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# A New Biologically Compatible Physical Sunscreen with Skin Firming Properties

## Abstract

**A** new skin-friendly micro-dispersed mineral active, belonging to the Hydroxyapatites group, has been discovered. It is able to enhance UV-B ray protection significantly. Its performances are comparable or even superior to those of Titanium dioxide, without any blue-white appearance and at a lower cost of the formula. Moreover, it does not react with dibenzoylmethane UVA sunscreens. It enhances the COLIPA standard P3 UV absorption by 10% and could replace Titanium dioxide perfectly in model W/O emulsions at SPF 30 and 50+. Due to its physiological components, it has also been tested in a series of evaluations related to anti-aging efficacy on a panel of 40-70 year-old women. Significant improvements have been obtained in terms of biologic elasticity and maximum wrinkledness parameters in comparison to the placebo. Moreover, the raw material provides a significant soft focus effect to the skin.

## ■ Background

It frequently happens that skin protective actives are not found to be as skin friendly as one could expect. It is well known that any foreign substance applied to the skin tends to diffuse in-depth and/or to interfere with the skin metabolism. Sometimes, this leads to problems of tolerability, mainly when the active ingredients are meant to work just while staying onto the skin surface. For example, in order to prevent the skin damage induced by UV rays, a large number of UVA and UVB filters are used. These perform broad range photo-protection.

Sunscreens block the penetration of UV rays through the epidermis by absorbing and/or reflecting them. UV rays effects on the skin include both acute responses, such as sunburn, pigmentation, hyperplasia, immuno-suppression and vitamin D synthesis, and chronic effects, primarily photo-carcinogenesis and photo-aging (1-4). Indeed, photo-protection strategies try to limit all the effects induced by sun exposure. Despite their efficiency, the use of organic filters could potentially produce harmful substances if they are illuminated while in contact with living cells (5-7). Some epidemiological studies indicate increased risks of malignant melanoma for the heavy sunscreen user (8-10), but so far, no medical association has published recommendations to avoid using sunblock. Different meta-analysis publications have concluded that the evidence is still not sufficient to claim a positive correlation between sunscreen use and malignant melanoma (11-12). Nevertheless, this

mainstream has induced the making of »skin friendly« sunscreen products, containing only inorganic filters. Indeed, the main inorganic ingredient of sunscreens, titanium dioxide (TiO<sub>2</sub>), has been used for years in consumer's products, and is generally considered to be inert and safe. However, as a semiconductor with a band gap in the region of 3.0 eV, corresponding to UV wavelengths below ~ 380 nm, TiO<sub>2</sub> is susceptible to excitation by UV rays. Photo-excited TiO<sub>2</sub> can generate a number of ROS in aqueous solutions (13-15). Some studies suggest that the frequent use of sunscreens containing microfine TiO<sub>2</sub> (30-220 nm) could result in the percutaneous absorption of titanium (16,17), penetration into the human stratum corneum and hair follicles (18) as well as inside cultured human skin fibroblasts (19).

HeLa cells, which are 'immortal' tumour cells often employed in scientific laboratories, were killed in the presence of TiO<sub>2</sub> following 10' UV rays irradiation (17). Irradiated TiO<sub>2</sub> causes oxidative damage of nucleic acids in cells (19) and strand breaks in isolated DNA (13, 15, 20).

Even if careful and stable coating with mineral or organic protective layers is a current method of avoiding most adverse reactions, many efforts are currently made to find out safer alternatives to Titanium dioxide.

## ■ Introduction

Hydroxyapatites are naturally occurring mineral forms of calcium apatite, with the general formula Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH). Hydroxyapatite is the hydroxyl endmember

## PHYSICAL SUNSCREEN

of the complex apatite group, where the OH<sup>-</sup> ion can be replaced by fluoride, chloride or carbonate ions. It crystallizes in the hexagonal crystal system (Fig. 1), has a specific gravity of 3.08 and occupies the 5<sup>th</sup> position in the Mohs hardness scale. Pure hydroxyapatite powder is white.

However, naturally occurring apatites can also be brown, yellow or green.

70% of bones are made of the inorganic mineral hydroxylapatite (21). Carbonated-calcium deficient hydroxylapatite is the main mineral of which dental enamel and dentin are composed.

Hydroxylapatite can be found in teeth and bones of the human body. Therefore, many studies report that it is commonly used as a filler to replace an amputated bone or as a coating to promote bone ingrowth into prosthetic implants. Many

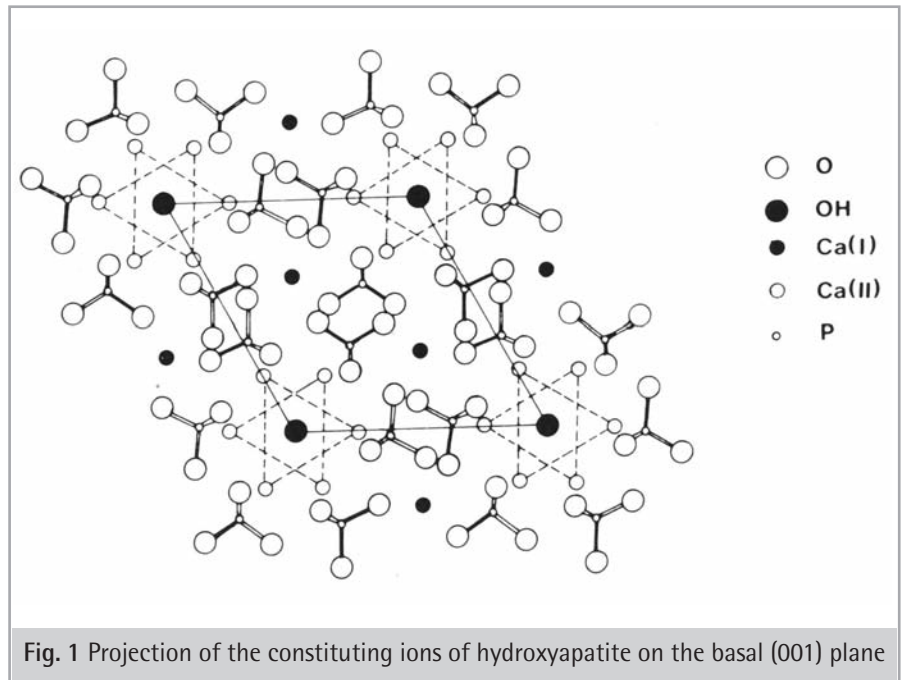


Fig. 1 Projection of the constituting ions of hydroxyapatite on the basal (001) plane

modern implants are coated with hydroxyapatite, and it has been suggested that this material may promote ossification (22-24). Recently, hydroxyapatite has been frequently used as a semi-permanent filler in non-surgical options in skin rejuvenation for treating wrinkles and textural changes. On the other hand, as the main component of dental enamel, it is reported to protect from acid erosion (25-27) and to exhibit enamel restoring effects, as well as an anti-plaque and antistain activity. As a slow-release source of phosphate and Calcium ions to the skin cells, it has been suggested as an anti-aging ingredient for aged skin. Considering the above literature, the anti-aging activity of one grade of this raw material and its property for a biocompatible delivery system has been tested. Surprisingly, it was discovered to also have sunscreen properties.

### ■ Experimental

The starting material is a micro-fine white stable suspension of hydroxyapatite particles (< 100 nm) activated with trace elements: (Zn, Mn, Mg) (Ca)<sub>5</sub> (PO<sub>4</sub>)<sub>3</sub>(OH) (Lactate) with a pH of about 7. The active substance in suspension is about 30%. The raw material suspension is preserved with phenoxyethanol, methylparaben and ethylparaben; the suspending agent is a blend of Xanthan Gum and of a synthetic ammonium acryloyldimethyl taurate/ VP copolymer. Commercial name is Apalight®.

#### In-vivo studies

##### *Anti-wrinkle efficacy*

The aim of the study was to evaluate the influence of that hydroxyapatite variant on the antiwrinkle efficacy of a functional anti-aging cream. Parameters like skin hydration, skin sebum, elasticity and skin roughness after long-term use were measured and compared with the values of the placebo without the apatite. Ten female volunteers (age range: 40-70 years old) were selected for the study and applied the products twice a day, each one on one side of the face, at home, for 4 weeks.

At the beginning of the study, instrumental measurements of skin hydration (Corneometer CM 825, Courage and Khazaka, Germany), skin sebum (Sebumeter SM 810, Courage and Khazaka, Germany), elasticity (Cutometer SEM 575, Courage and Khazaka, Germany) and roughness (Skin Surface Replicas and Image Analysis) were taken, on the left and on the right side of the face and were marked out in a reproducible way. The side of application on the face (left or right) of the two creams was randomised. At the end of the treatment, the subjects returned to the laboratory where the instrumental measurements were repeated. All the measurements were carried out in a bioclimatic room (24 °C; 50% rh). In the final calculation, the following key parameters were considered:  $U_f$  = maximal deformation of the skin, referred to as  $R_0$  parameter;  $U_a / U_f$  = overall elasticity referred to as parameter  $R_2$ ;  $U_v / U_e$  = viscoelastic ratio referred to as  $R_6$  parameter. As for skin sebum evaluation, the instrument reads an opaque tape before and after the contact of the probe with the skin: the increased transparency is related to the amount of lipids on the skin, expressed in  $\mu\text{g}/\text{cm}^2$ . In order to obtain negative imprints of skin surface (replicas), a fast hardening synthetic polymer (SILFLO – Flexico Ltd, United Kingdom) and adhesive discs were used. The skin replicas were then analysed by a designed image processing software (Quantilines, Monaderm, Monaco) which allows a global data analysis of relief parameters, according to the method described by Corcuff. The silicone replica of the cutaneous surface was illuminated by a light source at an incident angle of 35 °C. An image covering a 12 x 9 mm of each skin replica surface was acquired through a video-camera (High Performance CCD camera, COHU, Monaco). This software enables us to measure the following parameters: Ra: mean roughness value, Rz: maximum roughness value (deep wrinkles). The change of anti-wrinkle efficacy is proved by the decrease in Ra and/or Rz values at the end of the treatment.

Moreover, digital photos (Fotofinder Dermoscope Ver. 2.0, Teachsreen, Bad Birnbach, Germany) were taken in order to evaluate the soft-focus effect.

#### *Skin Cell Renewal*

Ten female volunteers (age range: 18-55 years old) were selected for the study. Before starting the treatment, a suspension of dansyl chloride (in petrolatum at 5%) was applied to the test sites of the volar forearm under a 24-hour occlusive patch. After the patch removal, stains were verified with a Quartz mineral lamp and a score was assigned. The volunteers had to use the products (reference and placebo) twice a day till the disappearance of stains, while an untreated area acted as a control. The intensity of the stains was also assessed 5 days after product application. The number of days required for the total disappearance of the fluorescent stains was also considered.

The amount of skin cells before and after the treatment was also assessed; skin corneocytes were collected by pressing a tape-coated slide (D-squame) to the skin surface. The number of corneocytes adhering to the tape was then measured with a colorimeter (Chroma Meter).

#### *Delivery system in deodorant efficacy*

Aim of the study was to verify the capacity of apatite to increase the deodorant power of chlorhexidine in a suspension where such active had been encapsulated (at 4%) in the apatite. A group of 12 subjects (average age: 40) were selected. The volunteers were asked to use an odourless cleansing product for 7 days in order to condition their armpit before the test.

After this period, a sniff test was carried out by a trained panel of 3 who scored the smell on a scale ranging from 0 to 10. Then, for a period of 2 weeks the volunteers applied two products (suspension and placebo) on their armpit. After the treatment a sniff test was repeated.

Delivery system in sunscreen power, International SPF test method, UVAPF Colipa 2007 In another study, our aim was to verify if a standard emulsion (Colipa Standard P3) showed different sunscreen power if one of the filters, ensulizole, was introduced, as is, in the formula with 6% of HA (hydroxyapatite) or if it was introduced encapsulated in HA. The two formulas were evaluated using the International SPF test Method Coli-

pa, CTFA-SA, JCIA and CTFA, 2006 in comparison with the unchanged standard Colipa formula P3. Subsequently, considering the obtained results, 3 additional formulas (Colipa Standard P3 and two W/O emulsions) were tested: a Sunscreen formula at SPF 30 and a Sunscreen formula at SPF 50+). Each of the 3 formulas was realized with the same percentage of HA or, respectively, Titanium Dioxide (respectively 6% in the Colipa Standard P3, 4% in the SPF 30 and 5% in the SPF 50+). The first couple of formulas (P3) was tested according to the International SPF method; the remaining two couples of formulas were tested according to the International SPF method and the UVAPF Colipa 2007 method.

■ Results and Discussion

In-vivo Efficacy

The results showed a nonsignificant difference between the test product and the placebo in the case of skin cell renewal, moisturization and skin sebum. Remarkable results were found, instead, in the case of skin roughness, where Rz shows a -9.5% variation ( $p < 0.05$ ) (Fig. 2). Also the skin elasticity results (Fig. 3) were significant, especially the 'Biologic elasticity'  $R_2$ : +10.3%,  $p < 0.05$  and the 'visco-elasticity coefficient'  $R_6$ : -17.1%,  $p < 0.01$ . Interesting results shows Fig. 4, where the soft-focus effect of HA is evident. Particularly, 6 volunteers out of 10 showed a decrease in the fine lines, while 3 volunteers out of 10 showed a decrease in the deep wrinkles volume.

Delivery system trials

The obtained results of apatite concerning its potential delivery system power were rather disappointing. Both deodorant efficacy and sun screen capacity did not show any significant difference in comparison with the respective placebo. However, in the evaluation of the data we found out that that Colipa Standard P3 formula with 6% of HA showed a remarkable increase in the SPF value in comparison with the same formula without HA. In detail, the SPF value of the Colipa Standard was 15 and the SPF val-

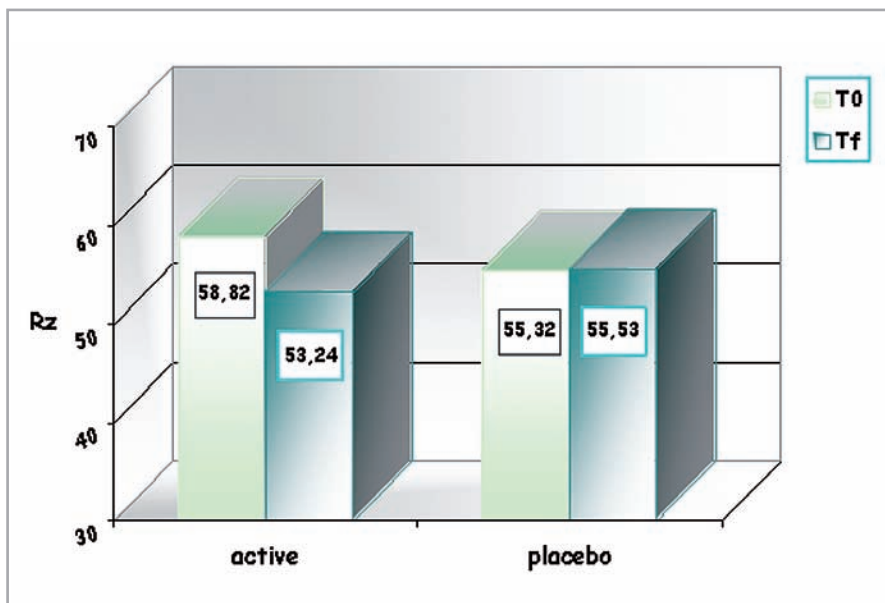


Fig. 2 9.5% average decrease in skin roughness is measured 4 weeks after use

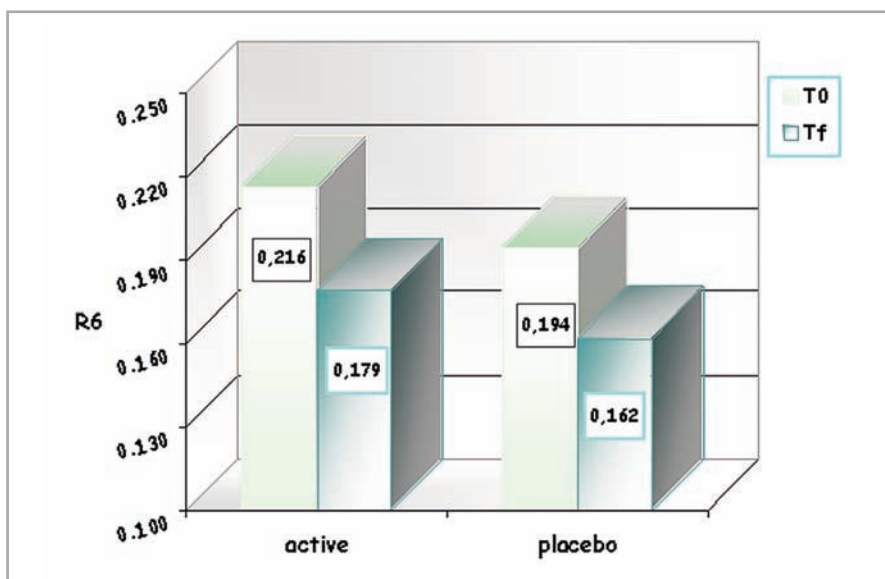


Fig. 3 Improvement of some skin viscoelastic parameters after a 4-week use of a suspension containing Apatite



Fig. 4 Evident soft focus effect of apatite containing emulsion after 4 week use

PHYSICAL SUNSCREEN

ue of the same formula containing 6% of HA was 17.5%.

**Sun screen efficacy**

Considering the abovereported results, 3 formulas were tested then: the Colipa Standard P3 and two W/O emulsions: a Sunscreen product with SPF 30 and a Sunscreen product with SPF 50+. Each of the 3 formulas was realized with the same percentage of HA and Titanium Dioxide (respectively 6% in the Colipa Standard P3, 4% in the SPF 30 and 5% in the SPF 50+. **Table 1** shows others filters in each formula. The obtained results were very significant (**Table 2** and **3**). In all cases, the evaluations showed an increase in the SPF values. In detail, in the case of Colipa Standard the increase

Sunscreen Filters	%		
	P3 SPF15	SPF 30	SPF 50+
Ethylhexyl Methoxycinnamate	3	10	10
Butyl Methoxydibenzoylmethane	0.5	2	2
Ensulizole	2.78	-	-
Ethylhexyl Salicylate	-	4	4
Diethylamino Hydroxybenzoyl Hexyl Benzoate	-	7	10
Ethylhexyl Triazone	-	1.5	3

**Table 1** Filters combined with HA or Titanium Dioxide

was by approximately 9%, for SPF 30 by approximately 18% and for SPF 50+ by approximately 2%. In the case of UVAPF the differences were negligible.

However, the requested UVA/UVB ratio was reached.

■ Conclusion

From the cosmetic point of view, Nature is sometimes seen through a nostalgic eye as 'Paradise Lost'. At times real discoveries can be made by simply observing the biological world around us.

With the use of a special grade of hydroxyapatite, a material similar to the bone and teeth structure, we found it possible to employ this active as a physiological sunscreen, which also improves some parameters of aged skin.

Additional tests are now being performed in order to develop interesting further applications.

In our research we still let ourselves be guided by the human physiology.

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	P3	P3 + 6% TiO <sub>2</sub>	P3 + 6% HA
SPF	15	16	17.5

**Table 2** SPF increase obtained by adding TiO<sub>2</sub> or alternatively hydroxyapatite to the COLIPA standard P3

	Sunscreen SPF 50+		Sunscreen SPF 30	
	SPF	UVA/UVB	SPF	UVA/UVB
TiO <sub>2</sub>	65.00	0.38	34.10	0.50
HA	66.20	0.33	40.30	0.35

**Table 3** Effect of replacing microfine TiO<sub>2</sub> with microfine Apatite: considerable increases in SPF values were always found

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