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Methodology to reach full spectral photo-protection by selecting the best combination of pigments and antioxidants

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Introduction

Despite the large number of skin-friendly, UV-absorbing sunscreens, the incidence of skin cancer has been rising sharply for decades. *In vivo* studies on human skin demonstrate, that UV light generates most of the radicals, followed by visible (VIS) and near infrared (NIR) irradiation. During sun exposure around 60% of all free radicals are produced by UV, the remaining 40% are formed by VIS and NIR for skin types I-III (Lohan et al., Exp.Derm 25 (2016), Zastrow et al. Skin Pharmacol Physiol. 22, 2009). For darker skin types, the percentage in the NIR spectral range even increases (Albrecht et al., Br J Dermatol 180, 2019) (Figure 1). Due to the use of UV absorbing sunscreens, most consumers extend their sun exposure time without considering that they are not protected in the VIS/NIR spectral region, promoting an enhanced radical formation in deeper skin layers. Antioxidants can reduce free radical formation where typical UV chemical filters may no longer be effective (Gabros et al., in: StatPearls, Treasure Island (FL) 2021). Interestingly, in contrast to the synergetic effect of chemical and physical filters in the UV region, we observed that the addition of TiO_2 and ZnO nanoparticles reduce the activity of the antioxidants added to the formulation, which is often not due to the lack of antioxidant analysis of the final product. In this paper, a general method is presented to preselect optimum combinations of antioxidants and pigments.

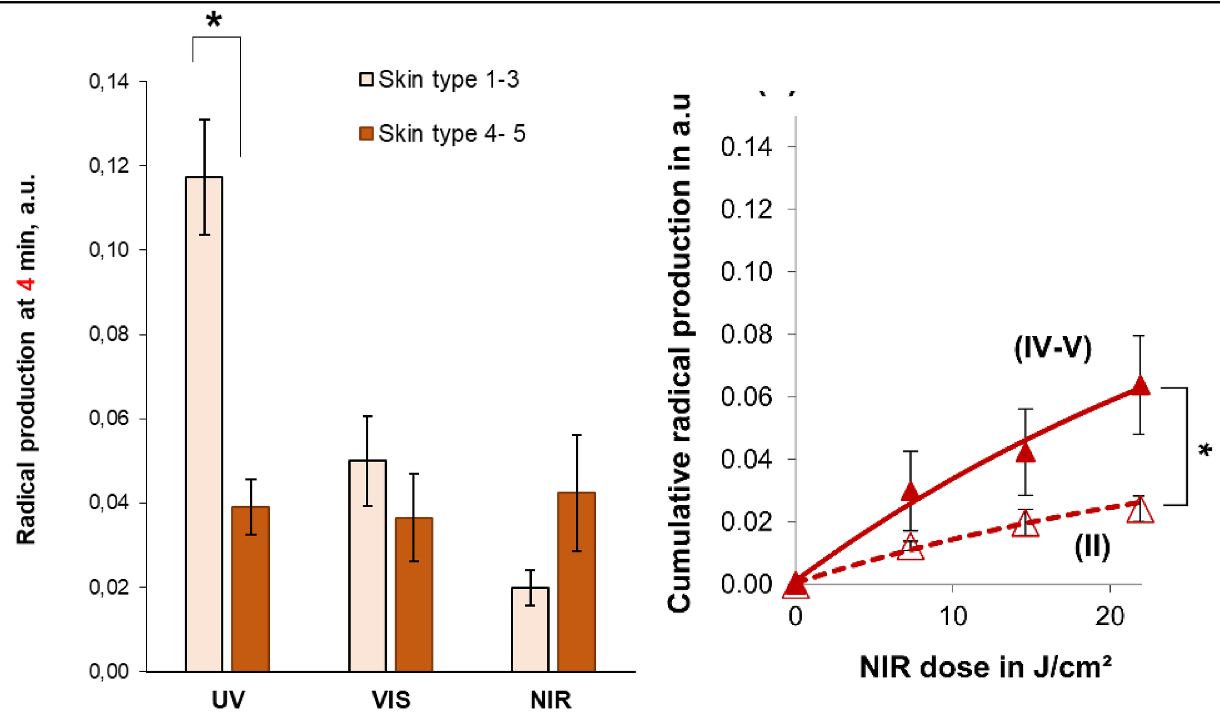


Figure 1 Radical production in skin for different skin types and wavelengths (Albrecht et al., Br J Dermatol 180 (2019).

Materials

Analyzed pigments and antioxidants

Table 1: Cream formulations codes with implemented pigments in % and the size of the pigments.

Cream Code/ Pigments	TiO_2-AlOH Titanium Dioxide, Aluminum Hydroxide, Isostearic Acid	ZnOtriet25 Zinc Oxide, Triethoxy-caprylylsilane	ZnOtriet45 Zinc Oxide, Triethoxy-caprylylsilane	ZnOpolymyr Zinc Oxide, Polydimethyl-siloxane, Myristic Acid
Diameter in nm*	10	25	25	35
No-pigm	**0**	**0**	**0**	**0**
ZnOtriet			**10**	
ZnOpolymyr				**10**
TiO2AlOH+ZnOtriet		**10**		

To counteract the radical formation, the following antioxidants were investigated in solution:

- Epigallocatechin Gallate EGCG
- Alpha-Tocoperol/Vitamin E (VE)
- Ferulic acid (FA)
- Emblica
- Rosemarinic acid
- Sodium metabisulfite (SM)

Epigallocatechin Gallate (EGCG)

Vitamin E (VE)

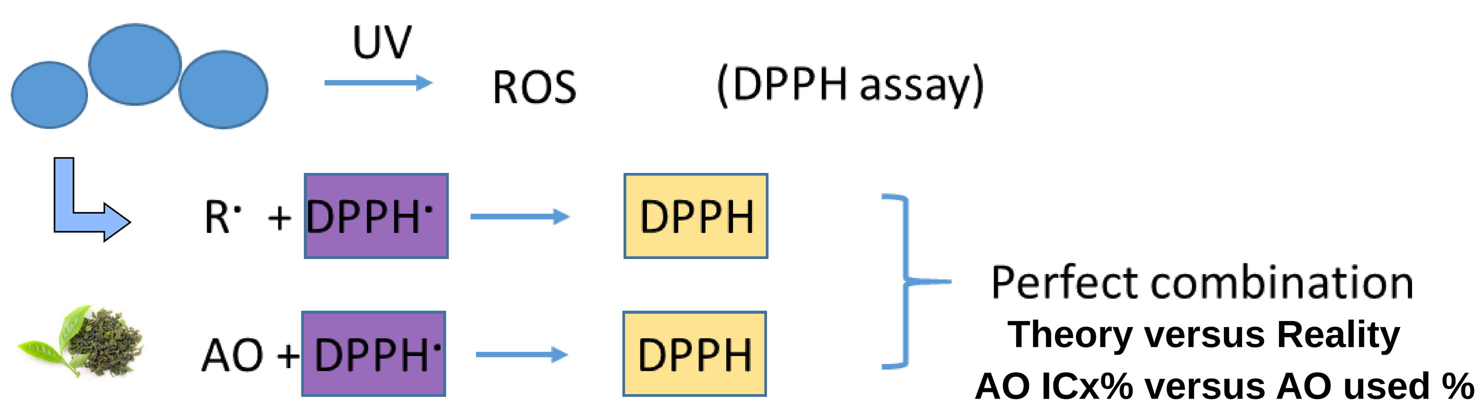
Sodium metabisulfite (SM)

Methods

Determination of the optimize ratio between physical filters and antioxidants

Step 1

- Ratio finding between pigments and AOs in solution for mixing a promising cream formulation



Prerequisite for the best combination:

- Low radical formation of pigments during irradiation
- Low amount of consumption of AO
- Low costs of antioxidants
- High availability and reproducibility of production of AO
- Various chemical properties (hydrophilic/lipophilic/protein for optimal combinations)

Step 2

- One promising AO mixture was selected and combined with different pigments in cream formulations
- Investigation of the cream formulations for their antioxidant capacity (radical protection factor, RPF) by electron paramagnetic resonance (EPR) spectroscopy
- Transmittance and reflectance measurements of selected cream formulations by VIS spectroscopy and calculation of absoption μ_a and scattering coeffcient μ_s' by inverse Monte Carlo simulation

Conclusion

- Reactions in solution are not perfectly transferrable to cream formulations, but preselection in solution is helpful to screen AO candidates
- Radical formation by pigments during UV radiation in solution mainly influence the RPF value in cream
- Combinations of antioxidants are recommendable to cover different requirements.
- High scattering properties are correlated with low antioxidant properties.

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Results

Pre-selection of promising pigments and AOs in solution

- Different pigments have different capacities to generate radicals during UV irradiation. The higher the scavenged DPPH, the more radicals were generated by a pigment under the same experimental condition (table 2, first line).
- Zinc oxide and ZnOpolymyr showed the lowest radical-generating capacity and ZnOtriet25 the most.

Table 2: Scavenged DPPH in % by different pigments during UV irradiation in solution, theoretically calculated amount of selected antioxidants ICx to counteract this radical formation, used amount of selected antioxidants to counteract the radical formation and the factor X between calculated and used amount of selected antioxidants (EGCG= Epigallocatechin Gallate, VE = Alpha-Tocopherol/Vitamin E, SM= Sodium metabisulfite).

Pigment	TiO_2-AlOH	ZnOtriet25	ZnOtriet45	ZnOpolymyr
Scavenged DPPH (%)	45	53	42	23
EGCG ICx%	0.000047	0.000062	0.000043	0.000018
EGCG used %	0.000050	0.000010	0.000015	0.000250
X_{EGCG}	0.90	6.20	2.90	0.10
VE ICx%	0.000792	0.001401	0.000681	0.000224
VE used %	0.00	0.00	0.00	0.001
X_{VE}	1.0	1.4	0.9	0.2
SM ICx%	0.04068	0.05383	0.03669	0.01287
SM used %	0.0075	0.05	0.0025	0.025
X_{SM}	5.4	1.1	14.7	0.5

- The antioxidant capacities of six antioxidants against DPPH were analyzed.
- Comparing their **IC50** against DPPH: **EGCG > Ferulic acid > Rosemarinic acid > Vitamin E > Emblica > Metabisulfite.**

Three AO were selected to be combined into a cream formulation **+ Careosine**:

- **EGCG:** low amount is necessary to counteract radicals formed by all pigments
- **VE:** strong lipophilic AO, easily available; stabilization of cream formulation
- **SM:** low-cost, yet effective AO; can prevent colour change in cream formulations

Mix of selected AOs to cream formulations containing **three different pigments**:

- **ZnOtriet:** very good scattering parameters; frequently used
- **ZnOpolymyr:** produces the lowest radical load
- **Mixture of TiO_2AlOH+ ZnOtriet:** very good scattering parameters, frequently used

Characterization of promising pigments mixed with selected AOs in cream formulations

All samples provide high RPF values:

- No-pigm (containing no pigments but **EGCG, VE, SM and carneosine**) provided the highest RPF

→ Reduction of this high value by addition of different pigments

→ ZnOpolymyr reduced the RPF only by 33% to 240.

Table 3: Determination of the RPF of different cream formulations and the scattering coefficient μ_s' at 400 and 800 nm of the investigated formulation

Cream formulation	RPF in 10^{14} radicals /mg	μ_s' at 400 nm in 1/mm	μ_s' at 800 nm in 1/mm
No-pigm	**360 ± 14**	4.2	3.4
ZnOtriet	160 ± 9	**41.1**	**16.3**
ZnOpolymyr	**240 ± 2**	30.4	15.5
TiO_2+AlOH+ZnOtriet	180 ± 13	**43.0**	15.5

- Scattering properties increase with particles up to a factor of 10 at 400 nm (Table 3).
- The sample TiO_2AlOH +ZnOtriet containing scattering particles with a concentration of 14% provide the highest µs', closely followed by ZNOtriet25 using only 10% of pigments.
- Scattering is negatively correlated with RPF

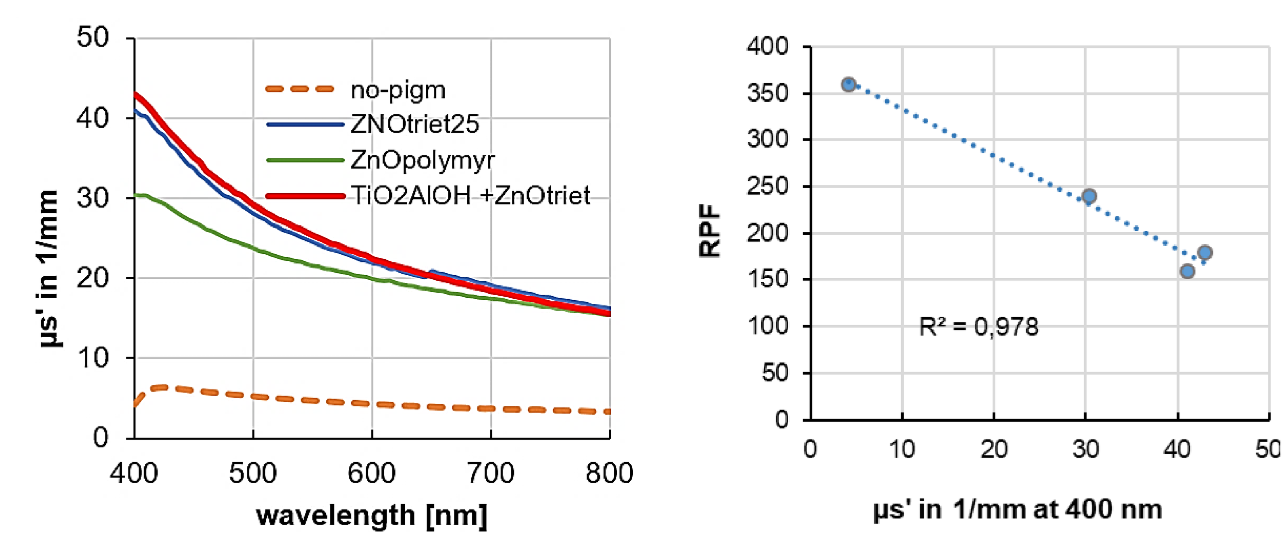

Figure 2: Scattering coefficient μ_s' for all creams and the correlation of the RPR and µs'.