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Nicoleta Badea [a], Maria Giurginca [a] & Aurelia Meghea [a]

[a] University Politehnica of Bucharest, Bucharest, Romania

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Complex Effects of Sunscreen Agents and Flavonoid Antioxidants Devoted to Enhance Photoprotection of Dermal Tissues

Nicoleta Badea, Maria Giurginca, and Aurelia Meghea
University Politehnica of Bucharest, Bucharest, Romania

In the collagen base, the screen compounds (TiO_2 and ZnO) and natural antioxidants (flavonoids) are introduced in 2–8% proportion. Photoprotective effects of creams have been followed in vitro by sun protection factor (SPF) determination, in correlation with the antioxidant activity. The screen substances tested into collagen base show the influence of the particle size on the SPF. During the irradiation process, the couples TiO_2 + flavonoids produce the amplification of the SPF value, due to the photocatalitic effect of the TiO_2 pigment and bis- ethylhexyloxiphenol – metoxiphenyltriazine (BEMT). The cream with TiO_2, BEMT and flavonoids offer photoprotection on both UVA and UVB domains.

Keywords: collagen; flavonoid antioxidants; spectral methods; SPF index; sunscreen agents

INTRODUCTION

During the last decades, a growing tendency was noted to replace synthesis products by selective plant extracts as a result of the secondary effects caused by some medicines, foods and cosmetics generated in time following the action of several destructive factors affecting the biomolecules and the internal protection mechanisms. Among these factors, the main role belongs to free radicals of oxygen and the ultraviolet radiation. The latter produces disfunctions of the protection and natural skin regeneration mechanisms, starting with a

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Address corresponding to Aurelia Meghea, University Politehnica of Bucharest, 1 Polizu Street, Bucharest, 011061, Romania. E-mail: a_meghea@gmail.com; a_meghea@chim.upb.ro

simple erythema, continuing with a keratosis and excess production of the melanine and ending in cancer [1–9].

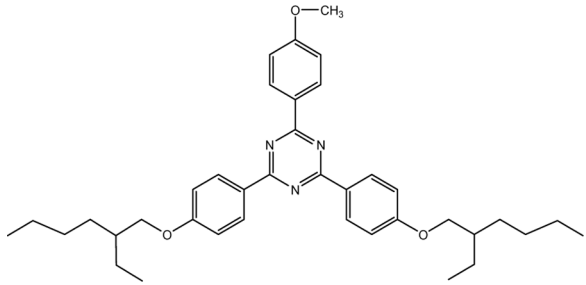
All these effects may be neutralized and/or limited by using some dermato-cosmetic treatments. With this aim, chemists and pharmaceutical chemists have created numerous types of lotions and creams for skin protection and regeneration of the affected cells using inorganic and organic substances coming from natural sources.

The main aim of this study is to obtain some pharmaceutical formulations with photoprotective and antioxidant properties and to point out their behaviour at the UV radiation; by UV and chemiluminescence tests we selected compounds with enhanced anti-UV and antioxidant capacity, and then these have been imbedded into the creams devoted to skin protection against the dehydration and photo-destruction.

EXPERIMENTAL

Materials

- Collagen base with antioxidant activity AA% = 56.4 and SPF index = 1.2;
- Screen substances: TiO_2 (Kemira, M161, M170 and M212 with specific surface area of 70, 80, and 60 m^2/g respectively) and ZnO (Nanox TM 200 and Zn Clear S60CCT with specific surface area 17 m^2/g);
- Filter substance: BEMT with triazinic structure:

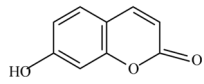


Bis – ethylhexyloxiphenol metoxiphenyl triazine

- Flavones antioxidants with characteristics presented in Table 1.

For the characterization of the resulting compositions and their components, spectral absorbtion techniques (UV-VIS spectroscopy) and chemiluminescence tests have been used.

TABLE 1 Antioxidant Characteristics of Flavones

Flavon/Chemical structure	AA %	k (s^{-1})
Quercetine	92.8	0.080
Rutin	77.4	0.095
Umbelipferone 	51.6	0.077

Equipment

- Jasco UV-V is spectrometer, model V-570 with 200–2500 nm range and a diffuse reflectance system; the Sun Protection Factor (SPF index) was determined by SPF – JASCO software;
- Chemiluminometer TD 20/20 (Turner Design, SUA);
- Jasco FT – IR 620 spectrometer with 4000–400 cm^{-1} range;
- Ultra – lum (USA) iradiation sourse 290–400 nm.

Reagents

- for SPF index, the diffuse reflectance device and a support TRANSPOR™ 3 M with a structure similar with the natural skin (on which the standard quantity of 2 mg/cm^2 cream was applied) were used;
- for chemiluminescence (CL), the generating couple: luminol + H_2O_2, $[LH_2] = 10^{-5}$ M; $[H_2O_2] = 0.2$ M in Tris – HCl, at pH = 8.6 was used; the luminol was dissolved in DMSO (Merck, Germany);
- for IR spectral analysis, KBr of spectral purity (Merck, Germany);
- for the preparation of the cosmetic formulations, a series of flavones such as Quercitin, Rutin and Umbeliferona (Sigma-Aldrich, Germany) were used. The screening substances used were TiO_2 and ZnO (Kemira Pigments Oy, Finland), while *Tinosorb S (BEMT)* (CIBA, Switzerland) was used as a filtering agent.

Sample Preparation

For the spectral analysis in the IR and UV-VIS domains, the samples were prepared according to the technique used:

- Pellet for the IR domains;
- For the chemiluminescence, solutions of 10^{-5} mol/L in double distilled water or organic solvents (DMSO) were prepared;
- In the UV domain, the cream was applied as a film on TRANSPORE™ 3 M, using as a standard a surface from TRANSPORE™ 3 M. According to the literature, this *in vitro* analysis method is well correlated with the *in vivo* analysis method [10].

The basic cream used for testing the selected agents with antioxidant and photoprotective properties, has a relatively simple structure, containing stearic acid, lanoline, parafinic oil, bees wax, cetilic alcohol and a phenolic additive, all included in collagen.

In the basic cream, the screening substances were added in 1–4% amounts by suspension in propilenglicol, its quantity being maximum 10% in order to avoid altering the rheological properties of the cream samples.

Computing Procedures

The spectral and chemiluminescence data were used to investigate some characteristics of the studied biomolecules:

- redox characteristics from the chemiluminescence CL = f(t) curve, showing the variation of the chemiluminescent signal in time, expressed as initial speed or at a certain time of the process with the equation:

$$v = \frac{I_5}{t}[s^{-1}],$$

where: v – reaction rate, time unit (s^{-1});
I_5 – intensity of the chemiluminescent signal after the first 5 s;
t – time corresponding to the intensity (s).

- reaction rate constant and the reaction order were established by verifying the kinetic equation or by the initial rate method [11];

- anti/prooxidant activity was evaluated with the equation:

$$AA\% = \frac{I_0 - I}{I_0} \cdot 100$$

where: I_0 – signal intensity of the standard at t = 5 seconds

I – signal intensity of the sample at t = 5 seconds

- SPF index was computed with the JASCO software using the method of Diffey & Robson [12], by the following equation:

$$SPF = \frac{\Sigma_{(400-290)} \cdot E_\lambda \cdot B_\lambda}{\Sigma_{(400-290)} \frac{E_\lambda \cdot B_\lambda}{MPF_\lambda}}$$

where: E_λ – normal radiation spectrum of the sun (more exactly from 20° to 40°N latitude);

B_λ – absorption relative efficiency for each wavelength;

MPF_λ (**M**onocromatic **P**rotection **F**actor) – average spectrum of the sample signal (support + cream), minus the reference (support).

RESULTS AND DISCUSSION

The photoprotective substances used can act in two ways: by the absorption of the UV radiation and by screening/dispersing it. Usually, the combination of these two types is used in order to prevent the UV radiations to alter the skin cell functions [13,14]. The basic structure exhibits a low SPF factor.

Testing the Creams with Screening Substances

Three creams were prepared with the TiO_2 (M212, M161, M170 Kemira) and their initial SPF factor and also after 120 minute UV exposure have been investigated. Its variation showed that the size of the TiO_2 particles and the specific surface area influence the UV screening capacity of the cream (Fig. 1): at small particles size and large specific surface the SPF factor has greater values. Among the three samples, we selected for further tests the Kemira M170 with the finest particle size and the highest surface area, this being quite easily included, giving a homogeneous cream, with a superior protection factor.

With the basic cream two variants of formulations containing ZnO (Nanox 20 and ZnClear S60CCT) were prepared. After 120 minutes

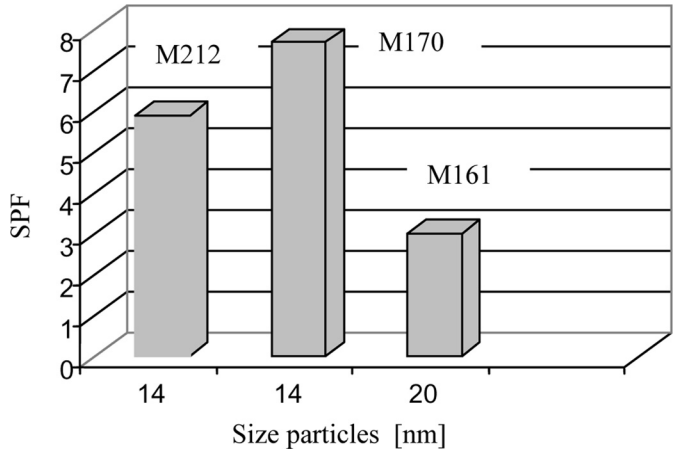


FIGURE 1 Variation of SPF index with particle size of TiO_2.

UV exposure, a better stability of the variant containing Nanox 200 was noticed, although its photoprotective capacity is reduced in comparison to the TiO_2 variant (SPF = 4.1 – 4.6).

The inclusion of both substances in the basic cream revealed that the two oxides cover different zones in the UV domain and, even though there is no improvement of the photoprotection, the protection action covers an extended spectral domain (Fig. 2).

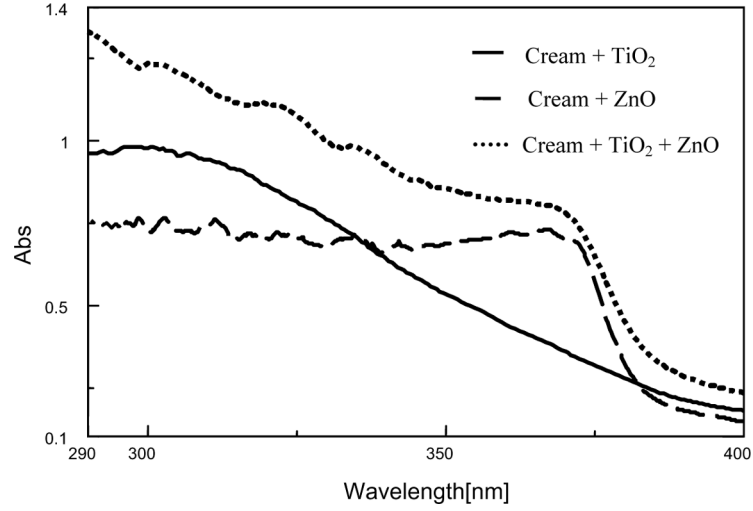


FIGURE 2 UV spectra for three types of cream.

Testing the Screening Substances (BEMT) in the Collagen Cream

The substance with a triazinic structure included in the collagen cream, presents an increase in the SPF value after 20 min, reaching a maximum at 40–100 min (Fig. 3).

Testing the Couple of Filter + Screening Substances

If in the basic cream a screening substance (TiO_2) and a filter substance (BEMT) are included, there is a significant increase of the SPF factor even during the irradiation process (Fig. 4).

Testing the Couples Photoprotector-Antioxidant

Several flavones (quercitin, rutin and umbeliferona) were included in the basic creams, after embedding TiO_2, ZnO and BEMT at 2–4% as suspension. The SPF factor, the UVA retention factor and the UVA/UVB ratio (showing the radiation retained in the irradiation process for 120 min) were evaluated. The variation of these parameters is presented in Table 2.

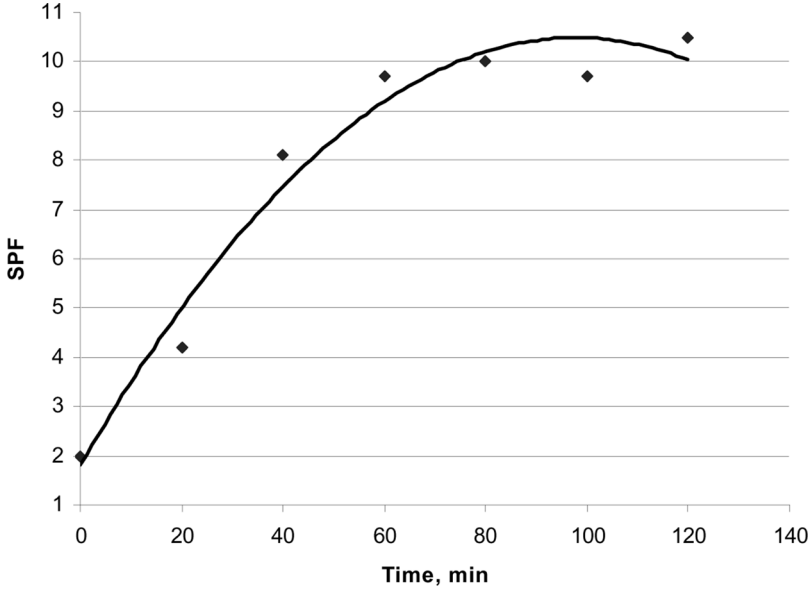


FIGURE 3 Variation of SPF index for BEMT in time.

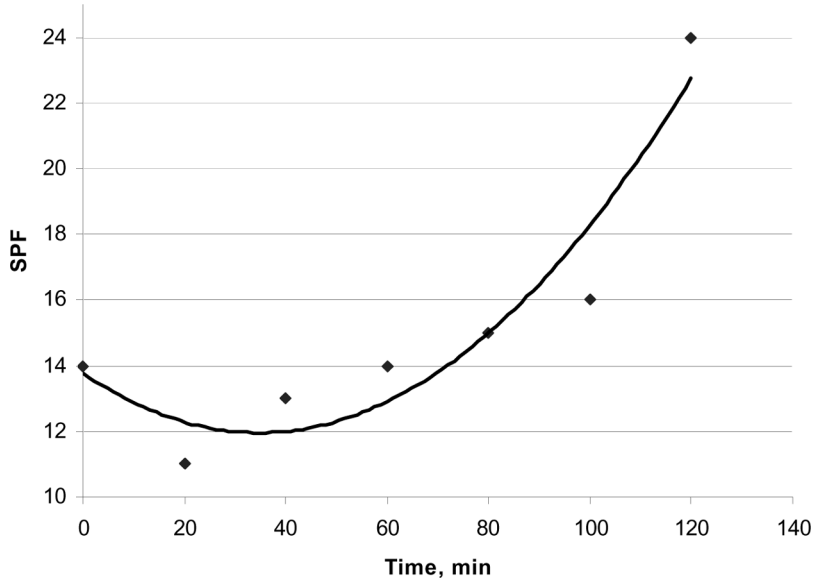


FIGURE 4 Variation of SPF index for BEMT + TiO_2 in time.

The data obtained emphasize the following aspects:

- during the irradiation process, the flavones diminish the SPF factor, especially the quercitin and the umbeliferona, while the rutin enhances it but only after 40 min. The association of two flavones (rutin and umbeliferona) leads to a significant increase of the SPF

TABLE 2 SPF Variation for Cream with TiO_2-M170 and Flavones

	SPF			
Irradiation time [min]	TiO_2 + Quercitine (4%)	TiO_2 + Rutine (2%)	TiO_2 + Umbelipherone (4%)	TiO_2 + Rutine (2%) + Umbelipherone (4%)
0	7.5	6.6	7.2	18.7
20	6.0	6.6	5.5	18.7
40	6.0	8.0	5.2	20.0
60	6.0	7.0	5.0	23.5
80	6.3	6.8	5.6	25.5
100	6.1	7.3	4.9	25.9
120	6.1	7.3	4.9	19.0

factor, the effect being maximum up to 100 minutes of exposure and having a synergic character ($\theta = 2.1$). We had also noticed an increase of the UVA values, while the UVA/UVB ratio had a value of 1.04 ± 0.03.

- the same flavones were included in the ZnO-Nanox 200 cream. After UV irradiation, in all cases the values of the SPF were comparable with those of the cream containing only ZnO.

The inclusion of the flavones in the cream with $TiO_2 + ZnO$ has maintained the characteristics of the systems containing separately TiO_2 and ZnO, leading to the fact that ZnO is an inhibitor of the photocatalitic effect of the TiO_2 and of the flavones. In the absorbtion spectra of the inorganic-flavones couples only the covering of the entire UV domain was observed as in the case of the $TiO_2 + ZnO$ couple.

There was no satisfying result on the anti-UV protection effect when the flavones were coupled with the filter substance BEMT. Nevertheless, when in the basic collagen cream $TiO_2 + BEMT$ (filter + screen substance) and the flavones (rutin + umbelipherona) are included, there was a strong increase in the SPF and UVA factors (Table 3).

With this system, the cream may be used for 120 minutes, the maximum protection from 60 and 100 minutes belonging to the flavones and BEMT, which by the extended conjugated systems enhance the photocatalitic action of TiO_2. We note the protection given by the small concentrations of flavones (2–4%) in comparison with literature data [13] the photoprotection being usualy achieved at concentrations of 25% maximum.

TABLE 3 Comparative Values of the SPF for the Creams with TiO_2 and BEMT

	$TiO_2 + BEMT$		$TiO_2 + BEMT + Flavone$	
Irradiation time	SPF	UVA	SPF	UVA
0	13.6	6.7	18	10.5
20	11.5	6.8	16.3	12.5
40	13.6	7.8	17.9	15.5
60	14.6	9.7	21.3	16.1
80	15.1	10.9	19.6	13.7
100	17.1	11.4	30.4	19.2
120	24.4	16.3	21.7	17.1

CONCLUSION

This article is devoted to obtain cosmeceuticals with photoprotective and antioxidant properties. Two screening (TiO_2 and ZnO) and one filter (BEMT) substances and three antioxidant flavones were used. According to the experiments, the following can be concluded:

- the particle size of the inorganic pigment influences the anti-UV protection capacity. The smaller the size of the particle and the higher the specific surface, the better the photoprotective capacity;
- including the flavones in the cream with TiO_2 leads to an increase in SPF factor, while there are no significant changes for ZnO;
- when the two screening substances are combined, we note an increase of the anti-UV protection domain (290–380 nm); when the flavone complex was added to the respective cream, the inhibitor role of the ZnO on the flavones was evidenced;
- our experiments demonstrated that the combination of TiO_2 with a filter substance (BEMT) in the presence of the flavones, leads to an optimal protective action at smaller additive concentrations (2–4%).

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