COSMETIC COMPOSITION FOR BLOCKING SKIN PIGMENTATION INDUCED BY UV RADIATION

The subject of the invention is a topical cosmetic composition for blocking pigmentation of the skin induced by UV radiation. In a first embodiment, this composition is used in the absence of UVA and UVB filters, on lighter coloured skins when it is wished to limit the phenomenon of pigmentation as much as possible. In this case, the composition is intended particularly for Asian populations. In a second embodiment, the composition in addition contains UVA and UVB filters and has at least two indications. The first indication is its use as an anti-tanning agent. Here again, the main market is the Asian market but the North African and Middle-Eastern markets are also concerned. The second indication is for skin developing hyperpigmentation phenomena in response to the effects of UV radiation.

Melanins are the pigments responsible for coloration of the skin. They are obtained by the mix of variable quantities of two types of pigment: phaeomelanin and eumelanin. Eumelanins (true melanins) are black or brown coloured pigments, completely absorbing the light, thus having a real photoprotective function. The phaeomelanins (red melanins) are orange-yellow pigments which provide less protection. Very fair skins, those of red-headed people (phototype I), contain mainly phaeomelanins.

The melanins are produced from tyrosine, in the presence of tyrosinase in the melanocytes, which occur in the basal layer of the epidermis. This is the process of melanogenesis. The synthesis of melanins occurs particularly in melanosomes, which are carried to the ends of the dendrites of the melanocytes then transferred into keratinocytes. Through the presence of melanins on the surface of the skin part of the visible and UV radiation is absorbed.

In Asian populations, it is the custom to keep the skin as light as possible. Even before being concerned by the effect of UV radiation, above all, they wish to prevent pigmentation of the skin, i.e. to inhibit melanin production to produce a lighter complexion. Many compositions particularly based on hydroquinone have been proposed for this purpose, hydroquinone acting directly on melanogenesis by stopping

melanin synthesis by inhibiting tyrosinase activity. This molecule has the disadvantage of being poorly tolerated and causes irritation when used to excess. In addition, it can destroy the melanocytes, leading to totally white depigmented areas. These changes are irreversible. Since January 2001, the cosmetic use of hydroquinone in Europe has been restricted because of its toxicity and it can only be used on medical prescription.

The applicant has now perfected a new cosmetic composition which differs from products of the prior art by containing substances stopping melanogenesis not only:

- directly, by inhibiting melanin synthesis by inhibiting tyrosinase activity,
- but also indirectly, by inhibiting the production of mediators by the keratinocytes, in as far as certain mediators, the production of which by the keratinocytes is stimulated by UV radiation, also play a role in melanogenesis.

These mediators are as follows:

Firstly, α-MSH: (alpha-melanocyte stimulating hormone), a neuropeptide hormone produced by the irradiated keratinocytes, which plays a major role in photo-induced pigmentation. When it binds to the MC-1 receptors on the surface of melanocytes, it induces proliferation of the melanocytes, accentuates their dendricity, increases tyrosinase activity and induces the production of eumelanins.

Secondly there is endothelin-1 (ET-1) which is a 21 amino-acid peptide which has strong vasoconstrictor activity. It also acts on melanocyte receptors (ETA and ETB) inducing increased melanocyte proliferation and dendricity, and stimulating melanogenesis.

Finally there is the Stem Cell Factor (SCF) which is produced by the keratinocytes and binds to its c-kit receptor on the surface of the melanocytes. SCF expression is increased during UV irradiation, inducing an increase in melanocyte number and dendricity, and melanogenesis.

It has also been shown that ET-1 and SCF act synergistically in the activation of melanogenesis.

To the applicant's knowledge no one has thought to approach the problem of photo-induced pigmentation by inhibiting keratinocyte stimulation (indirect action) combined with classic inhibition of tyrosinase activity (direct action).

Thus, in the first instance, the invention concerns a cosmetic composition characterised in that it comprises, in a cosmetic carrier:

- at least one agent blocking the binding of α-MSH to the MC-1 receptors present on the surface of melanocytes,
- at least one agent blocking the synthesis of ET-1,
- at least one agent inhibiting the synthesis of SCF,
- at least one agent inhibiting tyrosinase activity.

Those skilled in the art will know how to decide on potential candidates fulfilling the functions envisaged by performing appropriate tests. For α-MSH, it may be a matter of measuring the inhibitory effect of the candidate molecule on the stimulation of melanin synthesis in normal human melanocyte cultures. For ET-1 and SCF, it may be a matter of measuring the inhibitory effect of the candidate molecule on the secretion of ET-1 and SCF by normal human keratinocytes. For tyrosinase, the classic tests are performed in the test tube, i.e. without cells: tyrosinase is put into the presence of a substrate which changes colour when the tyrosinase is activated. If an inhibitory substance is present no (or less) colour change occurs.

This composition is more particularly used as an anti-pigmenting agent.

In an advantageous embodiment, the agent blocking the binding of α-MSH to the MC-1 receptors is the biomimetic hexapeptide-2 and/or nonapeptide-1 obtained by chemical synthesis, which have an affinity for the α-MSH receptor. In practice, the agent blocking α-MSH binding, in particular the two peptides mentioned above, forms

between 0.00001 and 1% by weight of the composition, preferably between 0.0001 and 0.0004% by weight.

Likewise, the agent blocking ET-1 synthesis is to advantage andrographolide preferably in the form of a leaf extract of *Andrographis paniculata.* In practice, it makes up between 0.001 and 10%, preferably between 0.01 and 0.1% by weight of the composition.

Likewise, the agent inhibiting SCF synthesis and tyrosinase activity is the same and is to advantage an aqueous extract of an alga called *Palmaria palmata,* preferably containing between 5 and 50% by weight of dry matter. In practice the extract represents between 0.01 and 25% by weight of the composition, ideally between 1 and 4% by weight.

Moreover, and this is a second aspect of the invention, to the applicant's knowledge, none of the non-pigmenting compositions of the prior art limited or stopped tanning or the hyperpigmentation phenomenon induced by UV radiation. This hyperpigmentation appears particularly as brown lentigo or "liver spots".

To resolve this problem, the cosmetic composition of the invention contains in addition at least one UVB and UVA filter and/or a UVA/UVB filter and possibly at least one mineral screen.

In other words the invention consists in this case of blocking the penetration of the major part of UV radiation using UVA and UVB filters, or a mineral screen, then inhibiting directly (inhibition of the stimulation of tyrosinase activity) and indirectly (inhibition of melanocyte stimulation by mediators secreted by the keratinocytes) the production and expression of melanin triggered by the remaining part of the UV radiation penetrating through the skin.

By acting on the main factors involved in melanogenesis, the composition of the invention can be used as an anti-tanning product for little or slightly pigmented skins,

in particular for the Asian market, or as a product to combat UV photo-induced hyperpigmentation.

The mineral screens and the UVB or UVA/UVB filters are intended to limit the penetration of UVB radiation into the skin. Depending on the nature of the filters, from 70 to 98% of the UVA irradiation will be stopped. The skin protection capacity relative to UVB radiation is characterised by the SPF index. For a given UVB irradiation, after application of a UVB filter or UVB/UVA, this index is the ratio between the UV dose triggering erythema on protected skin and the UV dose triggering erythema on unprotected skin. As an example, an SPF index of 20 means that 20 times more UV irradiation is required to trigger erythema, i.e. 95% of UVB rays have been stopped. An index of 50 means that 98% of UVB rays are stopped. In the invention, the UVB and/or UVA/UVB filters are selected to give the composition an SPF index of at least 20, to advantage at least 50.

Those skilled in the art will be in a position to select the appropriate constituents and their proportions to obtain such indices in accordance with current international norms (Colipa, FDA), in particular organic UVB or UVA filters selected from the group including:

- Para-aminobenzoic acid derivatives:
    - PABA,
    - Ethyl PABA,
    - Ethyl dihydroxypropyl PABA (Amerscreen® P sold by Amerchol),
    - Ethylhexyl dimethyl PABA sold particularly under the name of "ESCALOL® 507",
    - Glyceryl PABA,
    - PEG-25 PABA sold under the name of "UVINUL® P25"
- Salicylic acid derivatives:
    - Homosalate sold under the name of "EUSOLEX® HMS",
    - Ethylhexyl salicylate sold under the name of "NEO HELIOPAN® OS",
    - Dipropyleneglycol salicylate sold under the name of "DIPSAL ®",
    - TEA salicylate sold under the name of "NEO HELIOPAN® TS"

- Dibenzoylmethane derivatives:
    - Butyl methoxydibenzoylmethane,
    - Isopropyl dibenzoylmethane.
- Cinnamic derivatives:
    - Ethylhexyl methoxycinnamate sold particularly under the trade names "ESCALOL 557", "NEOHELIOPAN TYPEAV", "UVINUL MC80"
    - Isopropyl methoxycinnamate,
    - Isoamyl methoxycinnamate sold under the trade name "NEO HELIOPAN® E 1000",
    - Cinoxate,
    - DEA methoxycinnamate,
    - Glyceryl ethylhexanoate dimethoxycinnamate.

β-β'-diphenylacrylate derivatives:

- Octocrylene sold particularly under the trade name "UVINUL® N539",
- Etocrylene sold particularly under the trade name "UVINUL® N35"

- Benzophenone derivatives:
    - Benzophenone-1 sold under the trade name "UVINUL® 400",
    - Benzophenone-2 sold under the trade name "UVINUL® D50",
    - Benzophenone-3 or oxybenzone sold under the trade name "UVINUL® M40",
    - Benzophenone-4 sold under the trade name "UVINUL® MS40",
    - Benzophenone-5,
    - Benzophenone-6 sold under the trade name "HELISORB® 11",
    - Benzophenone-8 sold under the trade name "SPECTRA-SORB® UV 24",
    - Benzophenone-9 sold under the trade name "UNIVUL® DS-49",
    - Benzophenone-12.
- Benzylidene camphor derivatives:
    - 4-Methylbenzylidene camphor sold under the name of "EUSOLEX® 6300",
- Benzimidazole derivatives:
    - Phenylbenzimidazole sulphonic acid sold particularly under the trade name "EUSOLEX® 232",

- Disodium phenyl dibenzimidazole tetra-sulphonate sold under the trade name "NEO HELIOPAN® AP"

- Triazine derivatives:
  - Ethylhexyl triazone sold particularly under the trade name "UVINUL® T150",
  - Diethylhexyl butamido triazone sold under the trade name "UVASORB® HEB",
  - (Bis-ethylhexyloxyphenol methoxyphenyl triazine) Tinosorb S

- Benzotriazole derivatives:
  - Drometrizole trisiloxane sold under the name of "SILATRIZOLE®",
  - Methylene bis-benzotriazol tetramethylbutylphenol sold in solid form under the trade name "MIXXIM ®BB/100" or in micronised form in an aqueous dispersion under the trade name "TINOSORB® M"

- Anthranilic derivatives:
  - Menthyl anthranilate sold under the trade name "NEO HELIOPAN® MA"

- Imidazoline derivatives:
  - Ethylhexyl dimethoxybenzylidene dioxoimidazoline propionate.

  Benzalmalonate derivatives:
  - Polyorganosiloxane with benzalmalonate functional groups sold under the trade name "PARSOL® SLX"

- Diethylamino hydroxybenzoyl hexyl benzocaine (sold under the trade name Uvinul® A+)
- and mixtures of them.

The composition of the invention may also include pearls, pigments, or even nano-pigments (mean size of primary particles: generally between 5 nm and 100 nm, preferably between 10 nm and 50 nm) of metal oxides, enrobed or not, e.g. nano-pigments of oxides of titanium (amorphous or crystallised in the form of rutile and/or anatase), iron, zinc, zirconium or cerium and mixtures of them which are all individually well known UV photoprotective agents. Classic enrobing agents are moreover alumina and/or aluminium stearate.

In practice, these filters represent between 2 and 40% by weight of the composition, preferably between 15 and 30% by weight of the composition.

In all cases, the UVA/UVB filters are to advantage selected from the group consisting of:

- Bis-ethylhexyloxyphenol methoxyphenyl triazine: Tinosorb S
- Methylene bis-benzotriazol tetramethylbutylphenol: Tinosorb M

The mineral screens are to advantage selected from the group:

- TiO2
- ZnO

The cosmetic composition of the invention is generally applied topically.

It can be presented in all pharmaceutical forms normally used for topical application on the skin, particularly as an oil-in-water or water-in-oil or multiple emulsion, a siliconised emulsion, a microemulsion or nanoemulsion. This composition can be more or less liquid and have the appearance non-exclusively of a white or coloured cream, an ointment, milk, lotion, serum or gel.

The composition of the invention can contain the carriers usually used in the area of cosmetics and dermatological preparations, such as lipids, emulsifiers and co-emulsifiers, hydrophilic or lipophilic gelling agents, hydrophilic or lipophilic substances, preservatives, antioxidants, solvents, perfumes, fillers, hydrophilic and lipophilic filters, colouring materials, neutralisers, pro-penetrating agents, and polymers.

The invention also concerns the cosmetic treatment processes aiming to depigment the skin, stop tanning or to block hyperpigmentation induced by UV rays by topical application of the composition described earlier.

The invention and the advantages resulting from it are well illustrated by the following examples.

Example 1: Demonstration of the depigmenting activity of a cosmetic formulation on living human skin explants

OBJECTIVE OF THE STUDY

The aim of this study was to assess the depigmenting activity of the formulation according to the invention after chronic UVA/B irradiation (sun simulator) on living human skin explants. The depigmenting effect of the products to be tested was shown by spectrocolorimeter measurements.

FORMULATION TESTED

Purified water	qs 100%
Dipropylene glycol	4
Polyacrylamide	0.8
C13-14 isoparaffin	0.4
Laureth-7	0.11
Andrographis paniculata	0.05
Hexapeptide 2	0.0004
Palmaria palmata	2
Preservative	qs

PROTOCOL

1. Preparation of the explants

15 human skin explants (of intermediate phototype) were prepared and kept alive in a special medium. The explants were divided as follows (3 explants per condition):

- Control "**C**": untreated, not irradiated
- Formulation control "**F**": treated, not irradiated

- Control + UV "**C UV**": untreated, irradiated
- Formulation 1 + UV " **F UV**": treated, irradiated

2. Treatment

The product (1.5g/cm^2) was applied topically to the explants using a small spatula or filter paper discs. The explants were treated every two days.

3. Chronic UVA/B irradiation

Each day, for 8 days, the "C UV", "F UV" batches received UVA irradiation of 1.125 J/cm^2 (6-8% of UVB) corresponding to the spectrum of solar irradiation.

The irradiation was performed 30 minutes after treatment with the product, to avoid a possible filter effect.

4. Spectrocolorimeter measurements

The intensity of skin coloration was measured on D0 then on D8, by colorimetry. The results are expressed by calculating the variation in the ITA index:

ITA° = [ArcTan ((L* - 50) / b*)] x 180 / 3.1415

With: - L*: representing the lightness (from dark to pale)

- b* : representing the range from blues to yellows

This value allows different skin colours to be classified (the higher the ITA, the lighter the skin):

- Light coloured skin: 55>ITA>41
- Intermediate skin: 41>ITA>28
- Olive skin: 28>ITA>10

RESULTS

The table below shows the results obtained:

	Pigmentation D0	Pigmentation D8	Variation (%)
Control C	36.8 ± 1.3	36.7 ± 1.3	-0.4%
Formulation F	34.0 ± 1.6	37.6 ± 1.2	+11% **(A)**
Irradiated control CUV	38.1 ± 1.4	21.2 ± 1.2	-44%

Irradiated formulation FUV	36.9 ± 1.6	37.3 ± 1.8	+1% **(B)**

- The colour of the control explants did not vary.
- Treated by the formulation according to the invention, their tint lightened significantly (+11%) **(A).**
- Chronic UV irradiation stimulated the appearance of tanning, hence much greater pigmentation after 8 days (the skin changed from an intermediate colour to an olive colour). Treated with the invention, the explants kept their original tint **(B).**

By "upsetting" the process of melanogenesis, the formulation according to the invention blocked pigmentation of the explants induced by exposure to sun **(B),** and depigmented the pigmented areas (reversible effect) **(A).**

Example 2: Pharmaceutical formulations

1: Lightening sun cream

Purified water	qs 100%
Ethylhexyl methoxycinnamate (Parsol® MCX)	7.00
Butyl methoxydibenzoylmethane (Eusolex® 9020)	3.00
Micronised TiO2 (MT100 TZ).....	5.00
Bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb®S)	2.00
Methylene bis-benzotriazol tetramethylbutylphenol (Tinosorb® M)	4.00
Andrographis paniculata.....	0.10
Hexapeptide 2.....	0.0004
Palmaria palmata.....	1.50
Liquid paraffin	16.00
Cetostearyl ethylhexanoate.....	4.00
Sorbitan sesquioleate.....	1.00
Stearic acid.....	3.00

Stearic alcohol	1.70
Glycerol stearate	1.50
Beeswax	1.00
Triethanolamine	0.88
Acrylic polymer	0.20
Preservative	qs
Perfume	qs

2: Lightening serum

Purified water	qs 100%
Capryl glycerides/ethoxylated capric alcohols 6 moles	3.50
Xanthan gum	0.50
Polysorbate 20	1.20
Andrographis paniculata	0.10
Hexapeptide 2	0.002
Palmaria palmata	1.00
Sodium hyaluronate	0.10
Preservative	qs
Perfume	qs

3: Depigmenting cream

Purified water	qs 100%
Modified maize starch	5.50
Andrographis paniculata	0.10
Nonapeptide-1	0.005
Palmaria palmata	1.20
Isododecane	5.00
Methyl methacrylate polymer	1.925
Propylene glycol dicaprylate/dicaprate	1.52
Isodecyl neopentanoate	1.50
Glycerol stearate	1.30
Ethoxylated PEG stearate 100 moles	1.00
Stearic acid	1.00

Pyridoxine HCl	0.50
Zinc gluconate	0.50
Sodium hydroxide	0.32
Preservative	qs
Perfume	qs

CLAIMS

1. Topical cosmetic composition characterised in that it comprises in a cosmetic carrier:

- at least one agent blocking the binding of α-MSH onto MC-1 receptors,
- at least one agent blocking the synthesis of ET-1,
- at least one agent inhibiting the synthesis of SCF,
- at least one agent inhibiting tyrosinase activity.

2. Cosmetic composition according to claim 1, characterised in that the agent blocking the binding of α-MSH onto the MC-1 receptors is hexapeptide 2 and/or nonapeptide 1.

3. Cosmetic composition according to claim 1 characterised in that the agent blocking α-MSH binding to the MC-1 receptor forms between 0.00001 and 1% by weight of the composition, preferably between 0.0001 and 0.0004% by weight.

4. Cosmetic composition according to claim 1, characterised in that the agent blocking the synthesis of ET-1 is andrographolide.

5. Cosmetic composition according to claim 4, characterised in that the andrographolide is in the form of an extract of *Andrographis paniculata* leaves.

6. Cosmetic composition according to claim 5, characterised in that the *Andrographis paniculata* leaf extract forms between 0.001 and 10% by weight of the composition.

7. Cosmetic composition according to claim 1, characterised in that the agent inhibiting the synthesis of SCF and melanin activity is the same and is an aqueous extract of *Palmaria palmata.*

8. Cosmetic composition according to claim 7, characterised in that the aqueous extract of *Palmaria palmata* forms between 0.01 and 25% by weight of the composition.

9. Composition according to claim 1, characterised in that:
   - the agent blocking α-MSH binding onto the MC-1 receptor is hexapeptide 2
   - the agent blocking ET-1 synthesis is an *Andrographis paniculata* leaf extract,
   - the agent inhibiting the SCF synthesis and melanin activity is the same and is an aqueous extract of *Palmaria palmata.*

10. Cosmetic composition according to one of claims 1 to 9, characterised in that it comprises in addition:
   - at least one UVB and/or UVB/UVA filter,
   - at least one UVA filter,
   - possibly at least one mineral screen.

11. Cosmetic composition according to claim 10, characterised in that the nature of the organic UVB or UVA/UVB filters and their proportion is selected to give the composition an SPF of at least 20, to advantage at least 50.

12. Cosmetic composition according to claim 10, characterised in that the organic UVA or UVB filters are selected from the group including para-aminobenzoic acid derivatives, salicylic acid derivatives, dibenzoylmethane derivatives, cinnamic derivatives, benzophenone derivatives, benzylidene camphor derivatives, benzimidazole derivatives, triazine derivatives, benzotriazole derivatives, anthranilic derivatives, imidazoline derivatives, diethylamino hydroxybenzoyl hexyl benzoate and mixes of them, these filters forming between 2 and 40% by weight of the composition.

13. Cosmetic composition according to claim 10, characterised in that the mineral screens is selected from the group including TiO_2 and ZnO.

14. Cosmetic use of the composition forming the subject of one of the claims 1 to 9 as an anti-pigmenting agent.

15. Cosmetic use of the composition forming the subject of one of the claims 10 to 13 as an anti-tanning agent.

16. Cosmetic use of the composition forming the subject of one of the claims 10 to 13 as an agent preventing UV induced hyperpigmentation.