-N-oxyl |
| PUFA: | poly unsaturated fatty acid |
| LRF: | Lipid Radical Factor |
| RSF: | Radical Status Factor |
| k : | rate constant |
| ESR: | Electron Spin Resonance |
| UVA: | UV radiation 320-400 nm |
| UVB: | UV radiation 280- 320 nm |

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