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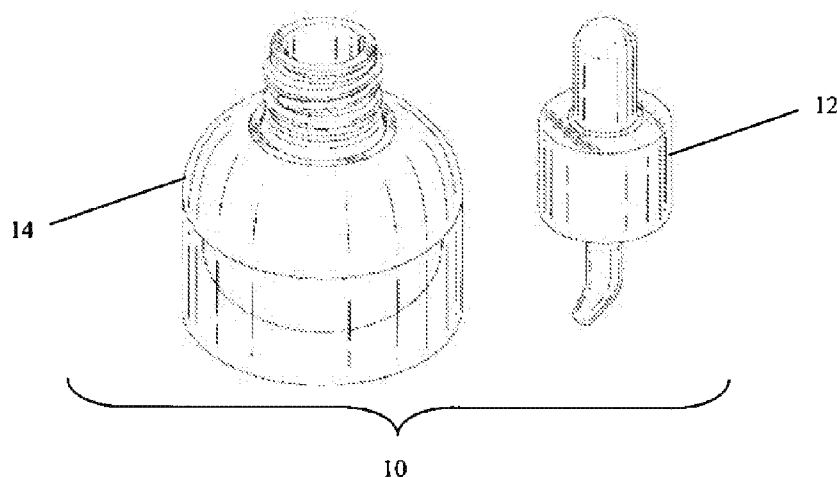


Fig. 1

(57) Abstract: A method of improving the appearance of a hyperpigmented spot and improving skin tone may comprise the steps of applying a safe and effective amount of a Laminaria Saccharina extract to a hyperpigmented spot on a skin surface, wherein the Laminaria Saccharina extract is applied for a period of time sufficient to improve the appearance of the hyperpigmented spot; and of applying a skin tone agent to the skin surface, wherein said tone agent is a vitamin B3 compound and wherein the tone agent is applied for a period of time sufficient to improve skin tone.

LAMINARIA SACCHARINA EXTRACT AND VITAMIN B3 AS WHITENING AGENTS

FIELD OF THE INVENTION

5 The present invention relates to methods for improving the appearance of hyperpigmented spots and skin tone.

BACKGROUND OF THE INVENTION

10 Human skin comprises three principal layers: the epidermis, the dermis, and the subcutaneous fat layer. The epidermis comprises four layers (from top to bottom): the stratum corneum, the granular layer, the spiny layer, and the basal layer. A separate fifth layer, the stratum lucidum, is often present between the stratum corneum and granular layer. The basal layer produces cells which gradually migrate upward to form the other epidermal layers. As these cells migrate upward, they lose their central nucleus and start to produce skin proteins
15 (keratins) and fats (lipids). These cells are identified as keratinocytes when present in the upper layers of the epidermis. Melanocytes are another class of cells located in the basal layer of the epidermis. Melanocytes are responsible for the production of melanin, which is primary factor in skin pigmentation and hyperpigmentation. There are approximately 1500 gene products expressed in melanosomes with 600 of them being expressed at any given time and 100 of them
20 believed to be unique to the melanosome. In addition, there are many regulatory elements involved in signaling, in the transport of melanosomes within the melanocyte, and in the transfer of melanosomes to the keratinocytes.

 Melanin is produced by a complex set of reactions within the melanocyte involving, at a basic level, the enzyme tyrosinase and the protein L-tyrosine. Tyrosinase catalyzes the conversion
25 of L-tyrosine to DOPA (L-3,4-dihydroxyphenylalanine) and of DOPA to dopaquinone. Dopaquinone undergoes further conversion to form melanin. Melanin aggregates in organelles known as the melanosomes which are transferred to keratinocytes along slender filaments of the melanocyte known as dendrites.

 The production of melanin can be triggered by a variety of external and internal events.
30 For example, melanocytes produce additional melanin when skin is subjected to UV radiation. The melanin is then transported via melanosomes to the keratinocytes, which then leaves the skin with a "tanned" appearance. Once the UV light is removed the melanocytes return to normal levels of melanin production. Inflammation may initiate hyperpigmentation by direct stimulation

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of the melanocytes by mediators such as IL-1, endothelin-1, and/or stem cell factor. Reactive oxygen species, such as superoxide and nitric oxide, generated in damaged skin or released as by-products from inflammatory cells may be stimulators of melanocytes.

Over time, chronic UV exposure and other intrinsic and extrinsic aging factors may lead to permanent gene expression changes in keratinocytes and/or melanocytes resulting in age-related hyperpigmented spots. The mRNA levels of some melanogenesis associated genes (for example, tyrosinase, TYRP1) appear to be increased in actinic lentigos (age spots). There may also be accentuation of the epidermal endothelin cascade and a role for stem cell factor in hyperpigmentation. These changes can result in overproduction of melanin and resultant hyperpigmented spots that persist even when an insult, such as UV exposure, is avoided. Even beyond hyperpigmented spots, chronic UV exposure and other intrinsic and extrinsic aging factors may lead to more subtle changes in skin tone. Often these changes are described as uneven tone or as a mottled appearance. At least one study suggests that age spots can sometimes add 10 to 12 years of perceived age to a person and that melanin distribution can drive tone dependent age perception.

Furthermore, there is a need to develop compositions for addressing hyperpigmented spots as well as address overall skin tone. Users want a composition that yields quick, noticeable improvement of hyperpigmented spots. The compositions must also improve skin tone to counteract what is disparagingly described as a mottled skin appearance. Thus, compositions and methods of treatment that result in a reduction in the size and/or contrast of hyperpigmented spots and improve overall skin tone are a continuing desire in the cosmetic field.

Furthermore, there is a need for a composition that addresses and regulates seasonal skin darkening. Seasonal skin darkening is the natural darkening (*i.e.*, tanning) that occurs seasonally due to increased sunlight and UV exposure. Seasonal skin darkening can manifest itself in overall tone darkening or the darkening of hyperpigmented spots.

Extracts of *Laminaria Saccharina*, a species of brown algae, are known in the art. One example is sold under the tradename Phlorogine by Biotech Marine, France. Phlorogine is known as anti-seborrhoeic agent that can regulate the activity of sebaceous glands, as described for example in United States Patent Application Publication No. 2008/0119527A1. Extraction methods for brown algae are also known. European Patent No. 1074262B1 describes an extraction method for the class *Phaeophyceae* and the species *Laminaria Ochroleuca*. These extracts are described as being used in cosmetic compositions as an osmoprotector, free-radical scavenger, or against the effects of skin aging effects. A cosmetic composition sold under the

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brand name SK-II Facial Clear Solution (Procter & Gamble, Cincinnati, OH) has a concentration of Phlorogine of about 1.25%. The SK-II Facial Clear Solution is marketed as a gel hydrator that moisturizes the skin without increasing oily shine.

5 SUMMARY OF THE INVENTION

A method of improving the appearance of a hyperpigmented spot and improving skin tone comprising the steps of applying a safe and effective amount of a *Laminaria Saccharina* extract to a hyperpigmented spot on a skin surface, wherein the *Laminaria Saccharina* extract is applied for a period of time sufficient to improve the appearance of the hyperpigmented spot; and
10 applying a skin tone agent to the skin surface, wherein said tone agent is a vitamin B3 compound and wherein the tone agent is applied for a period of time sufficient to improve skin tone.

A method of regulating seasonal skin darkening, the method comprising the step of applying a composition to a facial skin surface at least daily from a start date to an end date, wherein the composition comprises a *Laminaria Saccharina* extract and vitamin B3, and wherein
15 day length of the end date is longer than day length of the start date with the proviso that no day between the start date and end date is shorter than the start date.

In response to the technical problems identified in the background, the present invention may take other forms. Further forms of the present invention will be appreciated in the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

It is believed that the present invention will be better understood from the following description taken in conjunction with the accompanying drawings. The referenced drawings are not to be construed as limiting the scope of present invention.

Figure 1 is an exemplary applicator in the form of a dropper.

Figure 2 is an exemplary applicator in the form of a wand.

Figure 3 is an exemplary applicator in the form of a narrow-tip tube.

20 Figure 4 is a full color image of a participant.

Figure 5 is a melanin concentration map of the same participant as shown in Figure 4.

DETAILED DESCRIPTION OF THE INVENTION

All percentages and ratios used herein are by weight of the total composition and all measurements made are at 25°C, unless otherwise designated. All numeric ranges are inclusive

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of narrower ranges; delineated upper and lower range limits are interchangeable to create further ranges not explicitly delineated.

The compositions of the present invention can comprise, consist essentially of, or consist of, the essential components as well as optional ingredients described herein. As used herein, 5 “consisting essentially of” means that the composition or component may include additional ingredients, but only if the additional ingredients do not materially alter the basic and novel characteristics of the claimed compositions or methods.

The term “apply” or “application”, as used in reference to a composition, means to apply or spread the compositions of the present invention onto a human skin surface such as the 10 epidermis.

The term “dermatologically acceptable,” as used herein, means that the compositions or components described are suitable for use in contact with human skin tissue without undue toxicity, incompatibility, instability, allergic response, and the like.

The term “safe and effective amount” as used herein means an amount of a compound or 15 composition sufficient to significantly induce a positive benefit.

The term “post-inflammatory hyperpigmentation” as used herein refers to an acute to chronic increase in pigmentation as a response to a transient inflammatory event. Post-inflammatory hyperpigmentation is particularly prevalent in, but not limited to, dark skin subjects. Post-inflammatory hyperpigmentation typically subsides once the transient 20 inflammatory event dissipates. Examples of transient inflammatory events include, but are not limited to, acne lesions, ingrown hairs, scratches, insect bites, surfactant damage, and short-term UV exposure.

The term “hyperpigmented spot” as used herein refers to a defined area of skin wherein the pigmentation is greater than that of an adjacent area of skin due to localized and chronic or 25 systemic overproduction of melanin. Hyperpigmented spots typically are between about 2 mm and about 10 mm in diameter but smaller or larger spots are possible. Hyperpigmented spots can include one or more of age spots, sun spots, solar lentigos, hypo-melanotic lesions, freckles, and melasma spots.

The term “age spots” as used herein refers to a defined area of skin wherein the 30 pigmentation is greater than that of adjacent skin due to localized and chronic overproduction of melanin caused by intrinsic or extrinsic aging factors.

The term “skin tone agent” as used herein refers to an agent that regulates melanin production signals, synthesis of melanin, systemic transfer of melanin between the melanocyte

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and the keratinocyte, and/or melanin degradation. Skin tone agents can improve the appearance of uneven skin tone by acting as a lightening or pigmentation reduction cosmetic agent.

The term “skin tone” as used herein refers to the overall appearance of melanin in the skin caused by the systemic, rather than transient, synthesis of melanin. Skin tone is typically characterized over a larger area of the skin. The area ideally may be than 100 mm², but larger areas are envisioned such as the entirety of the facial skin or any of the facial skin surfaces. Skin tone can be measured by image analysis. For example, overall lightness can be measured by L* coordinate in L*a*b* color space (International Commission on Illumination). Chromophore mapping such as melanin mapping may be used as an indicator of overall skin tone. Mean melanin may be calculated from the chromophore map data. Additionally, skin tone evenness can be determined by melanin which also may be determined calculated from the chromophore map data. Suitable chromophore mapping techniques are discussed in the example below.

The term “facial skin surfaces” as used herein refers to one or more of forehead, periorbital, cheek, perioral, chin, and nose skin surfaces.

I. Compositions

The present invention relates to various compositions and, more specifically, to compositions for topical application to a skin surface. The compositions may be made into a wide variety of product forms that include, but are not limited to, solutions, suspensions, lotions, creams, gels, toners, sticks, pencil, sprays, aerosols, ointments, cleansing liquid washes and solid bars, shampoos and hair conditioners, pastes, foams, powders, mousses, shaving creams, wipes, strips, patches, electrically-powered patches, wound dressing and adhesive bandages, hydrogels, film-forming products, facial and skin masks (with and without insoluble sheet), make-up such as foundations, eye liners, and eye shadows, and the like. The composition form may follow from the particular dermatologically acceptable carrier chosen, if present in the composition.

A. *Laminaria Saccharina* Extract

Compositions of the present invention include a safe and effective amount of *Laminaria Saccharina* extract, a brown algae extract. A preferred extract is Phlorogine and/or Phlorogine BG, which is available from Marine Biotech, France. Another suitable *Laminaria Saccharina* extract is available via product code HG 657 from Ennagram, France. The composition may contain Phlorogine and/or Phlorogine BG in an amount from 0.008% to 50%, in one embodiment from about 0.04% to 20%, in another embodiment from 0.2% to 10%, by weight of the composition. In yet another embodiment the composition comprises from 1% to 5%, and in yet

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another embodiment from 1% to 3% Phlorogine and/or Phlorogine BG by weight of the total composition.

The *Laminaria Saccharina* extract may include other compounds, such as, for example water, thickeners, humectants, solvents and solubilizers, etc. For example, Phlorogine and/or
5 Phlorogine BG contain approximately about 1% to about 2.5% dry extract with the remaining material being inert carrier. The composition of the present invention therefore may contain a *Laminaria Saccharina* extract in an amount from about 0.00008% to about 1.25%, in one embodiment from about 0.0004% to about 0.5%, in another embodiment from about 0.002% to about 0.25%, by weight of the composition. In yet another embodiment the composition
10 comprises from about 0.01% to about 0.125%, and in yet another embodiment from 0.01% to 0.075% *Laminaria Saccharina* extract by weight of the total composition. The *Laminaria Saccharina* extract can be prepared by processes known in the art, such as, for example, described in European Patent No. 1074262B1.

B. Skin Tone Agent

15 In some embodiments, it may be desirable to include at least one skin tone agent in the composition in combination with the *Laminaria Saccharina* extract. The skin tone agents can be included to further improve overall skin tone. When present, the compositions of the present invention contain up to about 50%, 40%, 30%, 20%, 10%, 5%, or 3%, by weight of the composition, of the skin tone agent. When present, the compositions of the present invention
20 contain at least about 0.001%, 0.01%, 0.1%, 0.2%, 0.5%, or 1%, by weight of the composition, of the skin tone agent. Suitable ranges include any combination of the lower and upper limits including suitable ranges from about 0.1% to about 50%; from about 0.2% to about 20%; or from about 1% to about 10%, by weight of the composition, of the additional skin tone agent. The amounts listed herein are only to be used as a guide, as the optimum amount of the additional
25 skin tone agent will depend on the specific agent selected since their potency does vary considerably.

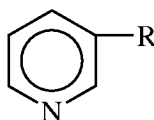
Suitable skin tone agents include, but are not limited to, sugar amines, vitamin B3 compounds, arbutin, deoxyarbutin, sucrose dilaurate, bakuchiol (4-[(1E, 3S)-3-ethenyl-3,7-dimethyl - 1,6 octadienyl] phenol or monoterpene phenol), pyrenoine (available from Biotech
30 Marine, France), panicum miliaceum seed extract, arlatone dioic acid, cinnamic acid, ferulic acid, achromaxyl, methyl nicotinamide, oil soluble licorice extract, folic acid, undecylenic acid (i.e., undecenoic acid), zinc undecylenate, thiamine (Vitamin B1) and its hydrochloride, L-tryptophan, hexylrescorcinol, helianthus annuus (sunflower) and vitis vinifera (grape) leaf extract, carnosine

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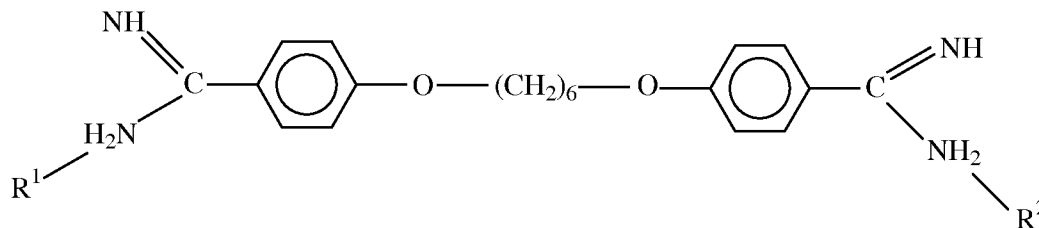
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(i.e., dragosine), methyl gentisate, 1,2-hexandiol and 1,2-octandiol (i.e., combination sold as Symdiol 68 by Symrise AG, Germany), inositol, decylenoylphenylalanine (e.g., sold under the tradename Sepiwhite by Seppic, France), kojic acid, hexamidine compounds, salicylic acid, and retinoids including retinol and retinyl propionate. Furthermore, *Laminaria Saccharina* extract
 5 may also be used as a skin tone agent.

In certain embodiments, the additional skin tone agent is selected from vitamin B₃ compounds, sugar amines, hexamidine compounds, salicylic acid, and retinoids. As used herein, "vitamin B₃ compound" means a compound having the formula:



10 wherein R is - CONH₂ (i.e., niacinamide), - COOH (i.e., nicotinic acid) or - CH₂OH (i.e., nicotinyl alcohol); derivatives thereof; and salts of any of the foregoing. As used herein, "sugar amine" includes isomers and tautomers of such and its salts (e.g., HCl salt) and its derivatives. Examples of sugar amines include glucosamine, N-acetyl glucosamine, mannosamine, N-acetyl mannosamine, galactosamine, N-acetyl galactosamine, their isomers (e.g., stereoisomers), and
 15 their salts (e.g., HCl salt). As used herein, "hexaminide compound" means a compound having the formula:



wherein R¹ and R² are optional or are organic acids (e.g., sulfonic acids, etc.). In one embodiment, hexamidine compound includes hexamidine diisethionate.

20 C. Anti-Inflammatory Agents

Hyperpigmentation may result from skin inflammation. Transient inflammatory events triggering hyperpigmentation and, more specifically, post-inflammatory hyperpigmentation include, but are not limited to, acne lesions, ingrown hairs, scratches, insect bites, surfactant damage, and short-term UV exposure. Inflammation induced hyperpigmentation including post-
 25 inflammatory hyperpigmentation may be managed by incorporating into the compositions of the present invention an anti-inflammatory agent. When present, the compositions of the present invention contain up to about 20%, 10%, 5%, 3%, or 1% by weight of the composition, of the

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anti-inflammatory agent. When present, the compositions of the present invention contain at least about 0.001%, 0.01%, 0.1%, 0.2%, 0.3%, 0.5%, or 1%, by weight of the composition, of the anti-inflammatory agent. Suitable ranges include any combination of the lower and upper limits. Suitable anti-inflammatory agents include, but are not limited to nonsteroidal anti-inflammatory agents (NSAIDS including but not limited to ibuprofen, naproxen, flufenamic acid, etofenamate, aspirin, mefenamic acid, meclofenamic acid, piroxicam and felbinac), glycyrrhizic acid (also known as glycyrrhizin, glycyrrhixinic acid, and glycyrrhetinic acid glycoside) and salts such as dipotassium glycyrrhizate, glycyrrhetenic acid, licorice extracts, bisabolol (e.g., alpha bisabolol), manjistha (extracted from plants in the genus *Rubia*, particularly *Rubia cordifolia*), and guggal (extracted from plants in the genus *Commiphora*, particularly *Commiphora mukul*), kola extract, chamomile, red clover extract, and sea whip extract, derivatives of any of the foregoing, and mixtures thereof.

D. Sunscreen Actives

The compositions of the subject invention may comprise one or more sunscreen actives (or sunscreen agents) and/or ultraviolet light absorbers. Herein, "sunscreen active" includes both sunscreen agents and physical sunblocks. Sunscreen actives and ultraviolet light absorbers may be organic or inorganic. Examples of suitable sunscreen actives and ultraviolet light absorbers are disclosed in Personal Care Product Council's *International Cosmetic Ingredient Dictionary and Handbook*, Thirteenth Edition, as "sunscreen agents." Particularly suitable sunscreen actives are 2-ethylhexyl-p-methoxycinnamate (commercially available as PARSOL™ MCX), 4,4'-t-butyl methoxydibenzoyl-methane (commercially available as PARSOL™ 1789), 2-hydroxy-4-methoxybenzophenone, octyldimethyl-p-aminobenzoic acid, digalloyltrioleate, 2,2-dihydroxy-4-methoxybenzophenone, ethyl-4-(bis(hydroxypropyl))aminobenzoate, 2-ethylhexyl-2-cyano-3,3-diphenylacrylate, 2-ethylhexyl-salicylate, glyceryl-p-aminobenzoate, 3,3,5-trimethylcyclohexylsalicylate, menthyl anthranilate, p-dimethyl-aminobenzoic acid or aminobenzoate, 2-ethylhexyl-p-dimethyl-amino-benzoate, 2-phenylbenzimidazole-5-sulfonic acid, 2-(p-dimethylaminophenyl)-5-sulfonicbenzoxazoic acid, octocrylene, zinc oxide, benzylidene camphor and derivatives thereof, titanium dioxide, and mixtures thereof.

In one embodiment, the composition may comprise from about 1% to about 20%, and alternatively from about 2% to about 10% by weight of the composition, of the sunscreen active and/or ultraviolet light absorber. Exact amounts will vary depending upon the chosen sunscreen active and/or ultraviolet light absorber and the desired Sun Protection Factor (SPF), and are within the knowledge and judgment of one of skill in the art.

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E. Optional Components

The compositions of the present invention may contain a variety of other ingredients that are conventionally used in given product types provided that they do not unacceptably alter the benefits of the invention. When present, compositions of the present invention may contain from
5 about 0.0001% to about 50%; from about 0.001% to about 20%; or, alternately, from about 0.01% to about 10%, by weight of the composition, of the optional components. The amounts listed herein are only to be used as a guide, as the optimum amount of the optional components used in a composition will depend on the specific active selected since their potency does vary considerably. Hence, the amount of some optional components useful in the present invention
10 may be outside the ranges listed herein.

The optional components, when incorporated into the composition, should be suitable for use in contact with human skin tissue without undue toxicity, incompatibility, instability, allergic response, and the like within the scope of sound judgment. The compositions of the present invention may include optional components such as anti-acne actives, desquamation actives, anti-
15 cellulite agents, chelating agents, flavonoids, tanning active, non-vitamin antioxidants and radical scavengers, hair growth regulators, anti-wrinkle actives, anti-atrophy actives, minerals, phytosterols and/or plant hormones, N-acyl amino acid compounds, antimicrobial or antifungal actives, and other useful skin care actives, which are described in further detail in U.S. application publication No. US2006/0275237A1 and US2004/0175347A1.

The Personal Care Product Council's *International Cosmetic Ingredient Dictionary and Handbook*, Thirteenth Edition, describes a wide variety of non-limiting cosmetic and pharmaceutical ingredients commonly used in the skin care industry, which are suitable optional components for use in the compositions of the present invention. Examples of these ingredient classes include: abrasives, absorbents, aesthetic components such as fragrances, pigments,
25 colorings/colorants, essential oils, anti-caking agents, antifoaming agents, binders, biological additives, buffering agents, bulking agents, chelating agents, chemical additives, colorants, cosmetic astringents, cosmetic biocides, denaturants, drug astringents, external analgesics, film formers or materials, e.g., polymers, for aiding the film-forming properties and substantivity of the composition (e.g., copolymer of eicosene and vinyl pyrrolidone), opacifying agents, pH
30 adjusters, propellants, reducing agents, sequestrants, and thickeners.

F. Dermatologically Acceptable Carrier

The compositions of the present invention may also comprise a dermatologically acceptable carrier ("carrier") for the composition. . The phrase "dermatologically acceptable

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carrier", as used herein, means that the carrier is suitable for topical application to the keratinous tissue, has good aesthetic properties, is compatible with the actives of the present and will not cause any safety or toxicity concerns. In one embodiment, the carrier is present at a level of from about 50% to about 99%, about 60% to about 98%, about 70% to about 98%, or, alternatively,
5 from about 80% to about 95%, by weight of the composition.

The carrier can be in a wide variety of forms. Non-limiting examples include simple solutions (aqueous or oil based), emulsions, and solid forms (gels, sticks, flowable solids, amorphous materials). In certain embodiments, the dermatologically acceptable carrier is in the form of an emulsion. Emulsion may be generally classified as having a continuous aqueous
10 phase (*e.g.*, oil-in-water and water-in-oil-in-water) or a continuous oil phase (*e.g.*, water-in-oil and oil-in-water-in-oil). The oil phase of the present invention may comprise silicone oils, non-silicone oils such as hydrocarbon oils, esters, ethers, and the like, and mixtures thereof.

The aqueous phase typically comprises water. However, in other embodiments, the aqueous phase may comprise components other than water (non-water components), including
15 but not limited to water-soluble moisturizing agents, conditioning agents, anti-microbials, humectants and/or other water-soluble skin care actives. In one embodiment, the non-water component of the composition comprises a humectant such as glycerin and/or other polyols. However, it should be recognized that the composition may be substantially (*i.e.*, less than 1% water) or fully anhydrous.

20 A suitable carrier is selected to yield a desired product form. Furthermore, the solubility or dispersibility of the compositions components (*e.g.*, *Laminaria Saccharina* extract, sunscreen active, additional components) may dictate the form and composition of the carrier. In one embodiment, oil-in-water or water-in-oil emulsions are preferred.

Emulsions may further comprise an emulsifier. The composition may comprise any
25 suitable percentage of emulsifier to sufficiently emulsify the carrier. Suitable weight ranges include from about 0.1% to about 10% or about 0.2% to about 5% of an emulsifier, based on the weight of the composition. Emulsifiers may be nonionic, anionic or cationic. Suitable emulsifiers are disclosed in, for example, U.S. Patent 3,755,560, U.S. Patent 4,421,769, and McCutcheon's Detergents and Emulsifiers, North American Edition, pages 317-324 (1986).
30 Suitable emulsions may have a wide range of viscosities, depending on the desired product form.

The carrier may further comprise a thickening agent as are well known in the art to provide compositions having a suitable viscosity and rheological character.

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II. Exemplary Compositions

The following are non-limiting examples of the compositions of the present invention. The examples are given solely for the purpose of illustration and are not to be construed as limitations of the present invention, as many variations thereof are possible without departing from the spirit and scope of the invention, which would be recognized by one of ordinary skill in the art. In the examples, all concentrations are listed as weight percent, unless otherwise specified and may exclude minor materials such as diluents, filler, and so forth. The listed formulations, therefore, comprise the listed components and any minor materials associated with such components. As is apparent to one of ordinary skill in the art, the selection of these minor materials will vary depending on the physical and chemical characteristics of the particular ingredients selected to make the present invention as described herein.

Suitable compositions are provided below. Examples A-E may be used to improve the appearance of one or more hyperpigmented spots and to improve overall skin tone. The present invention may further relate to a regimen involving the general or localized treatment for one or more hyperpigmented spots by a first composition (*e.g.*, Examples A or B with or without the niacinamide) and a more general facial skin treatment by a second composition (*e.g.*, Examples A-E with or without the Phlorogine), which can be applied before or after the localized treatment to improve skin tone across the face.

Component	Ex. A	Ex. B	Ex. C	Ex. D	Ex. E
Phlorogine or Phlorogine BG *1	2.000	1.000	1.000	1.000	1.000
N-Acetylglucosamine	0	0	2.000	0	0
Hexamidine Diisethionate	0			0.090	0.090
Undecylenoyl-phenylalanine *2 (neutralized)	0	1.000	0.500	0	0
Dipotassium Glycyrrhizate	0	0.300	0.100	0.100	0.100
Niacinamide	5.000	5.000	5.000	5.000	5.000
Isohexadecane	3.000	3.000	3.000	3.000	3.000
Isopropyl isostearate	1.330	1.330	1.330	1.330	1.330
Cetearyl glucoside + cetearyl alcohol *3	0.200	0.200	0.200	0.200	0.200
Behenyl alcohol	0.400	0.400	0.400	0.400	0.400
Cetyl alcohol	0.320	0.320	0.320	0.320	0.320
Stearyl alcohol	0.480	0.480	0.480	0.480	0.480
Tocopheryl acetate	0.500	0.500	0.500	0.500	0.500
PEG-100 stearate	0.100	0.100	0.100	0.100	0.100
Glycerin	7.000	7.000	7.000	7.000	7.000
Polyacrylamide + C13-14 isoparaffin + laureth-7 *4	2.000	2.000	2.000	2.000	2.000
Disodium EDTA	0.100	0.100	0.100	0.100	0.100
Benzyl alcohol	0.400	0.400	0.400	0.400	0.400

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Dimethicone/ Dimethiconol *5	2.000	2.000	2.000	2.000	2.000
Homosalate	0	0	0	0	9.000
Avobenzone	0	0	0	0	3.000
Octocrylene	0	0	0	0	2.600
Oxybenzone	0	0	0	0	1.000
Octisalate	0	0	0	0	4.500
Water	QS	QS	QS	QS	QS
TOTAL	100	100	100	100	100

*1 – Available from Biotech Marine, France.

*2 – Sepiwhite available from SEPPIC, France.

*3 – Emulgade PL 68/50 available from Cognis GmbH.

*4 – Sepigel 305, available from SEPPIC, France.

5 *5 – Dow Corning DC1503 available from Dow Corning, Inc., Midland, MI.

The compositions of the present invention are generally prepared by conventional methods such as are known in the art of making compositions and topical compositions. Such methods typically involve mixing of the ingredients in one or more steps to a relatively uniform state, with or without heating, cooling, application of vacuum, and the like. Typically, emulsions are prepared by first mixing the aqueous phase materials separately from the fatty phase materials and then combining the two phases as appropriate to yield the desired continuous phase. The compositions are preferably prepared such as to optimize stability (physical stability, chemical stability, photostability) and/or delivery of the active materials. This optimization may include appropriate pH (e.g., less than 7), exclusion of materials that can complex with the active agent and thus negatively impact stability or delivery (e.g., exclusion of contaminating iron), use of approaches to prevent complex formation (e.g., appropriate dispersing agents or dual compartment packaging), use of appropriate photostability approaches (e.g., incorporation of sunscreen/sunblock, use of opaque packaging), etc.

III. Methods of Treatment

Various methods of treatment may utilize the aforementioned compositions. In one embodiment, the method includes the step of identifying a hyperpigmented spot for treatment by the composition. The hyperpigmented spot may be identified by the user or a third party such as a dermatologist, cosmetician, or other caregiver. Identification may be done by visual inspection of the skin for hyperpigmented spots in need of treatment based on size and/or color. Identification may also be done by commercially available imaging devices such SIAScope V (available from Astron Clinica, Ltd., UK) or the VISIA® Complexion Analysis system (available from Canfield Scientific, Inc., Fairfield, NJ). Both devices are capable of collecting images of

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the skin and identifying hyperpigmented spots. In some instances, the method comprises the step of identifying a plurality of hyperpigmented spots for treatment by the composition

Identification of the hyperpigmented spot may occur on any skin surface of the body. Skin surfaces of the most concern tend to be those not typically covered by clothing such as facial skin surfaces, hand and arm skin surfaces, foot and leg skin surfaces, and neck and chest skin surfaces (e.g., décolletage). In particular, identification of the hyperpigmented spot of the facial skin surfaces includes the forehead, perioral, chin, periorbital, nose, and/or cheek skin surfaces.

The method may comprise the step of applying the composition to a hyperpigmented spot or spots, which may have been previously identified. Many regimens exist for the application of the composition to the hyperpigmented spot. The composition may be applied at least once a day, twice a day, or on a more frequent daily basis, during a treatment period. When applied twice daily, the first and second applications are separated by at least 1 to about 12 hours. Typically, the composition may be applied in the morning and/or in the evening before bed.

The treatment period is ideally of sufficient time to provide an improvement in the hyperpigmented spot and/or tone. The improvement may be a detectable reduction in size of the hyperpigmented spot, lightening of the hyperpigmented spot (e.g., lighter in color), a decrease in melanin, or improvement in melanin evenness. The treatment period may be at least about 1 week. The treatment period may last about 4 weeks or about 8 weeks. In certain embodiments, the treatment period will extend over multiple months (i.e., 3-12 months) or multiple years. In one embodiment the composition is applied least once a day during a treatment period of at least about 4 weeks or at least about 8 weeks. In one embodiment the composition is applied twice a day during a treatment period of at least about 4 weeks or 8 weeks.

The step of applying the composition may be done generally to a skin surface for spot or tone improvement. Suitable skin surfaces include those not typically covered by clothing such as facial skin surfaces, hand and arm skin surfaces, foot and leg skin surfaces, and neck and chest skin surfaces (e.g., décolletage). In particular, when applied more generally, the skin surface is facial skin surface. The composition can be applied to one or more of the forehead, perioral, chin, periorbital, nose, and cheek skin surfaces. In another embodiment, the first and second compositions are applied contemporaneously to at least the cheek, forehead, and chin/perioral skin surfaces. General application allows the *Laminaria Saccharina* extract to regulate the hyperpigmented spots while the tone agent works on overall skin tone.

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The step of topically applying the composition to the hyperpigmented spot or spots may be done by localized application. In reference to application of the composition, the term “localized”, “local”, or “locally” mean that the composition is delivered the hyperpigmented spot while minimizing delivery to skin surface not requiring treatment. It is recognized that localized application does allow for a reasonable amount of the composition to be applied to areas adjacent the hyperpigmented spot (*i.e.*, the composition is unlikely to be applied or to remain within the boundary of the hyperpigmented spot without some spreading). When applied locally, the form of the composition should be selected to facilitate localized application.

In some embodiments, the composition may be delivered by a variety of applicators that may be appropriate for localized and general application. The applicators are shown, by way of example, in Figures 1-3. A suitable applicator 10 may be a dropper 12 as shown in Figure 1 which is shown with a bottle 14 containing the composition. Figure 2 shows an applicator 20 as a wand 22 with a housing 24 containing the composition. The wand 22 may comprise a handle 26, a stem 27, and an applicator head 28. The applicator head 28 may comprise fibers, foam, cotton, or any other suitable material that may releasably hold the composition. Figure 3 shows an applicator 30 as a narrow-tip tube 32 with a body 34 and narrow dispensing tip 36. The composition is stored within the body 34 and dispensed through the pointed tip 36. Other applicators that can apply first composition locally to the hyperpigmented spot may also be used such as a cotton swab. Other suitable applicators include SH-0127 pen applicator available from Shya Hsin Plastic Works, Inc., Taiwan and either the Xpress Tip or liquid filled swab available from SwabPlus, Inc., China. The applicator may be configured to easily apply the composition to hyperpigmented spots having an approximate diameter between about 2 mm and about 10 mm and allowing for a dosed amount of the composition of between about 1 to about 50 $\mu\text{L}/\text{cm}^2$ or between about 1 to about 5 $\mu\text{L}/\text{cm}^2$. In another embodiment, the composition is applied to the one or more hyperpigmented spots and more generally to one or more facial skin surfaces contemporaneously (*i.e.*, over a period of less than 30 minutes or, more typically, less than 5 minutes).

While some methods described herein contemplate applying the compositions of the present invention with an applicator, it will be appreciated that applicators are not required and the compositions of the present invention can also be applied directly by using one's finger or in other conventional manners.

Suitable methods may comprise any one or more of the abovementioned steps. All of the aforementioned steps are applicable to both a single hyperpigmented spot as well as a plurality of

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hyperpigmented spots. Likewise, the exemplary methods that follow and are applicable to both a single hyperpigmented spot as well as a plurality of hyperpigmented spots.

In one embodiment, the method comprises the steps of applying a safe and effective amount of a *Laminaria Saccharina* extract to a hyperpigmented spot on a facial skin surface and
5 of applying a skin tone agent to the facial skin, wherein said tone agent is a vitamin B3 compound. The *Laminaria Saccharina* extract is applied for a period of time sufficient to improve the appearance of the hyperpigmented spot and the tone agent is applied for a period of time sufficient to improve skin tone.

In one embodiment, the method comprises the steps of applying a first composition
10 comprising a *Laminaria Saccharina* extract to a hyperpigmented spot or a plurality of hyperpigmented spots on a skin surface and of applying a second composition comprising a tone agent to the skin surface, before or after the first composition. The first composition is applied for a period of time sufficient to improve the appearance of the hyperpigmented spot and the second composition is applied for a period of time sufficient to improve skin tone. The first and
15 second composition may comprise one or more sunscreen actives, an anti-inflammatories, or optional components. Furthermore, the second composition may also comprise a safe and effective amount of *Laminaria Saccharina* extract. The second composition may be locally applied to the hyperpigmented spot or a plurality of hyperpigmented spots to which the first composition is applied or the second composition may be applied more generally to the skin
20 surface. Given the larger surface area to which the second composition may be applied compared to the localized treatment of the hyperpigmented spot, the dosed amount of the first or second composition may be between about 1 to about 50 uL/cm² per application (*i.e.*, per single application to the skin surfaces). In particular, when applied more generally, the skin surface is facial skin surface. The second composition can be applied to one or more of the forehead,
25 perioral, chin, periorbital, nose, and cheek skin surfaces. In another embodiment, the first and second compositions are applied contemporaneously to at least the cheek, forehead, and chin/perioral skin surfaces.

Another suitable method relates to regulating the impact of seasonal skin darkening. Seasonal skin darkening is the natural darkening (*i.e.*, tanning) that occurs seasonally due to
30 increased sunlight and UV exposure. Seasonal skin darkening can occur during a period having a specified start date and end date, wherein the length of day of the end date is longer than the length of day of the start date (with the provision that no day between the start date and end date

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is shorter than the start date). Compositions of the present invention may be used to help mitigate the effects of seasonal skin darkening.

A suitable method for regulating seasonal skin darkening comprises the step of applying a composition comprising a safe and effective amount of a *Laminaria Saccharina* extract and a tone agent to a skin surface on a start date having a daylight length and applying the composition at least daily until an end date having a daylight length, wherein the daylight length of the end date is greater than the daylight length of the start date with the proviso that no day between the start date and end date has a daylight length less than the start date.

10 IV. Test Methods

The following methods are provided to illustrate certain features and advantages of various embodiments of the invention and should not be construed as limiting the scope thereof.

A. Melanin Synthesis Assay

A B16-F1 mouse melanoma cell line is employed in the assay. The B16-F1 cells are obtained from American Tissue Culture Collection, Virginia, USA. The cell culture medium used in the assay comprises 500 mL of Dulbecco's Modified Eagle's Medium (DMEM), 50 mL Fetal Bovine Serum (FBS), and 5 mL of penicillin-streptomycin liquid. B16-F1 cells that are cultured in this medium and grown to greater than 90% confluency synthesize melanin. While not intending to be bound by any theory, it is hypothesized that the melanin synthesis is stimulated by the culture medium and/or stress induced by growth to a high confluency. The DMEM and FBS can be obtained from American Tissue Culture Collection and the penicillin-streptomycin liquid can be obtained from Invitrogen, Inc., California, USA. Equipment used in the assay include a CO₂ incubator, such as a Forma Series Model 3110 by Thermo Scientific, Massachusetts, USA; a Hemocytometer, such as a Bright Line model by Hauser Scientific, Pennsylvania, USA; and a UV-Visible Spectrum Plate Reader, such as a SpectraMax250 from Molecular Devices, California, USA. The assay steps include:

1. Day 0 - Cell Growth: Warm the cell culture medium to 37°C and place 29 mL into a T-150 flask. Add approximately 1×10^6 of B16-F1 passage 1 mouse cells to the T-150 flask and incubate for 3 days at 37°C, 5% CO₂, 90% relative humidity, until about 80% confluency.
2. Day 3 - Initiate a 96 Well Plate: At day 3, trypsinize the cells from the T-150 flask and determine the concentration of cells using the Hemacytometer. Initiate a 96 well plate

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with 2,500 cells per well in 100 uL of cell culture medium. Incubate the plate at 37°C, 5% CO₂, 90% relative humidity for 2 days until at least 20% to 40% confluent.

3. Day 5 – Remove the cell culture medium from the plate and replace with fresh culture medium (100uL per well). Add 1uL of Phlorogine diluted in water. Multiple dilution ratios may be tested in order to generate a dose response curve, wherein preferably three wells are treated with each dilution ratio. Controls comprise wells having the cell culture medium, B16-F1 cells, and the solvent (control #1); wells comprising the cell culture medium and the solvent (control #2); and optionally wells comprising the cell culture medium, solvent and [test compound] when necessary to control for the [test compound] background color (control #3).

4. Day 7 – Measure Melanin Production: Cells should have a confluency greater than about 80%. If not, this data point is not used. Add 100 uL of a 0.75% sodium hydroxide solution to each well. Read the 96 well plate using the UV-Vis Plate Reader at 410 nm to optically measure the amount of melanin produced between wells that are treated with [test compound] and control wells that are not. Wells in which melanin is produced appear brownish in color. Wells in which little melanin is produced appear clear to light purple in color. Percentage of melanin synthesis inhibition is calculated by the following equation:

$$\frac{100 - [\text{OD}_{410} \text{ Test Compound} - \text{OD}_{410} \text{ Control \#2}]}{(\text{OD}_{410} \text{ Control \#1} - \text{OD}_{410} \text{ Control \#2})} \times 100$$

Where OD₄₁₀ is the Optical Density at 410 nm as measured by the UV-Vis Spectrum Plate Reader.

When Control #3 is used, the formula for percentage melanin synthesis inhibition is:

$$\frac{100 - [\text{OD}_{410} \text{ Test Compound} - \text{OD}_{410} \text{ Control \#3}]}{(\text{OD}_{410} \text{ Control \#1} - \text{OD}_{410} \text{ Control \#2})} \times 100$$

Using generally the assay outlined above, melanin synthesis in Phlorogine treated B16-F1 cells was inhibited as compared to control cells as shown below in Table 1.

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Table 1

Phlorogine Concentration (w/v%)	1%	0.2%	0.04%	0.008%	0.0016%	0.000064%
approximate <i>Laminaria Saccharina</i> extract concentration	0.025%-0.01%	0.005%-0.002%	0.001%-0.0004%	0.0002%-0.00008%	0.00004%-0.000016%	0.0000016%-0.00000064%
Percentage melanin synthesis inhibition	48.1%	11.4%	5.5%	5.5%	-3%	-1.6%
Confluency (visual inspection)	>90%	>90%	>90%	>90%	>90%	>90%

B. *In Vivo* Testing for Hyperpigmented Spot Reduction and Melanin Evenness

A 9 week *in vivo* study was conducted using a round robin, vehicle controlled, split face design including a 1 week normalization period with 270 subjects. The 270 subjects were screened according to inclusion/exclusion criteria which included the following:

Inclusion

- Has hyperpigmented spots around cheek and/or periorbital area on both sides of the face.
- Has at least 1 hyperpigmented spot of 8-10 mm diameter, 4 spots of 4-6 mm or 10 spots of 2-3 mm diameter (sun spots, freckles, or melasma spots) or equivalent spot area in the cheek area on each side of their face.
- Is willing to refrain from sun exposure by using supplied UV lotion and physical UV blocks, such as a hat, to avoid facial sunburn, tanning or wind burn.

Exclusion

- Has been diagnosed as having atopy, eczema, psoriasis, or other chronic skin diseases.
- Has obvious signs of facial skin disease (e.g., more than 5 pimples, areas of red scaling skin, superficial thin blood vessels, etc.).
- Has significant areas of discoloration or scarring on the face.
- Has more than 3 prominent moles (> 3mm) on the face.

Two hundred and seventy subjects were recruited for the study. Approximately 60 subjects dropped during the course of the study.

Treatment Regimen – The regimen begins with a one week washout period. Each morning the subject is to wash her face with a suitable cleanser (e.g., Olay Purifying Mud Lathering Cleanser, available from The Procter & Gamble Company, Cincinnati, OH), gently dry

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with a towel, apply a stock moisturizer (*e.g.*, Vehicle as described in Table 2 with 3% glycerine, no panthenol, and 0.3% disodium EDTA) to the appropriate side of the face, wait 5 minutes, and then apply a UV blocking lotion (*e.g.*, Olay Natural White UV Moisturizing Lotion SPF 15, available from The Procter & Gamble Company, Cincinnati, OH). Each night the subject is to

5 wash her face with a suitable cleanser (*e.g.*, Olay Purifying Mud Lathering Cleanser, available from The Procter & Gamble Company, Cincinnati, OH), gently dry with a towel, and apply the stock moisturizer.

Each subject receives two coded test formulations for twice daily application to either the left or right side of the face. Each morning the subject is to wash her face with a suitable

10 cleanser (*e.g.*, Olay Purifying Mud Lathering Cleanser, available from The Procter & Gamble Company, Cincinnati, OH), gently dry with a towel, apply the test formulation to the appropriate side of the face, wait 5 minutes, and then apply a UV blocking lotion (*e.g.*, Olay Natural White UV Moisturizing Lotion SPF 15, available from The Procter & Gamble Company, Cincinnati, OH). Each night the subject is to wash her face with a suitable cleanser (*e.g.*, Olay Purifying

15 Mud Lathering Cleanser, available from The Procter & Gamble Company, Cincinnati, OH), gently dry with a towel, and apply the test formulation to the appropriate side of the face. Participants are to apply 0.5g of the appropriate test formulation on each side of the face. The test formulation should be applied with the fingers using gentle pressure and in a circular motion. Test formulations included a vehicle control, the vehicle + 1% Phlorogine, and the vehicle + 5%

20 vitamin B3. These test formulas are set forth in Table 2.

Table 2

	Vehicle	Vehicle + 1% Phlorogine	Vehicle + 5% Vitamin B3
Water	Q.S.	Q.S.	Q.S.
Phlorogine	---	1.000	---
Niacinamide	---	---	5.0000
Glycerin	10.0000	10.0000	10.0000
Isohexadecane	3.0000	3.0000	3.0000
Polyacrylamide(and)C13-14 Isoparaffin(and)Laureth-7 *A	2.0000	2.0000	2.0000
Dimethicone and Dimethiconol *B	2.0000	2.0000	2.0000
Isopropyl Isostearate	1.3300	1.3300	1.3300
Tocopheryl Acetate	0.5000	0.5000	0.5000
Panthenol	1.0000	1.0000	1.0000
Cetyl Alcohol	0.3200	0.3200	0.3200
Sucrose Polycottonseedate	0.6700	0.6700	0.6700
Cetearyl Glucoside/Cetearyl Alcohol *C	0.2000	0.2000	0.2000
Stearyl Alcohol	0.4800	0.4800	0.4800

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Behenyl Alcohol	0.4000	0.4000	0.4000
Polymethylsilsesquioxane *D	0.2500	0.2500	0.2500
Ethylparaben	0.2000	0.2000	0.2000
Propylparaben	0.1000	0.1000	0.1000
Disodium EDTA	0.1000	0.1000	0.1000
Benzyl Alcohol	0.4000	0.4000	0.4000
PEG-100 Stearate	0.1000	0.1000	0.1000

*A – Sepigel 305, available from SEPPIC, France.

*B – Dow Corning 1503 Fluid, available from Dow Corning, Midland, MI.

*C – Emulglade PL 68/50, available from Cognis GmbH, Germany.

*D – Tospearl 2000, available from Momentive Performance Materials, Albany, NY.

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Images of the facial treatment sites are captured at baseline (week 0), and after 4 and 8 weeks of treatment and analyzed for changes to skin color and spot size and color. Prior to image collection the participant's face is washed with the above referenced cleanser and allowed to dry (approximately 20 minutes). Images are collected of the right and left side of the participant's face. Images are collected using a digital camera (*e.g.*, Fuji F2 Pro digital SLR) equipped with a suitable lens for facial imaging (*e.g.*, 60mm Nikor lens). Images are saved in a suitable file format such as RAW format at a suitable camera resolution. Lighting is provided by a flash source (*e.g.*, 1000W strobe with color temperature of about 5600K). The camera and lighting are equipped with polarizing filters to reduce specular reflection. A fixed color chart captured in each images allowing for computerized color calibration and correct of the study images.

The image file is processed via algorithms to yield a grayscale concentration map of eumelanin. The algorithm analyses every pixel of the RAW image and calculates the concentration of eumelanin present. A suitable algorithm involves decompiling the red, green, and blue values for every pixel. The RGB values for each pixel are processed and compared to known standards to yield the melanin concentration. The melanin concentration for each pixel is assigned a grey scale value from 0 to 255. Upon recombining the pixel array, a parametric grayscale concentration map of eumelanin (and/or other chromophores such as oxyhemoglobin) is produced. Figure 4 depicts a full color image of a participant as collected. Figure 5 depicts a melanin concentration map of the same participant.

Suitable methods for image collection and melanin mapping via RGB algorithms are described in U.S. Patent Application Publication Nos. 2008/0075340A1 to Cotton et al. (published March 27, 2008) and 2007/0161910A1 to Preece et al. (published July 12, 2007). A description of chromophore mapping can also be found in Matts, P.J., *et al.*, "The Distribution of

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Melanin in Skin Determined *In Vivo*", British Journal of Dermatology, 156(4):620-628, April 2007.

Additional image analysis software, such as Optimas™ 6.5 (available from Media Cybernetics, Inc., Bethesda, MD), can be used to select a region of interest from the RAW image or from the resulting melanin map. A region of interest is selected to narrow the image to areas of the face where test formulation was targeted for application or to areas of the face of particular interest. For example, in the present study, a suitable region of interest of the facial skin surface includes the cheeks and periorbital region (*i.e.*, area bounded on one side approximately 1cm from hair line; bounded on the opposite side by a line parallel to the bridge of the nose along the upper edge and then along the nasolabial fold on the lower edge; bounded at the top along a line across the temple parallel to the upper eyelid and then approximately along lower edge of the orbital bone, and bounded at the bottom along a line parallel to the lower lip). The region of interest of the melanin map is further analyzed to calculate melanin spot area fraction and melanin evenness.

Another commercially available image collection and melanin mapping system is contact SIAscopy utilizing SIAscope V (available from Astron Clinica, Ltd., UK) or non-contact SIAscopy utilizing conventional digital imaging equipment (available from Astron Clinica, Ltd., UK). Another commercially available image collection and melanin mapping system is the VISIA® Complexion Analysis system utilizing the RBX™ technology (available from Canfield Scientific, Inc., Fairfield, NJ). The RBX™ technology transforms an image from RGB color into a RBX color-space where the red and brown channels represent hemoglobin and melanin distributions. The VISIA®/RBX™ is capable of spot area detection. See RBX™ Technology Overview White Paper, available at

<http://www.canfieldsci.com/FileLibrary/RBX%20tech%20overview-LoRz1.pdf>

or Canfield Imaging Systems, 253 Passaic Avenue, Fairfield, New Jersey 07004-2524.

Data from the melanin map are used to calculate Melanin Spot Area Fraction Percentage and Melanin Evenness. Melanin Spot Area Fraction Percentage (SAF) is calculated as the ratio of area occupied by melanin spots to the skin measurement area (*i.e.*, the region of interest) multiplied by 100. This percentage may be used to indicate size change of hyperpigmented areas. A lower percentage reflects smaller and/or fewer melanin spots.

Melanin Evenness is calculated as the standard deviation of the mean individual pixel grey scale value over the skin measurement area (*i.e.*, the region of interest). A lower value reflects more even melanin pigmentation. Further description of melanin spot area fraction and

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melanin evenness can also be found in Kinball, A.B., *et al.*, “Reduction in the appearance of facial hyperpigmentation after use of moisturizers with a combination of topical niacinamide and N-acetyl glucosamine: results of a randomized, double-blind, vehicle-controlled study”, British Journal of Dermatology, 162(2):435-441, February 2010.

- 5 Phlorogine was the best performer after 4 weeks, significantly ($p \leq 0.10$) reducing hyperpigmented spots better than the control and vitamin B3 compositions. After 8 weeks, the vitamin B3 composition was the best performer although the Phlorogine composition was also significantly better than the control at reducing hyperpigmented spots.

10 Table 3 summarizes the image analysis data, wherein “SAF” is the mean Spot Area Fraction and “ Δ SAF” is the mean change in Spot Area Fraction from an adjusted common baseline (week 0), and “ Δ SAF test leg - Δ SAF vehicle” is the difference of the mean change in Spot Area Fraction of the test formulation minus the mean change in Spot Area Fraction of the vehicle.

Table 3

	Week 0	Week 4				Week 8			
	SAF	SAF	Δ SAF	Δ SAF test leg - Δ SAF vehicle	P-Value ^{*1}	SAF	Δ SAF	Δ SAF test leg - Δ SAF vehicle	P-Value
Vehicle	12.111 ^{*2}	12.025	-0.085	---	---	12.693	0.582	---	---
1% Phlorogine		11.749	-0.361	-0.276	0.0159	12.372	0.262	-0.321	0.0064
5% Vitamin B3		12.000	-0.110	-0.026	0.8189	12.281	0.171	-0.412	0.0004

15 *1 – Statistically significant (Δ SAF test leg - Δ SAF vehicle) values in boldface. values in boldface.

*2 – Adjusted Common Baseline

Vitamin B3 was the best performer after an 8 week study of melanin evenness, significantly ($p \leq 0.10$) evening melanin better than the control and phlorogine compositions.

20 Table 4 summarizes the image analysis data, wherein “ME” is the mean Melanin Evenness and “ Δ ME” is the mean change in Melanin Evenness from an adjusted common baseline (week 0), and “ Δ ME test leg - Δ ME vehicle” is the difference of the mean change in Melanin Evenness of the test formulation minus the mean change in Melanin Evenness of the vehicle.

Table 4

	Week 0	Week 8			
	ME	ME	Δ ME	Δ ME test leg - Δ ME vehicle	P-Value ^{*1}
Vehicle	31.963 ^{*2}	32.378	0.415	0.000	---
1% Phlorogine		32.297	0.334	-0.081	0.3734
5% Vitamin B3		32.112	0.149	-0.266	0.0027

25 *1 – Statistically significant (Δ ME test leg - Δ ME) vehicle values in boldface.

*2 – Adjusted Common Baseline

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It is recognized that the SAF and ME for the vehicle and both test legs increased from baseline (week 0) to Week 8. This test was performed in Beijing, China from approximately January 21 to approximately March 21. In this time period, the length of day increases by approximate 2.5 hours. While a UV product was used during the test to reduce the impact of seasonal skin darkening, it is believed that the SAF increase from baseline to Week 8 is attributable to seasonal skin darkening. Seasonal skin darkening is the natural darkening (*i.e.*, tanning) that occurs seasonally due to increased sunlight and UV exposure. However, it is noted that phlorogine and naicinamide mitigated the effects of seasonal skin darkening when compared to the vehicle.

The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as "40 mm" is intended to mean "about 40 mm."

Every document cited herein, including any cross referenced or related patent or application, is hereby incorporated herein by reference in its entirety unless expressly excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

While particular embodiments of the present invention have been illustrated and described, it would be obvious to those skilled in the art that various other changes and modifications can be made without departing from the spirit and scope of the invention. It is therefore intended to cover in the appended claims all such changes and modifications that are within the scope of this invention.

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CLAIMS

What is claimed is:

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1. A method of improving the appearance of a hyperpigmented spot and improving skin tone, the method comprising the steps of:

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- a. applying a safe and effective amount, preferably between 0.00008% and 1.25% by weight of the composition, of a *Laminaria Saccharina* extract to a hyperpigmented spot on a skin surface, wherein the *Laminaria Saccharina* extract is applied for a period of time sufficient to improve the appearance of the hyperpigmented spot; and

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- b. applying a skin tone agent to the skin surface, wherein said tone agent is a vitamin B3 compound and wherein the tone agent is applied for a period of time sufficient to improve skin tone.

2. The method according to any one of the preceding claims further comprising the step of providing a composition comprising both the *Laminaria Saccharina* extract and the tone agent.

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3. The method of claim 2, wherein the composition further comprises a sunscreen active, an anti-inflammatory active, or combinations thereof.

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4. The method according to Claim 1 further comprising the steps of providing a first composition comprising the *Laminaria Saccharina* extract and providing a second composition comprising the tone agent.

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5. The method according to any one of the preceding claims, wherein the *Laminaria Saccharina* extract is applied to at least once or twice a day for at least four or eight weeks.

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6. The method according to any one of the preceding claims, wherein the improvement is a size reduction of the hyperpigmented spot, increased lightness of the hyperpigmented spot, or a reduction in melanin of the hyperpigmented spot.

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7. The method according to any one of the preceding claims, wherein the tone agent is applied to the skin surface at least once or twice a day for at least eight weeks.
- 5 8. The method according to any one of the preceding claims, wherein the improvement in skin tone is improved melanin evenness or a reduction in melanin.
9. The method according to any one of the preceding claims, wherein the *Laminaria Saccharina* extract is locally applied to the hyperpigmented spot by an applicator, preferably the applicator doses between about 1 to about 50 uL/cm² of the first
10 composition.
10. The method according to any one of the preceding claims further comprising the step of identifying a hyperpigmented spot on a skin surface, preferably by an imaging
15 device.
11. A method of regulating seasonal skin darkening, the method comprising the step of applying a composition to a facial skin surface at least daily from a start date to an end date,
20 wherein the composition comprises a *Laminaria Saccharina* extract and vitamin B3, and
wherein day length of the end date is longer than day length of the start date with the proviso that no day between the start date and end date is shorter than the start date.

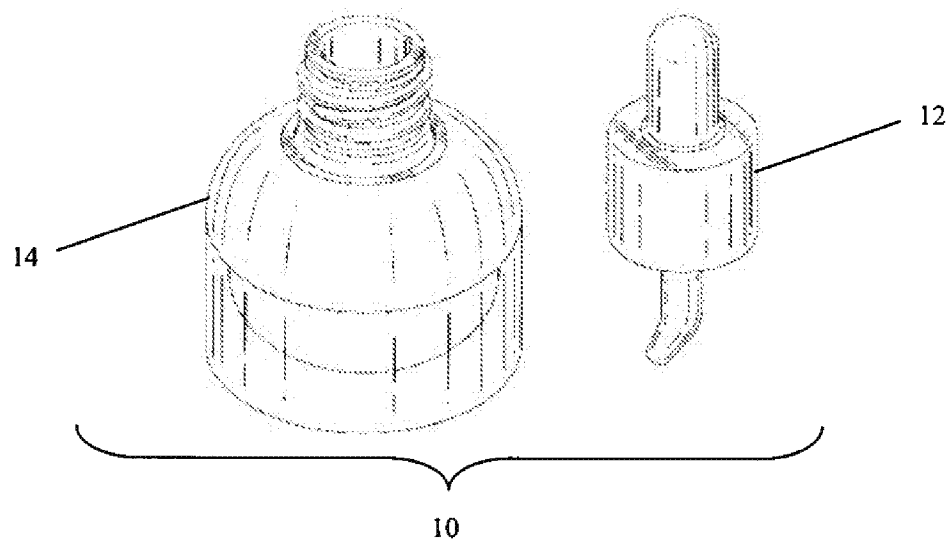


Fig. 1

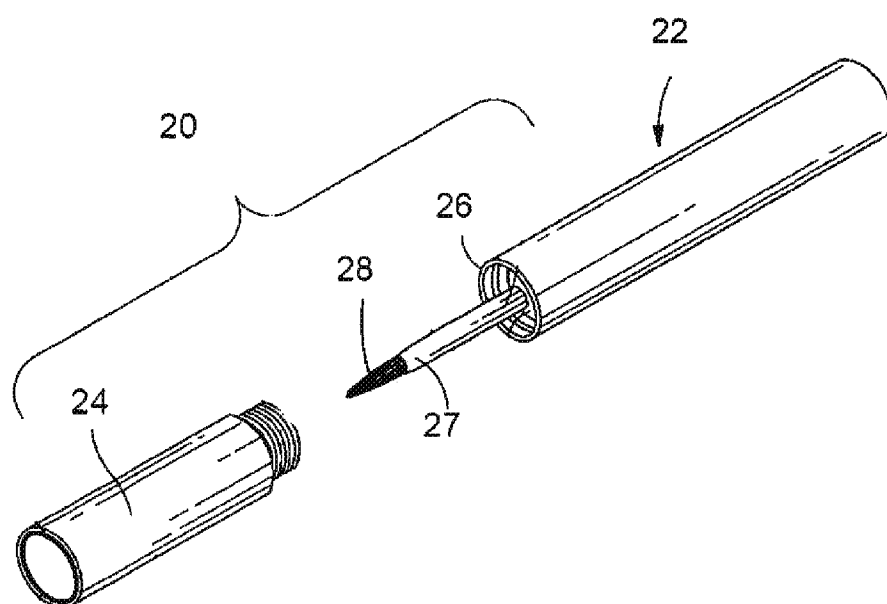


Fig. 2

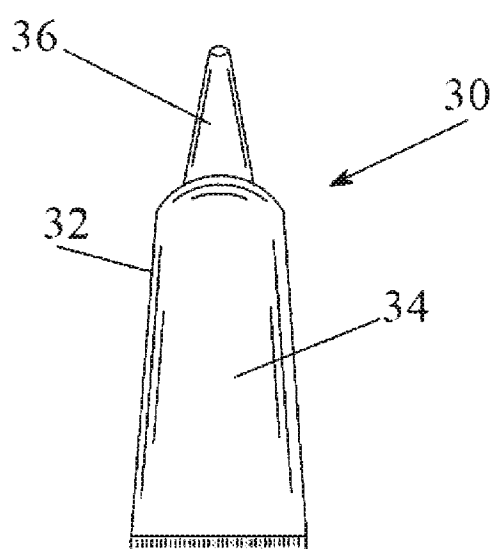


Fig. 3

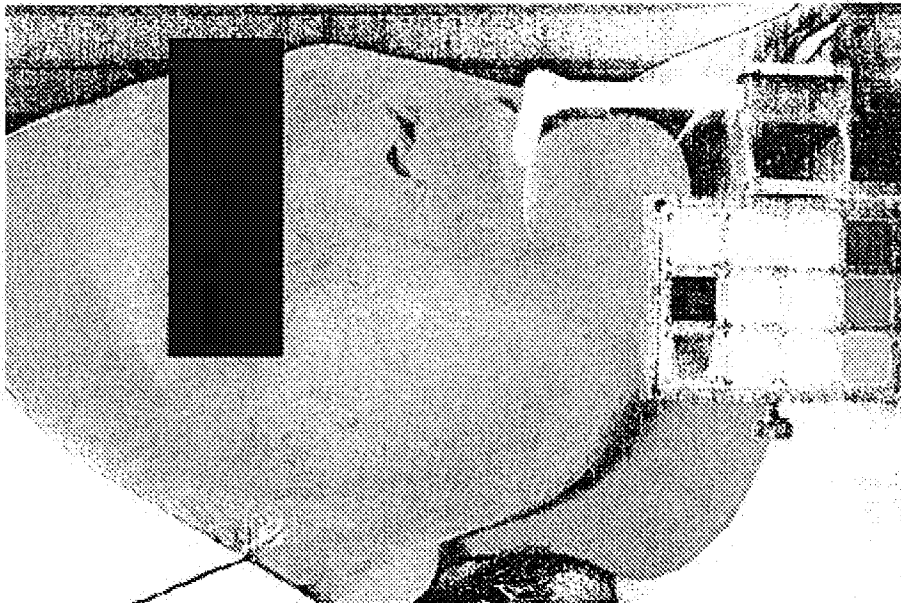


Fig. 5

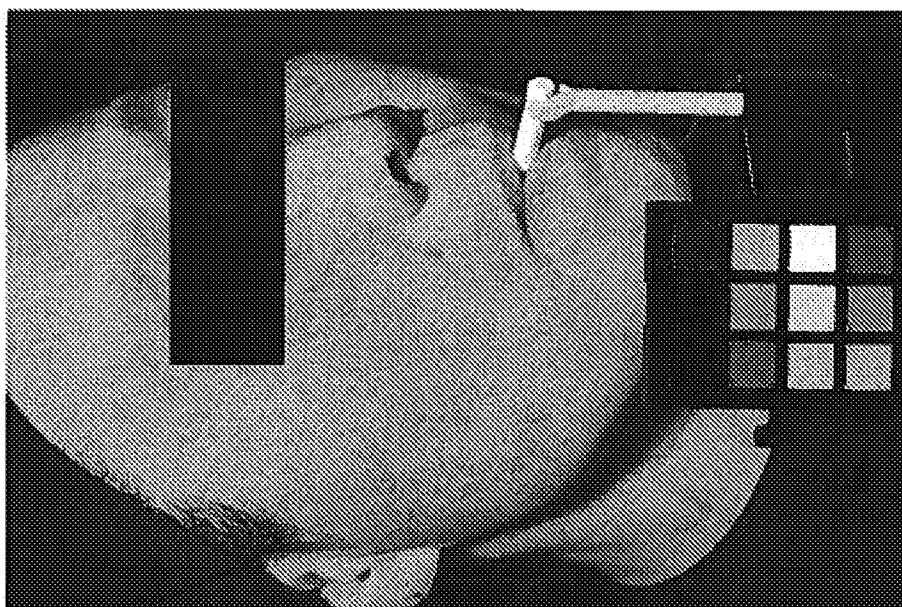


Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/042860

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K8/97 A61Q19/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2010/088225 A2 (PROCTER & GAMBLE [US]; HAKOZAKI TOMOHIRO [US]) 5 August 2010 (2010-08-05) page 5 - paragraphs 1,2 page 12, paragraph 4 - page 13, paragraph 3	1-11
Y	----- DATABASE GNPD [Online] Mintel; July 2008 (2008-07), anonymous: "Extra Brightening Moisture Lotion", XP002610254, retrieved from www.gnpd.com Database accession no. 940552 the whole document ----- -/--	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

10 March 2011

Date of mailing of the international search report

31/03/2011

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/042860

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE GNPD [Online] Mintel; April 2008 (2008-04), anonymous: "Extra Concentrated Brightening Essence", XP002610255, retrieved from www.gnpd.com Database accession no. 887317 the whole document -----	1-11
Y	DATABASE GNPD [Online] Mintel; July 2009 (2009-07), anonymous: "Brightening Moisture Gel Cream", XP002610256, retrieved from www.gnpd.com Database accession no. 1133334 the whole document -----	1-11
Y	DATABASE gnpd [Online] Mintel; August 2006 (2006-08), anonymous: "Moisture Milk Combination Oily to Oily Skin", XP002610257, retrieved from www.gnpd.com Database accession no. 573002 the whole document -----	1-11
Y	DATABASE gnpd [Online] Mintel; July 2008 (2008-07), anonymous: "Skin Shine Control Facial Foam", XP002610258, retrieved from www.gnpd.com Database accession no. 556086 the whole document -----	1-11
Y	DATABASE gnpd [Online] Mintel; September 2007 (2007-09), anonymous: "Bionic Serum 10", XP002610259, retrieved from www.gnpd.com Database accession no. 738557 the whole document -----	1-11
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/042860

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE gnpd [Online] Mintel; August 2005 (2005-08), anonymous: "Pore Minimizing Matifying Milk", XP002610260, retrieved from www.gnpd.com Database accession no. 384090 the whole document -----	1-11
Y	DATABASE gnpd [Online] Mintel; August 2004 (2004-08), anonymous: "Clear White Peel-Off T-Zone Mask", XP002610261, retrieved from www.gnpd.com Database accession no. 291044 the whole document -----	1-11
Y	DATABASE GNPd [Online] Mintel; April 2010 (2010-04), anonymous: "Toner", XP002610262, retrieved from www.gnpd.com Database accession no. 1309852 the whole document -----	1-11
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A	DATABASE gnpd [Online] Mintel; June 2010 (2010-06), anonymous: "Skin Lightening Body Milk", XP002610264, retrieved from www.gnpd.com Database accession no. 1348299 the whole document -----	1-11
A	DATABASE Gnpd [Online] Mintel; August 2004 (2004-08), anonymous: "SK-II Facial Whitening Mask", XP002610265, retrieved from www.gnpd.com Database accession no. 290213 the whole document -----	1-11
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/042860

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE gnpd [Online] Mintel; June 2009 (2009-06), anonymous: "Anti-Ageing Night Cream", XP002610266, retrieved from www.gnpd.com Database accession no. 1122741 the whole document -----	1-11
A	orangehrzn: "OIL CONTROL with Phlorogine - Smart Skin Care", 28 April 2005 (2005-04-28), pages 1-4, XP002610267, Retrieved from the Internet: URL:http://www.smartskincare.com/forum/vie wtopic.php?t=410 [retrieved on 2010-11-18] page 2 -----	1-11
A	US 2008/119527 A1 (BALDO FRANCINE [FR]) 22 May 2008 (2008-05-22) paragraph [0014] paragraph [0046] paragraph [0082] claim 1 claim 23 -----	1-11
A	Biotech Marine: "High-tech bioactive substances", 1 July 2009 (2009-07-01), pages 1-16, XP002610268, Retrieved from the Internet: URL:http://www.biotechmarine.com/admin/ima gescommunication/imagescatalogue/BiotechMa rine_Catalogue_2009.pdf [retrieved on 2010-11-18] pages 7,9,15 -----	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/042860

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010088225 A2	05-08-2010	US 2010189669 A1	29-07-2010
US 2008119527 A1	22-05-2008	EP 1900353 A1	19-03-2008
		FR 2905859 A1	21-03-2008