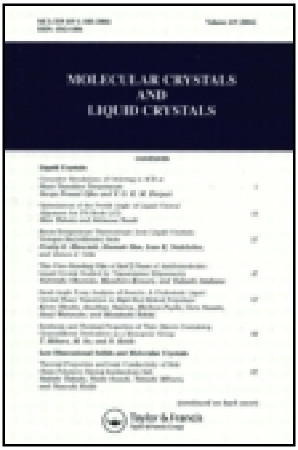

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New Nanostructured Complex Systems with Antioxidant and Photoprotective Activity

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It has been demonstrated that the alarming increase in UV radiation on the ground and intensive or repeated sun expositions cause systemic effects that can be shown as alterations of the general defense mechanisms against free radicals, increase of red blood cells fragility or a systemic immunosuppression.

This article is the first step in developing new cosmetics based on nanostructured complexes containing active principles extracted from animal (collagen) and vegetal (polyphenols) tissues, with sun protective and antioxidant activity. The antioxidant activity has been monitored by chemiluminescence, while the photoprotective capacity has been quantified by using diffuse reflectance spectroscopy (Sun Protection Factor).

Keywords: antioxidant activity; chemiluminescence; cosmetics; photoprotective capacity; plant extracts

INTRODUCTION

It was already accepted that Sun rays can produce harmful effects on the skin, even at short term, such as erythema, Langerhans cells

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depletion or local immunosuppression, or at wide term, by photoaging or, even, skin cancer. Furthermore, intensive or repeated sun expositions cause systemic effects that can be shown as alterations of the general defense mechanisms against free radicals, increase of red blood cells fragility or a systemic immunosuppression [1]. All these effects are defined by generic concept of "oxidative stress" [2].

UVB rays (290–320 nm) promote the formation of melanin in the melanocytes of the deepest epidermal layers and therefore also the darkening of the skin through delayed pigmentation. They are largely involved in the development of inflammatory reactions, such as sunburn and have immunosuppressant effect [3,4]. UVA rays (320–400 nm) penetrate the horny layer and reach the epidermis and dermis leading to immediate pigmentation. They have comparatively low effect on the triggering sunburn, but may cause pathological light reactions by means of irreversible oxidation of melanin precursors, initiating thus photosensitivity processes that generate radicalic species responsible for oxidative stress. As the light-related aging of the skin and the formation of tumors can be attributed both to UVB and UVA rays, a new generation of broad spectrum sunscreens should provide high protective capacity in both these domains [3,5].

To increase the resistance against photodestructive processes in special compositions for human use either some organic products (synthetic or vegetal) with anti-UV activity are introduced, or couples consisting in mixtures of antioxidants and photo-stabilizers with synergistic effects on the inhibition of some oxidative/auto-oxidative reactions. Such type of reactions are setting up at the double bounds of biomolecules, mainly unsaturated fatty acids, depending on the temperature, the dose of UV radiations accumulated, on the enzyme system present, and on some transitional ions existing in the biological environment.

Protection against photo-degradation can be achieved in various ways, as following:

- screening UV absorption and its reduction by the substrate, by using some UV absorbers like TiO_2, ZnO, etc;
- diminishing the initiation reaction rate by using quenchers for excited singlet/triplet states of complex groups;
- decay of hydro-peroxides into non-radical products;
- scavenger process of free radicals during their formation stage.

In order to meet these needs, special organic compounds are used having phenolic or polyphenolic structure, containing intramolecular hydrogen bonds, able for structural rearrangements, such as: hydroxy-benzophenones, salicilate, benzimidazoles, cinnamates, camphor, derivatives of para-aminobenzoic acid.

Therefore, the materials used as photostabilizers have to assure protection on the entire 290–400 nm range, specific to both UVA and UVB radiations present in terrestrial atmosphere.

This article is the first step in developing new cosmetics based on nanostructured complexes containing active principle extracted from animal (collagen) and vegetal (polyphenols) tissues with sun protective and antioxidant activity [6]. An increasingly favourable impact on human health is expected to be obtained by formulation of new nanostructures containing proteins, natural lipid compounds and selected active principles that could assure maxim antioxidant, photoprotective and immunostimulative effects, acting at the same time as controlled delivery systems.

EXPERIMENTAL

For preparation and characterization of new nanostructured complex systems the following materials have been used:

- basic structures of cosmetic nanoemulsions, containing various types of raw materials (Table 1);
- plant extracts in various organic solvents, containing active principles for antioxidative and photoprotective activity (Table 2);
- oil extracts from plants and fruits (Table 3);
- powders reach in flavones and polyphenols, obtained from various wood materials (Table 4);
- vegetal (from wheat and algae) and animal (collagen) proteins (Table 5);
- synthetic organic chemicals with anti-UV activity (Table 6).

The nanostructured complexes were obtained as nanoemulsion systems using the well-known hot homogenization technique [7,8], by

TABLE 1 Characteristics of the Basic Matrix of Nanoemulsions

Type	Composition	AA%
A	Stearats, glycerine, fatty alcohols, emulsifier, emollients, BHA (butylhydroxyanisole) antioxidant	51.8
B	Stearine, lanoline, parafinic oil, cetylic alcohol, phenolic antioxidant, bee wax, animal collagen	56.4

TABLE 2 Active Principles and Antioxidant Activity of Plant Extracts

Plants	Extractant	Active principles	AA%
Aloe vera	Propylenglycol	anthraquinone; glycoside	66.5
Hardhay	Sunflower oil	polyphenols; carotenoides; sesquiterpenes	48.5
Marigold	Olive oil	triterpenes; carotenoides; flavonoides	64.5
Leaf nut	Sunflower oil	organic acids (galic, elagic); polyphenols	43.0
Chestnut	Propylenglycol	triterpenes; flavones; polyphenols	72.9
Shepherd's thyme	Glyceroalcoholic	polyphenols; rosemary acid; flavones	94.7
Mint	Glyceroalcoholic	mentol; polyphenols flavonozides	93.9
Wormwood	Glyceroalcoholic	$\alpha + \beta$-pinene and sesquiterpenes	86.7

embedding the bioactive principles in aqueous or oil phase, depending on their solubility.

Their main structural characteristics have been evidenced by infrared spectroscopy (FT-IR 620, Jasco).

The antioxidant activity (AA%) has been monitored by chemiluminescence technique (Turner-Design TD 20/20, USA). As generator of chemiluminescence was used the system luminol and hydrogen peroxide in buffer Tris:HCl 0.2 M at pH = 8.6 [9a, b].

Photoprotective capacity is quantified by means of Sun Protection Factor (SPF index), currently determined by cosmetics producers by using *in vivo* Colipa test [10,11].

TABLE 3 Characteristics of the Vegetable Oils

Type	I_2 index (mg I/100 g)	Composition of fatty acids (%)	AA%
Avocado oil	80–95	palmitic, 5–20; oleic, 60–80; linoleic, 8–15	24.8
Apricot kernel oil	90–112	oleic, 54–80; linoleic, 12–39; palmitic, 4–9	39.8
Olive oil	83–97	oleic, 63–83; linoleic, 13–5; palmitic, 7–17; stearic, 2–4	63.1
Sweet almond oil	93–105	oleic, 62–86; linoleic, 10–30; palmitic, 4–9; stearic, 3	62.0
Sunflower oil	110	oleic, 30; linoleic, 60; palmitic, 4; stearic, 3; linolenic, 1	49.0
Jojoba oil	80–90	palmitic, 3; oleic, 5–15; gadolinic, 65–80; erucic, 10–20; nervonic, 3	39.9
Shea butter	53–73	oleic, 41–49; linoleic, 3–7; stearic, 38–48; palmitic, 3–6; arahidonic, 2.5	65.1
Seabuckthorn	67, 94	palmitic, 34.2; palmitoleic, 25.7; oleic, 31.5; linoleic, 5.5; linolenic, 2.0	72.4

TABLE 4 Characteristics of Some Powders with Flavonoide and Polyphenolic Structures

Powder	Active principles	AA%	Polyphenols content (%)
Hiprose	vitamin C, fatty acids, lecitine	67.1	–
Crab apple	flavones, volatile oils, vitamin C	87.6	–
Spirulina	vitamins, proteins, carotenoides, mineral salts	23.0	–
Chestnut	polyphenols (pyrogallol)	38.5	8–12
Oaktree	polyphenols (pyrogallol + pyrocatechine)	24.7	10
Mimosa	polyphenols (pyrogallol) + pyrocatechine	21.9	22–48
Quebracho	pyrocatechine	42.7	17–25
Valonea	pyrogallol	27.7	29

TABLE 5 Characteristics of Vegetal and Animal Proteins

Type	Composition	AA%
Vegetable collagen – Dermogene	Essential aminoacid – 45% phenolic compound for protection	50.5
Wheat placenta	Polypeptide, fatty acids, carbohydrates, phenols, B_1 and B_2 vitamins, mineral salts	35.5
Calf collagen	Proteins, 97–98%	51.9

Alternatively, an *in vitro* laboratory test of SPF index could be successfully obtained by using Diffey and Robson method [12] in which the sunscreen sample is prepared by applying a uniform layer of $2\,mg/cm^2$ on a UV transparent substrate, in this case, by using a transpore surgical tape. The sample is then measured in an instrument provided with a diffuse reflectance system, capable of detecting scattering from the sunscreen in the spectral region corresponding to the UVA and UVB regions (Jasco UV-Vis spectrometer, model V-570).

RESULTS AND DISCUSSION

In order to quantify the antioxidant and photoprotective characteristics of these complex compositions, in the first step the behavior to oxidative stress of individual components was established, and afterwards these components have been introduced into the basic structure of cosmetics and tested for their SPF index.

The basic structures of cosmetics initially analyzed by chemiluminescence (Table 1) revealed a medium and quite similar antioxidant activity, between 51.8% and 56.4%.

TABLE 6 SPF Characteristics of Nanoemulsions

No.	Sample	SPF (%)
1	Emulsion A	1.2
2	Emulsion B	1.2
3	Emulsion B + oaktree extract	8.9
4	Emulsion B + oxycinnamate	15.2
5	Emulsion B + oxycinnamate + benzophenone-3*	22.3
6	Emulsion B + oxycinnamate + benzophenone-4**	23.9
7	Emulsion A + oxycinnamate + benzophenone-3 + benzophenone-4	25.0
8	Emulsion B + oxycinnamate + benzophenone-3 + benzophenone-4	33.6
9	Emulsion B + oxycinnamate + benzophenone-3 + benzophenone-4 + oaktree extract	46.5

*3-metoxy-5-hydroxy-benzophenone.
**acid-2 hydroxy-4-metoxybenzophenon-sulphonic.

Among the plant extracts with recognized therapeutic effects due to their active principles, some representative species have been selected and presented in the Table 2, with the antioxidant activities ranging from 43% to 95%. The maximum activity is registered for mint and shepherd's thyme, while the main differences could be interpreted as being generated by the nature of the solvent host, by its interaction with the plant matrix, as well as by the variable amounts of polyphenols, flavones and carotenes present into the extracts.

Some oil fractions of plant extracts have been also selected for obtaining new nanostructured complexes containing various mono and polyunsaturated fatty acids (Table 3). Their antioxidant activity could be qualitatively correlated with the unsaturation degree of fatty acids (determined by iodine index), but it could be also influenced by the existence of some other extractible components, particularly vitamins and aminoacids. Indeed, the best antioxidant activity is noticed for seabuckthorn oil and shea butter, with high content of saturated acids, while the lowest values are for avocado and apricot kernel oils, with the highest content of unsaturated acids. This fact could be explained by a higher reactivity of hydrogen in alylic position (which is easily extractible) permiting a more rapid formation of oxygenated structures in comparison with saturated ones.

Although these oil extracts have complex composition, their investigation by IR spectroscopy is relevant for the main functional groups of their constituents (Fig. 1), fatty acids, their esters, and aminoacids.

Another group of experiments of spectral characterization and chemiluminescence have been also performed on powders obtained

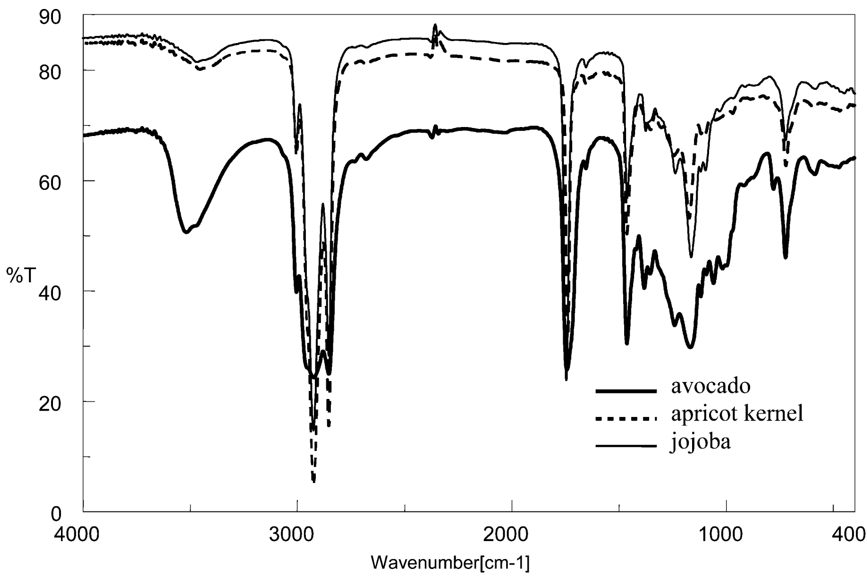

FIGURE 1 IR spectra of some oils.

from various wild fruits and vegetal materials, with high content of polyphenols and polysaccharides (Table 4).

Even though the last natural products are reach in polyphenolic constituents, the significantly higher antioxidant activity of crab apple and hiprose could be assessed to the synergistic effect of vitamin C combined with flavones and citric/uric acids.

A special group of interest is represented by some vegetal and animal proteins used either as basic structures of cosmetics or as nanostructurated additives. They have not so important antioxidant properties (Table 5), but for both collagen structures this activity is superior to that of wheat placenta, with some content in fatty acids.

For practical purpose the most important is the characterization of nanostructured complex systems containing these active principles into the basic matrix of cosmetics. In this respects, some variants of emulsions have been prepared and tested, containing 0.1–0.4% plant extracts (Fig. 2) and other containing 0.5–2% oils or polyphenolic powders (Fig. 3).

By analyzing these data, the following remarks could be obtained:

- the increase in antioxidant activity with the concentration of most additives (apricot, jojoba, almond), but not always proportional, or in similar extent;

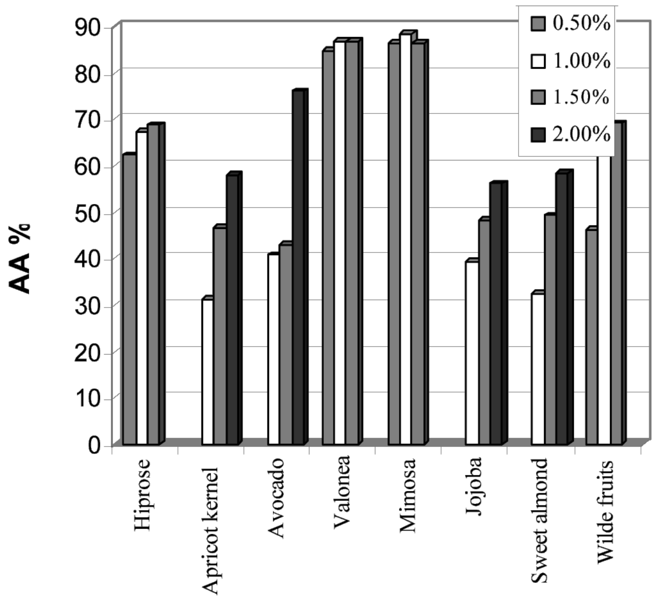

FIGURE 2 Antioxidant activity of some emulsions with oils and wild fruits.

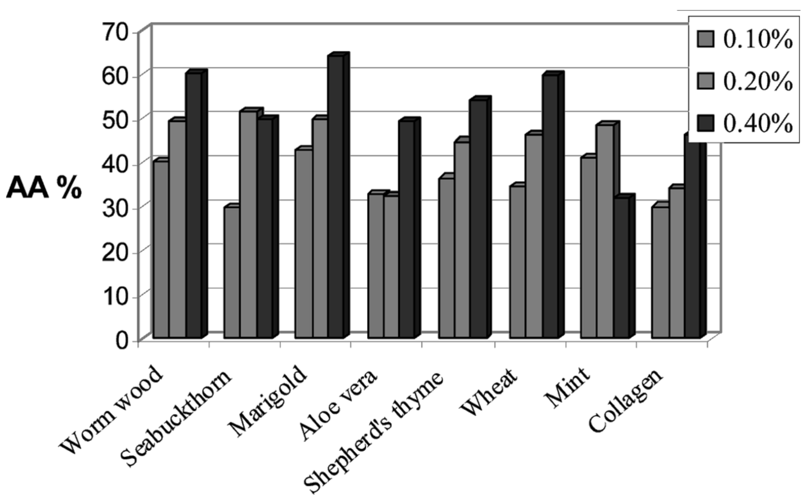

FIGURE 3 Antioxidant activity of some emulsions with active principles.

- there are some cases when the antioxidant activity is similar on the given concentration range (hiprose, valonea, mimose), or for pairs of concentrations (avocado, seabuckthorn, aloe vera, vegetable collagen);
- in case of mint extract, the antioxidant activity is going down with its concentration in the basic matrix of nanoemulsions.

Based on these results and taking into account for the therapeutic effects of other active principles existent in these plants, each of these nanostructured compositions could be recommended for specific cosmetic products.

As referring to photoprotective activity, characterized by SPF index, the main results are collected in the Table 6.

Firstly, it could be noticed that the basic structures of cosmetic products (with or without collagen), even though have a high hydration capacity, they have no significant photoprotective activity (SPF = 1.2).

By introducing some natural extracts, one of the most efficient for photoprotection being the oak extract, the SPF index is significantly improved, to 8,9. Nevertheless, due to the negative effect on the color of the final product, which is very important in cosmetics (the oak extract is brown), the concentration of these natural additives could not be raised at any limit. In order to counteract this disadvantage at this stage of research, and for obtaining higher values of SPF index, it is recommended to use both natural and synthetic additives. Among the variants tested, the maximum SPF index is obtained by using oak extract with oxicinnamate, benzophenone-3 (3-metoxy-5-hydroxy-benzophenone) and benzophenone-4 (acid-2 hydroxy-4-metoxybenzophenon-sulphonic). Such compositions, with SPF >35, are recommended for target population groups, children and blonde women, which have very sensitive skin to UV radiation.

CONCLUSION

A number of active principles from natural plant extracts have been characterized and tested for their antioxidant and photoprotective activity. They contain in various compositions vitamins, flavones, fatty acids, phenolic compounds, and proteins. Their comparative antioxidant capacity was discussed in relation to chemical structure and composition of the main types of active biomolecules contained.

These complex plant extracts have been included into the basic matrix of nanoemulsions in order to obtain specific nanostructured materials with special applications. Among them, the nanostructured complexes including polyphenolic species are the most efficient natural antioxidant, acting at the same time as controlled delivery systems.

Based on the antioxidant capacity, some of plant extracts have been selected for obtaining new compositions with improved photoprotective activity. At this stage, a combined composition containing both natural extracts and synthetic compounds has proved the maximum SPF index.

New tests are under investigation to correlate these antioxidant and photo-protective properties with immunostimulative activity of the complex nanostructured systems.

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