Example 2 Formula

(54) Title: HYDROQUINONE COMPOSITIONS FOR SKIN LIGHTENING

(57) Abstract: Compositions for lightening skin color (or for reducing skin darkening) and for repairing DNA damage are provided. The compositions comprise (a) one or more skin lightening agents (e.g., hydroquinone) in a combined amount effective to lighten skin (or to reduce darkening), (b) one or more DNA repair agents (e.g., one or more DNA repair enzymes in pH-sensitive liposomes) in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle. Methods for preparing and using the compositions are also provided.
Declarations under Rule 4.17:
— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
— of inventorship (Rule 4.17(iv))

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
HYDROQUINONE COMPOSITIONS FOR SKIN LIGHTENING

I. BACKGROUND OF THE INVENTION

A. Skin Lightening

Skin lightening, also called whitening, brightening, clarifying, fading or bleaching, is a major skincare product category around the world. These products are used to treat areas of hyperpigmentation which arise for many reasons. High pigment deposits in skin may be congenital, which we recognize in some cases as moles, or they may develop during growth and development as areas of uneven pigmentation. Melasma, a dark discoloration on the face with no apparent etiology, is most prevalent in young women with darker skin. Areas of hyperpigmentation may also arise during pregnancy under the influence of hormonal changes that affect pigment production. An especially widespread cause of hyperpigmentation is sun exposure, which causes freckling primarily in lightly pigmented skin, and tanning primarily in skin with darker tones. The sun also produces age spots, or solar lentigines, which are localized areas of hyperpigmentation that develop later in life after prolonged sun exposure. Another type of localized hyperpigmentation is called a liver spot and appears particularly on the hands of the elderly. It results from the sequestration of pigment debris, called lipofuscin, by phagocytic cells in the dermis. Post-inflammatory hyperpigmentation which appears after the inflammatory response subsides is a particular problem in people with darker skin color.

Hyperpigmentation is not painful or life-threatening but it causes psychological discomfort because it can be unsightly. It may also detract from personal feelings of beauty or attractiveness, and lower self esteem. It also runs counter to some cultural images of beauty or attractiveness, which emphasize unblemished, even-toned skin, and in some cases very pale or porcelain white skin.

Many treatments are in use for skin lightening, and they are reviewed in Skin & Aging, Suppl September 2005:1-22, as well as by Abramovits et al., Skinmed 4:371-
376, 2005. The most effective treatment for hyperpigmentation is topical application of 1,4-benzenediol, more commonly known as hydroquinone (HQ). Products containing HQ are widely used in the United States, with sales of approximately $50 million. Products with 2% HQ are classified as over-the-counter drugs and regulated by a monograph issued by the U.S. Food and Drug Administration. Products with 3% HQ, or more commonly 4% HQ, are sold as prescription drugs. Hydroquinone products are not approved for use in many countries in Europe because of concern about the potential for mutagenicity of HQ. Despite many years of use and widespread exposure to HQ, no studies in humans have shown an increased risk for any type of cancer among those using these products. The monobenzyl ether of hydroquinone is a severely toxic form of hydroquinone that produces irreversible depigmentation. Another compound in the phenol/catechol family of hydroquinone is N-acetyl-4-S-cysteaminylphenol.

Among the leading 4% HQ brand name products on the market today (with the distributor in parentheses) are TRI-LUMA (Galderma Laboratories), LUSTRA and LUSTRA AF (TaroPharma), CLARIPEL (Stiefel), GLYQUIN (Valeant), NUQUIN (Stratus) and EQUIQUIN MICRO (SkinMedica).

Some products contain active agents in addition to HQ to increase the skin whitening effect. Vitamins A (of the retinoid family) and C have been reported to inhibit pigmentation; TRILUMA contains prescription levels of retinoic acid; EPIQUIN contains both retinol and vitamin C, and US Patent 6,964,776 also describes combinations of HQ with retinol and vitamin C. Alpha hydroxy acids such as glycolic acid are reported to increase the desquamation of pigment granules and therefore speed depigmentation; LUSTRA contains glycolic acid and GLYQUIN contains glycolic acid as well as vitamins A and C. CLARIPEL contains an extract of Rumex occidentalis which is reported to inhibit the pigment producing enzyme, tyrosinase (see US Patent 6,521,267). Other lightening products suggested in the prior art include mulberry extract and kojic acid (see US patents 5,164,182 and 5,279,834), as well as azelaic acid, 4-hydroxyanisole, arbutin, glabridin, acrostaphylos plant extracts, melatonin, and vitamin C and its derivatives such as magnesium ascorbyl phosphate.

L-ergothioneine is an amino acid that is an antioxidant and a metal chelator. AGI Dermatics, the assignee of this application, disclosed to clients in 1997 that
concentrations of 20 to 200 micromolar (0.0005% to 0.005% w/v) inhibit tyrosinase and that these concentrations inhibit the UV-induced tanning response in mouse melanoma cells in culture. Some of those clients purchased L-ergothioneine (specifically, AGI Dermatic's THIOTAINE product) for use in skin whitening compositions manufactured in Japan. Both L-ergothioneine and hydroquinone are known in the cosmetic arts, and both are sometimes included in long lists of substances that may be added to compositions in this field (for examples see US patents 6,274,124, 6,846,812, 6,872,401, 6,964,954, and 6,974,583). U.S. patent publication 2005/0042233 discloses that ergothioneine may be used to stabilize hydroquinone and other oxygen-labile active agents in skin care products. US Patent 6,039,935 discloses various sunscreen formulations and contains a long list of "sunscreen agents," including hydroquinone. It gives 8 examples, each of which contains 0.1% ergothioneine and none of which contains hydroquinone as a sunscreen or in any other way. The patent contains no mention of DNA repair enzymes.

HQ, because it depigments the skin, increases sun sensitivity. This is of particular concern because this lowers the threshold for inducing the tanning response from further sun exposure, which undoes the effects of the HQ therapy. The additional active agents added to some of the HQ products, such as retinoids or glycolic acid, further increase sun sensitivity by thinning the outermost layer of skin, the stratum corneum. In fact, retinoic acid, retinol or glycolic acid, when formulated separately in anti-aging products, each carries a warning that the product increases sun sensitivity. The corticosteroid in TRILUMA further thins the skin, leaving it susceptible to sun damage.

The response to this problem for some HQ products is to add a sunscreen; see US Patents 4,136,166 and 6,699,464. For example, LUSTRA AF and CLARIPEL each contain a sunscreen. This is unsatisfactory because the products do not include enough sunscreen to be effective and, even if they did, people do not put enough HQ product containing sunscreen on the skin to afford significant sun protection. Indeed, neither LUSTRA AF nor CLARIPEL carries an FDA approved SPF number on the label.

Other HQ products simply recommend that sunscreens should be used in conjunction with the product.
B. DNA Repair Enzymes

Solar ultraviolet radiation (UVR) causes suppression of the immune system and impairs the ability of skin to respond to antigen presented to it in the first few days after exposure. DNA damage is known to be involved because it triggers the release by keratinocytes of immunosuppressive cytokines (reviewed by Yarosh et al in Methods 28: 55-62, 2002). Pigment and sunscreens attenuate the UVR that reaches the epidermis and the consequent damage. Once DNA damage has occurred, DNA repair enzymes can reverse the damage and thereby reduce or prevent immune suppression.

As one example, the effect of liposomal DNA repair enzymes on immune suppression was studied at the Department of Dermatology, Case Western Reserve University. Five subjects were recruited and randomly assigned to each test group. They were healthy volunteers, 18-60 years old, with readily sunburned Fitzpatrick skin types I-III, and with sensitivity to minimal erythema doses (MEDs) between 20-50 mJ/cm².

The test article consisted of a 1% ULTRASOMES (AGI Dermatics) formulation in a lotion vehicle. ULTRASOMES are comprised of a Micrococcus luteus extract containing DNA repair activity specific for UV damage to DNA. The extract was encapsulated in pH-sensitive liposomes. Sensitivity to pH imparts the liposomes with the ability to readily release the DNA repair enzymes upon entry into the interior of cells (US 5,190,762).

The MED for each volunteer was determined 17 days prior to the test date. Beginning three days prior to the test date, the test article was applied daily to a site on the upper arm and another site was left untreated. On the test date, the test article was again applied. Two hours later, the skin sites were exposed to solar simulating radiation (ssUVR) from a 1 KW xenon arc lamp at a dose of 75% of the volunteer’s MED. The test article was again applied immediately and 6 hours post-exposure.

Three days after the test date, the volunteers were sensitized to dinitrochlorobenzene (DNCB, 12.5 µg in 20 µl solution) at the sites of ssUVR exposure both treated and untreated by the test article. Fourteen days later (17 days after the test date) the sites were challenged with DNCB, and the following day (18 days after the test date) the contact hypersensitivity (CHS) reaction, which results in localized edema,
was measured by skin fold thickness. An increase in skin fold thickness represents the competent immunological response.

Unprotected ssUVR resulted in suppression of the CHS response by 65% compared to the response in unirradiated skin. Treatment with the lotion containing ULTRASOMES allowed for stimulation of the CHS response, despite the ssUVR exposure. Suppression of CHS was reduced to 35% at the ULTRASOME treated site, a statistically significant difference (p<0.05). The results demonstrate that treatment with an ULTRASOME formulation reduces UV-induced suppression of the contact hypersensitivity response in healthy human volunteers exposed to a sub-erythemal dose of ssUVR.

C. Hydroquinone Instability

The prior art has taught that HQ is difficult to stabilize against oxidation at pH values approaching neutral. Oxidation of HQ turns the product brown, which in small amounts makes the product unappealing and in large amounts compromises the product's efficacy.


This oxidation process [of hydroquinone] is affected by the pH of the product, by heavy metals and by oxygen. The pH of an aqueous solution of hydroquinone is 4.5. Stable preparations of hydroquinone can be prepared from pH 3.0 to 5.5, but the oxidation increases with increasing pH. Heavy metals serve to catalyze the oxidation process. Finally, oxygen available from entrained air during the manufacturing process may cause oxidation. Because light can cause solutions of hydroquinone to darken, it is best to manufacture hydroquinone-containing creams under subdued light.

In addition to keeping the pH below 5.5, Shevlin recommends an antioxidant system which is universally used in hydroquinone products, i.e., sodium sulfite combined with sodium metabisulfite.

US Patent No. 6,964,776 asserts that a combination of sodium metabisulfite and magnesium ascorbyl phosphate as antioxidants allows formulation of HQ in the pH range of 6.0 to 7.5. While this patent shows data for stabilization of HQ with each of these agents separately, no data is presented that demonstrate their stabilizing activity
when used together. As shown in Example 1 below, this combination of antioxidants was found not to produce a stable composition. US patent publication 2005/0042233 refers to combining ergothioneine with HQ, but provides no stability data for the HQ and no information on a desirable pH range.

II. SUMMARY OF THE INVENTION

In accordance with a first aspect, the invention provides a method for lightening skin color and repairing DNA damage by ultraviolet light comprising applying a topical composition to skin of a person in need of skin lightening wherein the composition comprises (a) one or more skin lightening agents (e.g., hydroquinone) in a combined amount effective to lighten skin and (b) one or more DNA repair agents (e.g., one or more DNA repair enzymes encapsulated in liposomes, e.g., pH-sensitive liposomes) in a combined amount effective to repair DNA damage by ultraviolet light.

In accordance with a second aspect, the invention provides a method for reducing skin darkening and repairing DNA damage by ultraviolet light comprising applying a topical composition to skin of a person in need of a reduction in skin darkening wherein the composition comprises (a) one or more skin lightening agents (e.g., hydroquinone) in a combined amount effective to reduce skin darkening and (b) one or more DNA repair agents (e.g., one or more DNA repair enzymes encapsulated in liposomes, e.g., pH-sensitive liposomes) in a combined amount effective to repair DNA damage by ultraviolet light.

In accordance with the above aspects, the topical composition can further comprise L-ergothioneine, an anti-inflammatory agent and/or an anti-nociceptive agent.

In accordance with a third aspect, the invention provides a method for preparing a skin lightening composition comprising:

(a) preparing an aqueous solution comprising hydroquinone;
(b) adding ergothioneine (e.g., L-ergothioneine) to the aqueous solution of step (a); and
(c) adding one or more lipid-containing components (e.g., pH-sensitive liposomes) to the aqueous solution of step (b).

This sequence of steps allows the ergothioneine to be oxidized by the hydroquinone prior to the addition of the lipid-containing components so that the
hydroquinone is reduced and will not attack (oxidize) the lipids in those components when they are added.

In accordance with a fourth aspect, the invention provides a composition for lightening skin color and repairing DNA damage by ultraviolet light comprising (a) one or more skin lightening agents (e.g., hydroquinone) in a combined amount effective to lighten skin, (b) one or more DNA repair agents (e.g., one or more DNA repair enzymes encapsulated in liposomes, e.g., pH-sensitive liposomes) in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle.

In accordance with a fifth aspect, the invention provides a composition for reducing skin darkening and repairing DNA damage by ultraviolet light comprising (a) one or more skin lightening agents (e.g., hydroquinone) in a combined amount effective to reduce skin darkening, (b) one or more DNA repair agents (e.g., one or more DNA repair enzymes encapsulated in liposomes, e.g., pH-sensitive liposomes) in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle.

In accordance with the fourth and fifth aspects, the composition can further comprise L-ergothioneine, an anti-inflammatory agent and/or an anti-nociceptive agent.

In accordance with a sixth aspect, the invention provides a composition for topical application to skin comprising:

(a) hydroquinone,
(b) ergothioneine, and
(c) a cosmetically or pharmaceutically acceptable vehicle,

wherein the composition has a pH which is greater than or equal to 6.2 and less than 6.8, said pH being measured in the absence of any ingredients which substantially interfere with the measurement of pH (e.g., in the absence of any silicone-containing ingredients which are known to interfere with the measurement of pH).

In accordance with each of the above aspects of the invention, the topical composition preferably does not include a cationic salt of an acidic ascorbyl ester (e.g., magnesium ascorbyl phosphate).

Additional features and advantages of the invention are set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art.
from that description or recognized by practicing the invention as described herein. It is to be understood that both the foregoing general description and the following detailed description are merely exemplary of the invention and are intended to provide an overview or framework for understanding the nature and character of the invention. It is also to be understood that the various aspects and features of the invention disclosed in this specification can be used in any and all combinations.

III. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows photomicrographs of human skin equivalents illustrating the effects of treatment with the lotion of Example 2 (FIG. 1B) and a commercial lightening lotion (TRI-LUMA; FIG. 1C). FIG. 1A shows the control (untreated) case.

IV. DESCRIPTION OF THE PREFERRED EMBODIMENTS

Without limiting the present invention by any theory or mechanism of action, the following specification details its preferred embodiments. The concentrations referred to herein are in weight percent of the composition unless otherwise indicated.

As will be evident from the foregoing, various aspects of the invention address the problem that agents that lighten the skin by their very nature increase the sensitivity of the skin to sunlight. They thereby increase the risk of the detrimental effects of the sun, which include tanning (which undoes the lightening effects), photoaging, and skin cancer. The problem is exacerbated by the fact that several popular agents chosen to enhance the lightening effects of hydroquinone, such as, retinoids and glycolic acid, further increase the sensitivity of the skin to sunlight by thinning the uppermost stratum corneum layer. The only approach used to date to reduce sun sensitivity is to add sunscreens to the formulation or recommend that patients concomitantly use sunscreens.

In accordance with certain aspects of the invention, it has been discovered that by combining one or more lightening agents with one or more DNA repair agents capable of repairing DNA damage by ultraviolet light, a lightening effect can be achieved along with a reduction in the accompanying sun sensitivity. In particular, the use of DNA repair enzymes encapsulated in liposomes as the DNA repair agent offers a particularly effective and practical approach to reducing sun sensitivity. This is particularly apparent at doses of UV less than that required to produce sunburn.
Significantly, the prior art teaches away from the application of liposomal DNA repair enzymes in skin lightening formulas because these enzymes are useful in compositions that increase pigmentation in the skin (US Patent 5,352,458). With regard to this patent, it should be noted that the tanning response described therein results from actively exposing the skin to enough UV radiation to get a natural tanning response; it is only then that the application of the DNA repair enzyme triggers more tanning. However, if this threshold is not reached then, as disclosed herein, the DNA repair enzyme can serve the beneficial function of protecting lightened skin from UV damage and does not result in undoing of the lightening effect.

As one particular, non-limiting example, a combination of hydroquinone (as a primary lightening agent) and L-ergothioneine with one or more liposomal DNA repair enzymes, e.g., the cyclobutane pyrimidine dimer (CPD) glycosylase from Micrococcus luteus, and 8-oxo-guanine glycosylase 1 (OGG1) from Arabidopsis thaliana, can accomplish the two goals of lightening skin color and reducing the accompanying sun sensitivity.

Other aspects of the invention address the problem of formulating a topical medication that maintains the stability of hydroquinone while gaining the advantages of DNA repair enzymes in pH-sensitive liposomes. To date, liposomal DNA repair enzymes have not been used to reduce sun sensitivity caused by HQ skin lightening formulations because HQ oxidizes lipids. This would lead to the breakdown of liposomes.

Moreover, an HQ product having DNA repair enzymes in pH-sensitive liposomes requires a pH above 5.0 (preferably, above 6.0) for preservation of the liposomes. Yet, as discussed above, the art teaches that HQ is most stable at a low pH. Accordingly, the art would view HQ and pH-sensitive liposomes as fundamentally incompatible. In accordance with certain aspects of this invention, this incompatibility is addressed and compositions having higher pH's are achieved. This is beneficial not only in formulations employing pH-sensitive liposomes but more generally since for some subjects, HQ administrated in an acidic pH vehicle is irritating to the skin.

Administration of HQ at a more neutral pH can be helpful to these subjects.

Skin lightening and/or a reduction in skin darkening is achieved through the use of one or more lightening agents to reduce pigmentation of the skin. As used herein,
the phrase a "skin lightening agent" is an agent that reduces existing color or reduces an increase in color of human skin when topically administered. The skin lightening agent(s) may work by any one, or a combination of means, such as by inhibiting the signaling of melanocytes to make melanin, e.g., by inhibiting the release of α-melanocyte stimulating hormone, or by inhibiting the biochemical pathways of melanin production, e.g., by inhibiting tyrosinase. Further, the agent may inhibit the production or transfer of melanosomes from melanocytes to keratinocytes or the uptake of melanosomes by keratinocytes. The agent may also increase the loss of melanosomes from the skin, by, for example, increasing cell turnover or thinning the epidermis. It may also directly bleach the melanin pigment to lighten the color. The amount of lightening agent in the composition will depend upon the agent used and typically will range from about 0.001% to about 20% (e.g., from about 0.01% to about 10%).

A potent lightening agent is hydroquinone (HQ), which may be used in the concentration range of 1% to 12%. In one preferred embodiment, hydroquinone is used in the range of about 2% to 4%. When used at 2% in the U.S. the formulation must follow the regulations of the FDA 21 CFR Part 358. Formulations with 5% HQ can cause irritation and contact dermatitis with prolonged use for some individuals. Derivatives of HQ have also been used for lightening, such as the tertiary butyldimethyl ether of hydroquinone and the monotertiary butylmonomethyl ether of hydroquinone.

Other agents that have been used to accelerate depigmentation of the skin and can be used as skin lightening agents in the practice of the invention include *Acrostaphylos* plant extracts, alpha hydroxy acids, beta hydroxy acids, poly hydroxy acids, arbutin, ascorbic acid (at high concentrations) and its salts and derivatives, ascorbyl palmitate, magnesium ascorbyl phosphate, tetrahexyldecyl ascorbate, azelaic acid, ergothioneine in the L- form or racemic mixture, glabridin, 4-hydroxyanisole, licorice extract, melatonin, mulberry extract, retinoids such as retinol, retinol palmitate and retinoic acid and *Rumex occidentalis* extract. These agents can be used in combinations, including in combinations with HQ.

Another embodiment of the invention uses ergothioneine to both stabilize the formula and increase the lightening effect. In terms of stability, the ergothioneine can be L-ergothioneine, D-ergothioneine, or a racemic mixture of the two forms. In terms of skin lightening, the ergothioneine is preferably L-ergothioneine. US 6,964,776
asserts that a combination of sodium metabisulfite (most preferably 0.1%) and magnesium ascorbyl phosphate (most preferably 0.5%) stabilize a 4% hydroquinone formula pH 6.5, although no examples of such effect are shown in the patent. However, as shown by the stability assay of Example 1, this combination still permits the extensive oxidation of HQ. Addition of 0.3% ergothionine to this mixture reduces but does not eliminate HQ oxidation. As illustrated in Example 1, it has been unexpectedly found that replacing the magnesium ascorbyl phosphate with 0.3% ergothionine virtually eliminates oxidation of HQ. As discussed above, the order in which ergothionine is added to the composition is important in achieving its antioxidative effects for HQ and other active agents. This order is used in Example 2 below.

Ergothionine (e.g., L-ergothionine) may be used in the range of about 0.03% to 10%. In practice, it has been found that concentrations above 0.03% increase the stability of hydroquinone in the composition, especially at above pH 6.0. In order to effectively depigment skin, L-ergothionine should be present in concentrations above 0.1%. Because of its expense, concentrations above 1% are impractical. In a preferred embodiment, L-ergothionine is used in the range of about 0.03% to 0.5% and most preferably about 0.3%.

As used herein the phrase "DNA repair agent" means an agent now known or subsequently developed which repairs DNA damage, i.e., an agent that removes or directly assists in the removal of unnatural sugars, bases, or phosphates from DNA. DNA repair may be achieved by enzymes that biochemically act on damaged DNA or by agents and methods that stimulate natural DNA repair responses. DNA repair enzymes that biochemically act on DNA include the photolyases (which are dependent for activation on exposure to light), the dark repair UV endonucleases such as found in Micrococcus luteus or in the bacteriophage T4 (T4 endonuclease V), 8-oxo-guanine glycosylase (OGG1) from Arabidopsis, Ultraviolet Damage Enzyme (UVDE) from yeast, and others now known or that may be discovered. These enzymes need to enter the cells of the skin by means of a delivery vehicle that traverses the stratum corneum, allows uptake into the living cells and releases the enzyme so that it can travel to the nucleus. This functionality can be achieved by encapsulating the DNA repair enzyme(s) (or other DNA repair agent(s)) in liposomes as discussed in, for example,
U.S. Patent No. 5,190,762. The preferred DNA repair liposomes are pH-sensitive liposomes. Preferred lipids for such liposomes are phosphatidylethanolamine, phosphatidylcholine, oleic acid and cholesteryl hemisuccinate.

Agents other than repair enzymes that are reported to enhance natural DNA repair responses and can be used in the practice of the invention include UNIPERTAN (composed of tyrosine, riboflavin, and collagen), niacinamide, niacin and its derivatives, Cat's Claw (*Uncaria tomentosa*), and various dinucleotides and oligonucleotides, some of which contain dTpT or sequences homologous to the telomerase binding sequence.

The amount of DNA repair agent in the composition will depend upon the agent used and typically will range from parts-per-billion to about 20% (e.g., from parts-per-million to about 5%). In one embodiment the composition comprises from about 50 nanograms/ml to about 10 milligrams/ml of DNA repair enzyme (e.g., purified T4 endonuclease V) or of an extract which contains DNA repair enzyme (e.g., an extract prepared from *Micrococcus luteus*) in the final composition as applied to the skin. The liposomal DNA repair enzymes may be used in protein concentrations in the final composition from about 0.01 microgram per ml to 10 milligrams per ml, depending on the particular purity and activity of the DNA repair enzymes. For a UV endonuclease extract prepared from *Micrococcus luteus*, the preferred protein concentration is about 1 milligram per ml, while the preferred protein concentration for T4 endonuclease V is about 1 microgram per ml. The preferred protein concentration for the *Arabidopsis thaliana* OGG1 glycosylase prepared from an extract of recombinant *E. coli* is about 50 micrograms per ml.

The base of the lotion may be made from many substances known in the pharmaceutical and cosmetic arts. These include silicones such as dimethicone, cyclopentasiloxane, phenyl trimethicone, hydrogels such as CARBOPOL 940, CARBOPOL 941, or mixtures of sepiogel polyacrylamide with isoparafin with laureth-7. The concentration of these bases may be adjusted to achieve a viscosity that is pleasant and allows application of a smooth film to the surface of the skin. The base lotion may be composed of an emulsion, such as an oil-in-water emulsion, or a water-in-oil emulsion, or a water-in-oil-in-water emulsion, or similar systems known in the art. Salts may be added to the water phase, such as sodium citrate or linoleic acid, to
optimize the osmolarity, the pH and/or improve the sensory feel. An example of a preferred embodiment of a base lotion is shown in Example 2.

The pH may be adjusted over a wide range, from approximately pH 3 to pH 12. Lowering the pH below 6.8 increases the stability of hydroquinone and lessens the dependence on other components of the formula to reduce or prevent its oxidation. However, when hydroquinone is combined with DNA repair liposomes that are sensitive to pH, such as ULTRASOMES (liposomal UV endonuclease; AGI Dermatics) and ROXISOMES, also known as ROXLIPOSOMES, (liposomal OGG1; AGI Dermatics), it is important that the pH is maintained at greater than 6.0, and most preferably at pH 6.2. The pH must be properly measured, since some components like silicone can interfere with standard pH measurement techniques. The pH must also be measured taking into account the mixing of sequence 2 ingredients with sequence 3 ingredients (see Example 2), since sequence 2 alone has a pH below 5.0, while the combination has a pH of 6.2.

Unexpectedly, the stability of HQ in the formula in Example 2 is extremely sensitive to pH. This formula may be identically prepared except for a change in step 2 of the manufacturing procedure of adjusting the pH of the sequence 2 ingredients to 6.8 using triethanolamine, which has a strong buffering capacity, rather than leaving those ingredients at their natural pH of 4.8. The final formula resulting from the sequence 2 ingredients whose pH has been adjusted to 6.8 looks identical to one whose pH has not been adjusted. DNA repair liposomes added to either one remain stable for many months, as determined by examining the formulas by scanning transmission electron microscopy. Both the original and the pH-adjusted formulas when filled in bottles remain white for 30 days at 40°C. However, the non-adjusted formula is stable for more than 5 months at room temperature in a glass jar with air, while the formula adjusted to pH 6.8 begins to turn brown at the top of the solution stored for just 2 months in a glass jar with air.

Lipids may be added to the formula as liposomes and as oil-in-water or water-in-oil emulsions or double emulsions. As discussed above, when DNA repair liposomes are used, the preferred lipids are phosphatidylethanolamine, phosphatidylcholine, oleic acid and cholesteryl hemisuccinate. Other lipids that may be included in the formulation are LECINOL S-10 at about 0.45%, and LIPOMULESE
165, composed of glyceryl stearate and PEG 100 stearate, at about 3.2%. In addition, vitamins may also be added. Typical vitamins that can be used are Vitamin E (tocopherol) acetate at about 0.75%, tocopherol at about 0.05%, ascorbic acid (Vitamin C) at about 0.1%, ascorbyl palmitate at about 1.25%, retinol at about 0.5% or VITAIN (retinol in liposomes; AGI Dermatics) at about 2.5%. Alcohols can have favorable effects on the feel of such a formula. Alcohol compounds that may be used are benzyl or cetyl alcohol at about 2.75%, COSMOWAX (cetearyl alcohol and ceteareth-20) at about 1.5% or SDA 40-20 at about 3%.

Hydroquinone produces a skin irritation in certain people. Accordingly, anti-inflammatory agents and/or anti-nociceptive agents may be added to the formula. These include steroids, corticosteroids, non-steroidal anti-inflammatories, bisabolol (the active component of chamomile), *Evodia* extract, lichocalcone, sea whip extract, indomethacin, prostaglandin inhibitors, or aspirin. In particular extracts of *Evodia rutaecarpa* that contain indole quinazoline alkaloids or purified evodiamine or rutaecarpine or limonene may be used. In a preferred embodiment EVODIOX (AGI Dermatics) is added at concentration of 0.004% - 50%, most preferably at 1.0%.

Any number of fragrances known in the art may be added to the formula, such as MAIDA-J-9145 at about 0.03%. Preservatives may be added to prevent microbial growth, such as 1% phenoxyethanol, 0.05% butylated hydroxytoluene, or 0.75%

OPTIPHEN plus.

Sunscreens may be added to increase sun protection. Such sunscreens include p-aminobenzoic acid (PABA), avobenzone, cinoxate, dioxybenzone, ensulizone, homosalate, menthol anthranilate, mexoryl SX and XL, octocrylene, octyl dimentyl PABA, octyl methoxycinnamate, octyl salicylate, oxybenzone, sulisobenzone, titanium dioxide, trolamine salicylate zinc oxide, and as yet unapproved sunscreens (i.e., sunscreens not yet approved by the FDA for listing as sunscreens but otherwise considered safe for use in cosmetic and pharmaceutical products) such as isoamyl methoxycinnamate, 4-methylbenzylidene camphor, octyl triazone bis-ethylhexyloxyphenol methoxyphenol triazine and methylene bis-benzotriazolyl
tetramethylbutyphenol.

The products of the present invention may be used by men or women of any age, but because pigmentation changes from childhood into adulthood and in view of
general health concerns associated with medicating children, it should, in general, preferably be used by people over 18 years of age. However, exceptions should be considered for reasons of abnormal pigmentation in childhood due to accident, injury with post-inflammatory hyperpigmentation or congenital defects. All racial groups may benefit from the use of the present invention, but the group that will experience the greatest benefit will be those who are normally lightly pigmented but who have areas of darker discoloration.

The products of the present invention can be applied anywhere on the body, but care should be taken near the eyes and mucous membranes because of the potential for irritation. The product can be applied in any amount that can be readily absorbed but preferably as a thin layer. The product should be applied preferably on a limited site, and no more than necessary to cover the hyperpigmentation. The product is preferably pretested on a small patch of skin for 24 hours to determine if the user has a potential for an allergic reaction.

The products of the present invention may be applied any time, preferably once per day at night, or twice daily in the morning and night. The period of application can be for any length of time, but because of the potential for irritation, it is recommended that the treatment be discontinued after 24 weeks. In many cases, a mild irritation develops after a few weeks that is followed by the lightening effect. Treatment should be discontinued if a more serious inflammation develops.

A person who is using a product of the present invention should avoid sun exposure that can provoke tanning or any type of irritant that can trigger repigmentation of the treated region. For example, the treated site on the skin is preferably always protected from sun exposure with a sunscreen, preferably a sunscreen with at least an SPF of 30 applied according to the directions of the manufacturer.

As discussed above and illustrated by the examples presented below, a preferred embodiment of the invention comprises a combination of hydroquinone (as a primary lightening agent) and L-ergothioneine with one or more liposomal DNA repair enzymes, e.g., the cyclobutane pyrimidine dimer (CPD) glycosylase from Micrococcus luteus, the CPD glycosylase T4 endonuclease V from bacteriophage T4, and/or 8-oxoguanine glycosylase 1 (OGG1) from Arabidopsis thaliana. The hydroquinone may be in the concentration of about 1 to 12%, but more preferably is in the range of about 2%
to 5% (most preferably 2% to 4%). The L-ergothioneine may be in the range of about
0.02% to 1%, but is more preferably in the range of about 0.045% to 0.5% and most
preferably about 0.3%. The one or more DNA repair enzymes encapsulated (either
separately or in combination) in pH-sensitive liposomes may be used in protein
concentrations in the final formula (combined protein concentration where more than
one DNA repair enzyme is used) from about 0.01 microgram per ml to 10 milligrams
per ml, depending on the particular purity and activity of the DNA repair enzyme or
enzymes. For a UV endonuclease extract prepared from Micrococcus luteus, the
preferred concentration is about 1 milligram per ml, while the preferred concentration
for T4 endonuclease V expressed in E. coli by bacteriophage or expressed by
recombinant E. coli, is about 1 microgram per ml. The preferred concentration for the
Arabidopsis thaliana OGG1 glycosylase prepared from an extract of recombinant E.
coli is about 50 micrograms per ml. In order to make use of a pH-sensitive liposome
delivery system for these DNA repair enzymes, the formula should be maintained at a
pH greater than 5.0 (preferably, greater than 6.0).

Without intending to restrict in any way the scope of the invention, the
following examples are presented to illustrate several of the invention’s aspects and its
use.

**Example 1.**

**Ergothioneine for Stabilization of a Hydroquinone Formula at pH 6.5**

To test the stability of a 4% hydroquinone solution, a 100 ml solution was
prepared of 0.18% sodium citrate and 4% hydroquinone, pH 6.5. To 10 ml aliquots of
this solution was added 0.25% sodium metabisulfite or 0.5% magnesium ascorbyl
phosphate, or 0.3% L-ergothioneine or various combinations. The aliquot was placed
in an orbital shaker with an air temperature of 40°C for 24 hrs, and then shaken for an
additional 24 hours. During the incubation 1-ml aliquots of the samples were removed
and the optical density at 475 nm was read in a spectrophotometer, with a final reading
at 48 hrs. Under these conditions, hydroquinone oxidized and turned dark brown which
can be measured by reading the optical density at 475 nm in a spectrophotometer.

To compare the ability of antioxidants to stabilize hydroquinone, the OD475 of
the unprotected 4% hydroquinone solution is set to 100% and the OD475 of each sample
is compared to it. The results are shown in Table 1.
As can be seen in this table, at a pH of 6.5, hydroquinone is easily oxidized. The addition of 0.1% sodium metabisulfite and 0.5% magnesium ascorbyl phosphate, as described in US 6,964,776, reduces the oxidation to 69% of control but does not eliminate it. Adding 0.3% L-ergothioneine to the mixture further reduces the oxidation to 40% of control. However, substituting 0.3% L-ergothioneine for the 0.5% magnesium ascorbyl phosphate reduces the oxidation of hydroquinone to 5%.

**Example 2.**

*A lotion comprising hydroquinone, L-ergothioneine.*

**DNA repair liposomes and *Evodia* biomimetic**

Table 2 sets forth a formulation for a 4% hydroquinone product which contains DNA repair liposomes (ULTRASOMES and ROXISOMES) and which can be readily prepared by those skilled in the art. The formulation remains within specifications for at least 30 days at 45° C.

The following order of procedures for manufacture of the composition was used so that HQ is reduced by the addition of L-ergothioneine to the sequence 2 mix prior to being exposed to the pH >6 oxidizing environment created by addition of the sequence 3 components, and so that the reduced HQ does not oxidize lipids in the sequence 3 DNA repair liposomes (i.e., ULTRASOMES and ROXISOMES).

**Manufacturing Procedure:**

1. In main kettle, combine Sequence #1 ingredients (oil phase ingredients) and mix until uniform.
2. In side kettle, combine Sequence #2 ingredients (aqueous phase ingredients), and heat to 40°C.
3. Very slowly add Sequence #2 to Sequence #1 and mix well. The rate of addition determines emulsion stability and viscosity. A lab scale batch may be made at a rate of addition of 2.5 to 3.0 grams per minute and a commercial batch can be made at an addition rate of 1.5 to 1.7 liters/minute.
4. Slowly add Sequence #3 ingredients, one at a time, mixing well after each addition.
5. Switch to a Silverson mixer, and mix for 1 – 3 minutes at approximately 2500 – 3500 rpm.
Specifications:  
Color: White
Hydroquinone: 4.0% ± 0.5%

**Example 3.**

*A lotion comprising hydroquinone, L-ergothioneine, DNA repair liposomes and* Evodia* biomimetic is stable for commercial purposes*

A formula was prepared as described in Example 2 and filled in 30 ml airless pump bottles. The bottle comprised two sub-assemblies: Sub-assembly A: a barrel, a piston, and a bottom and Sub-assembly B: a non-removable pump (crimp-on), a cap, an actuator and an overcap. The system operates with a combination of a non-vented pump and a deformable container. The pump does not let air into the container and each dose that is dispensed builds a vacuum inside the container. The vacuum is balanced by a reduction of the volume of the container by an upward movement of the piston. The vacuum filling technology requires equipment which performs three functions *in vacuo*: evacuation of air from the product, filling of the barrel with product, and crimping on the pump, thus protecting the formulation from exposure to air. The pump dispenses a metered dose of 1 ml. The filling equipment is available from Valois of America (Congers, New York).

The formula was dispensed from a bottle immediately after filling and analyzed for color and hydroquinone concentration. Filled bottles were placed in temperature controlled incubators set to 25° and 45°C. The 45°C elevated temperature is used for accelerated stability testing; in the pharmaceutical industry the standard is that a product stable for 30 days at 45°C has an equivalent stability at room temperature of one year. The formulas were prepared at 4.5% HQ, which is near or exceeds the upper limit of the specification, in order to maximize the sensitivity of the testing. The filled bottles were removed from the incubator at the times indicated in Table 3 and the formula was analyzed for color and hydroquinone content.

As shown in Table 3, the drug content remains within 1% of the initial hydroquinone concentration in the accelerated stability test. Therefore, the stability for the hydroquinone in the formula of Example 2 can be extrapolated to one year at room temperature.

The formula of Example 2 was further tested for stability by comparison to commercially available 4% HQ drugs. Small aliquots were dispensed from sample
tubes of each product onto a plastic tray, open to the air, and placed in a 45°C air incubator for 24 hr. The appearance of each of the formulas was examined and compared to its appearance at the beginning of the incubation. The results are shown in Table 4.

The results of this table show that under these test conditions, the commercially available 4% hydroquinone products do not maintain their color because of the oxidation of the hydroquinone. However, the formula of Example 2 does maintain its initial white color under these extreme conditions.

The formula of Example 2 was further characterized for the stability of the pH-sensitive liposomes included in the composition, i.e., the ULTRASOMES and ROXISOMES which contain DNA repair enzymes and were added as Sequence #3 ingredients. The liposome concentration was determined as liposome particles/ml using transmission electron microscopy. For this analysis, a sample of the formula was mixed with an equivalent volume of 50 nm latex beads for reference, originally at 2.3 x 10^{14}/ml but diluted 1/100. Ten microliter samples were applied to FORMVAR carbon-coated grids and the grids were negatively stained with 2% uranyl acetate. Grid openings from 5 grids were randomly selected for examination by a PHILIPS CM 12 transmission electron microscope. Representative areas containing both liposomes and latex spheres were photographed at a magnification of 35,000x and three micrographs were counted. The pH-sensitive liposomes were observed to be intact and were found to be present at an average concentration of 3.65 x 10^{12}/ml, as calculated from comparison to the latex beads of known concentration and averaged for 3 determinations (see Table 5).

**Example 4.**

*A lotion comprising a liposomal Micrococcus lysate reduces the sun sensitivity of skin*

This example was designed to be an extreme case of exposure to a very high dose of a laboratory source of UV-B in order to demonstrate a biological phenomenon. It is not intended to replicate a typical exposure from solar UV or to represent a realistic experience of a user of this invention.

A test lotion comprising 1% ULTRASOMES V (liposomal Micrococcus lysate with all components derived from vegetable sources; AGI Dermatics) in a vehicle
lotion composed of 0.54% CARBOPOL 981-NF and 1% phenoxyethanol in phosphate buffered saline (PBS) was prepared.

The subject was a 49-year old male with Type II skin (easily burns, does not readily tan). Three sites on the left volar forearm were irradiated with 3 MED or 1500 J/m\(^2\) of UV-B (305nm), and either left untreated, or treated with vehicle, or with test lotion. The vehicle and test lotions were applied before, immediately after and twice daily for 3 days following irradiation. Over a period of two weeks the changes in transepidermal water loss (TEWL) and erythema (E) were measured, and the sites were visually monitored for the appearance of peeling. TEWL is a measure of the integrity of the skin barrier and E is a measure of the sunburn reaction. The procedures used to measure TEWL and E can be found in the Handbook of Non-Invasive Methods and the Skin, (eds. J. Serup and G.B.E. Jemec, CRC Press, Boca Raton, 1995). In particular, J. Pinnagoda and R.A. Tupkor describe the procedure used to measure TEWL in Chapter 9.1 “Measurement of the Transdermal Water Loss” pages 173-178, and H. Takiwaki and J. Serup describe the procedure used to measure E in Chapter 16.2 “Measurement of Erythema and Melanin” pages 377-384.

In the untreated and vehicle treated sites, E values increased for 48 hrs and then recovered. At six hours, erythema was less at a statistically significant level at the site treated with ULTRASOMES than at the vehicle or untreated site and recovered more quickly following the peak value than at the vehicle or untreated sites. TEWL began to rise after 6 days and reached peak values on day 9 for the untreated and vehicle treated sites, while for site treated with ULTRASOMES, the peak was 25% less and occurred one day earlier on day 8 for TEWL.

By day 7 peeling began at sites left untreated or treated with vehicle. On day 8 the peeling was significantly less at the site treated with ULTRASOMES compared to the control sites.

These experimental results indicate that treatment of skin with a lotion containing a DNA repair enzyme (e.g., a liposomal Micrococcus lysate) following exposure to a real life dose of solar UV radiation (i.e., a much lower dose) will lessen skin sensitivity, including lessening erythema and barrier disruption, and reduce the extent of skin peeling.
Example 5.

Treatment of Normal Human Epidermal Keratinocytes with liposomal OGG1 enzyme increases DNA repair of 8-oxo-guanine

Solar UV striking the skin produces oxygen radicals that react with DNA in the cells. In addition, reactive oxygen species from environmental ozone or produced during normal metabolism also modify DNA. The most common adduct formed is the addition of oxygen to the guanine base to form 8-oxo-guanine (8oG), which interferes with the coding capacity of the DNA, and can lead to mutations. The DNA repair enzyme oxo-guanine glycosylase 1 (OGG1) initiates removal of 8oG by making a glycosylcic break that releases the damaged base. The apurinic site is then excised and resynthesized using the opposite strand as template.

The rate of repair of 8oG can be increased by introducing into the cell additional OGG1 enzyme. This can be accomplished in skin by topical application of pH-sensitive liposomes containing OGG1 enzyme, e.g., AGI Dermatics' ROXISOMES product. The enhanced repair provided by liposomal OGG1 enzyme is observed by staining treated cells with antibodies against 8oG and examining them by immunofluorescence microscopy.

Normal human epidermal keratinocytes (NHEK) were plated into 1-well glass chamber slides (Nalgene Nunc International, Naperville, IL) at 1x10⁵ cells per slide. NHEK were obtained from Cascade Biologics (Portland, OR). Cells were cultured in EPIFLIFE medium (Cascade Biologics) containing a human keratinocyte growth supplement (HKGS) kit (Cascade Biologics), and maintained at 37°C in a humidified atmosphere containing 5% CO₂ to approximately 80% confluency.

Stock solutions of ferrous sulfate and copper sulfate (Sigma, St. Louis, MO) were prepared in water at a concentration of 10 mM. The ferrous sulfate and copper sulfate stock solutions were then diluted to 0.1mM in NHEK EPIFLIFE medium. A 5mM hydrogen peroxide (H₂O₂) stock solution was also prepared in water. Medium containing 0.1mM ferrous sulfate and copper sulfate was transferred onto the NHEK and incubated for 10 minutes at 37°C. Following incubation, 5mM H₂O₂ stock solution was added to the EPIFLIFE medium containing ferrous sulfate and copper sulfate to a final H₂O₂ concentration of 100 μM. Upon addition of the H₂O₂-containing medium, NHEK were again incubated for 10 minutes at 37°C. Treatment medium was aspirated
after the final 10 minute incubation and replaced with fresh medium. ROXISOMES were then added to the fresh EPILOFE medium to obtain a final concentration of 1 ng/µL of OGG1 and incubated for 6 hours.

EPILOFE medium was aspirated from the 1-well chamber slides and the gasket was removed. Cells were fixed in methanol and acetic acid (3:1) and then dehydrated with increasing concentrations of ethanol (50%, 70%, 90%, 100% and 100%). Protein was denatured with 0.05N HCl (J.T. Baker, Phillipsburg, NJ). RNA was then removed by incubating slides in 1X SSC buffer (0.15 M NaCl, 15 mM Na citrate, pH 7.0) containing 0.1mg/mL RNase A (Sigma). DNA was denatured with 0.15N NaOH (Sigma). Slides were rehydrated and protein was removed with a 5 µg/ml solution of PROTEINASE K (Sigma) in Tris-EDTA. Slides were blocked with 20% FBS (fetal bovine serum)/Tween-20 for 30 minutes. Anti-8-oxo-dG clone 4E9 antibody (Trevigen, Gaithersburg, MD) was diluted 1:500 in 10mL of 20% FBS/Tween-20 block. Slides were transferred to a humidity chamber and the anti-8-oxo-dG antibody was applied before incubation for 1 hour at room temperature. Slides were washed twice in 1X PBS before application of FITC conjugated anti-mouse IgG (Santa Cruz Biotechnology Inc., Santa Cruz, CA) as secondary antibody. Secondary antibody was diluted 1:5000 in 15mL of 20% FBS/Tween-20 block and applied to the slides for 1 hour, in the dark. Slides were again washed twice with 1X PBS. Slides were then treated with a 500 nM solution of propidium iodide (Sigma) prepared in 2X SSC buffer (0.3M NaCl, 30 mM Na citrate pH 7.0) for 5 minutes in the dark. Following incubation, slides were washed twice in 2X SSC buffer. Coverslips were mounted using SLOWFADE (Molecular Probes, Eugene, OR) and allowed to dry at -20°C for at least 30 minutes. Slides were thawed for a minimum of 10 minutes before examination, in both brightfield and UV fluorescence, with an OLYMPUS BX51 microscope (Olympus America Inc., Melville, NY). The fluorescence intensity was scored on a five-point scale: 0, +, ++, ++++, +++++.

The results are shown in Table 6. As can be seen in this table, hydrogen peroxide treatment of NHEK cells produces 8oG detectible by antibody, and incubation with a DNA repair enzyme for six hours reduced the intensity of staining for 8oG in comparison to untreated NHEK, indicating repair enhanced over the endogenous level.
Example 6.

A lotion comprising a biomimetic of *Evodia* extract reduces pain

EVODIOX (AGI Dermatics) is a biomimetic of the traditional Chinese medicine Wu-Zhu-Yu, which is made from the unripe berries of the *Evodia rutaecarpa* bush. It is a mixture of indolequinazoline alkaloids and limonene in butylene glycol with butylated hydroxytoluene. EVODIOX, like the traditional medicine, has anti-inflammatory properties (WO 2006/023377). The traditional medicine also is reported to be anti-nociceptive, that is, reduces pain.

The neurometer (Neurotron Inc., Baltimore MD) is a testing device used to determine the threshold of sensation in skin. A current is passed between two gold electrodes taped to the skin of a subject. The sensory threshold is the lowest voltage at which a subject reports feeling a sensation. Three frequencies are used, one tuned to each of the three nerve fibers of the skin: 5 Hz for the C fibers that detect touch and pressure, 250 Hz for the Aδ fibers that detect cold temperature, fast/sharp pain and pressure, and 2000 Hz for the Aβ fibers that detect hot temperature and slow pain. Skin sensations related to sun overexposure and irritation are thought to be mediated by the Aβ fibers. The sensation threshold measurement is reproducible for each person, but may vary greatly among people.

Two male subjects in good health, one 53 years old and one 59 years old, were tested to establish baseline sensory threshold for each of the three nerve fiber types. A lotion comprising 1% EVODIOX and 1% CARBOPOL 981 NF hydrogel in phosphate buffered saline was prepared. Baseline measurements were taken and then the lotion was applied in a moderate layer over the skin sites where the measurements were made. After 30 minutes the measurements were repeated.

The results are shown in Table 7, with the sensory threshold expressed in arbitrary units. As shown in this table, for both subject 1 and 2, treatment with the EVODIOX lotion increased the sensory threshold for the Aβ fibers, stimulated by the 2000 Hz current, by 10-20%. This indicates an anti-nociceptive effect for the biomimetic of *Evodia* extract.

From the foregoing, it can be seen that in accordance with the preferred embodiments of the invention, a stabilized formula for a hydroquinone cream has been provided as well as methods to treat hyperpigmentation that reduce the consequent
increased sensitivity to sun exposure. The preferred formula comprises hydroquinone as the primary skin lightening agent, liposomal DNA enzymes for repair of photodamage, and L-ergothioneine for stabilization of hydroquinone and pH-sensitive liposomes at pH > 6. Other agents may be added to augment the lightening effect, reduce pain, swelling, and irritation, and to impart the cream with favorable feel and fragrance. Methods are also provided for the production of the cream.

**Example 7.**

**Efficacy and safety of the formula 2 lotion for lightening melanocytes in a human skin equivalent**

MELANODERM tissues from MatTek Corp. (Ashland, MA) consist of normal, human-derived epidermal keratinocytes and melanocytes which have been cultured to form a multilayered, highly differentiated model of the human epidermis. MELANODERM B tissue (MEL-300-B), derived from a black donor, was chosen for optimal demonstration of efficacies in a comparison study of skin lightening lotions.

Tissues were treated daily with 2 µl of the formula of Example 2 or the leading commercial product, TRI-LUMA from Galderma, for 7 days. Each day the tissues were rinsed twice with PBS before lotions were reapplied. Lotions were applied with the end of a glass rod. Tissues were incubated at 37°C in a 5% CO₂ atmosphere between lotion applications.

Photographs were taken on the 8th day at a magnification of 300X with a light microscope. Melanocytes in untreated MELANODERM tissues exhibited an abundant amount of melanin and extensive dendritic processes, indicative of normal black melanocytes (FIG. 1A). The melanocytes treated with the Example 2 formula lack melanin, but still retain the oblong shapes and dendritic processes of healthy melanocytes (FIG. 1B). The melanocytes treated with TRI-LUMA also have reduced amounts of melanin but to a lesser extent and they are round in shape with loss of dendritic processes, indicative of stress (FIG. 1C).

These results indicate that the lotion of Example 2 successfully reduced melanin levels while giving no evidence of having harmed the cells. In contrast, the leading commercial lotion, TRI-LUMA, was less effective in reducing melanin and may have harmed the cells as evidenced by the round shapes of the treated melanocytes and the loss of dendritic processes.
Although specific embodiments of the invention have been described and illustrated, it is to be understood that modifications can be made without departing from the invention's spirit and scope. The following claims are intended to cover the specific embodiments set forth herein as well as such modifications, variations, and equivalents.
Literature References


US Patents 4,136,166; 5,164,182; 5,279,834; 5,352,458; 5,516,793; 5,703,122;

5,190,762; 6,039,935; 6,274,124; 6,521,267; 6,699,464; 6,846,812; 6,872,401;
6,964,954; 6,974,583; 6,964,776

US Patent Publication 2005/0042233
PCT Patent Publication WO 2006/023377
### TABLE 1

<table>
<thead>
<tr>
<th>Sodium Citrate</th>
<th>Hydroquinone</th>
<th>pH</th>
<th>Sodium Metabisulfite</th>
<th>Magnesium Ascorbyl Phosphate</th>
<th>L-ergothioneine</th>
<th>OD&lt;sub&gt;475&lt;/sub&gt; @ 48 h</th>
<th>Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.18%</td>
<td>4%</td>
<td>6.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.44</td>
<td>100%</td>
</tr>
<tr>
<td>0.18%</td>
<td>4%</td>
<td>6.5</td>
<td>0.1%</td>
<td>0.5%</td>
<td>0</td>
<td>1.69</td>
<td>69%</td>
</tr>
<tr>
<td>0.18%</td>
<td>4%</td>
<td>6.5</td>
<td>0.1%</td>
<td>0.5%</td>
<td>0.3%</td>
<td>0.97</td>
<td>40%</td>
</tr>
<tr>
<td>0.18%</td>
<td>4%</td>
<td>6.5</td>
<td>0.1%</td>
<td>0</td>
<td>0.3%</td>
<td>0.13</td>
<td>5%</td>
</tr>
</tbody>
</table>

### TABLE 2

<table>
<thead>
<tr>
<th>SEQ.</th>
<th>Percent</th>
<th>INGREDIENT</th>
<th>INCI NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.00</td>
<td>DC 345 Fluid</td>
<td>Cyclopentasiloxane</td>
</tr>
<tr>
<td>1</td>
<td>10.00</td>
<td>DC 5225C Formulation Aid</td>
<td>Cyclopentasiloxane (and) PEG/PPG-18/18 Dimethicone</td>
</tr>
<tr>
<td>1</td>
<td>2.00</td>
<td>DC 200/100cs.</td>
<td>Dimethicone</td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>LIPOSORB O-20</td>
<td>Polysorbate 80</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>BOTANISTAT PF-64</td>
<td>Phenoxyethanol (and) Caprylyl Glycol (and) Ethylhexylglycerin (and) Hexylene Glycol</td>
</tr>
<tr>
<td>2</td>
<td>66.10</td>
<td>Deionized Water</td>
<td>Water</td>
</tr>
<tr>
<td>2</td>
<td>6.00</td>
<td>Glycerin</td>
<td>Glycerin</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>DISSOLVINE Na2S</td>
<td>Disodium EDTA</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>Sodium Metabisulfite</td>
<td>Sodium Metabisulfite</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>Hydroquinone</td>
<td>Hydroquinone</td>
</tr>
<tr>
<td>2</td>
<td>0.30</td>
<td>L-ergothioneine</td>
<td>Ergothioneine</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>ULTRASOMES</td>
<td>Micrococcus Lysate (and) Lecithin (and) Water (and) Phenoxyethanol</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>ROXISOMES</td>
<td>Arabidopsis Extract (and) Lecithin (and) Water (and) Phenoxyethanol</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>EVODIOX</td>
<td>Evodia Rutaecarpa Fruit Extract (and) Butylene Glycol (and) Phenoxyethanol</td>
</tr>
</tbody>
</table>
### TABLE 3

<table>
<thead>
<tr>
<th>Incubation (Days)</th>
<th>Temperature (°C)</th>
<th>Hydroquinone concentration</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>--</td>
<td>4.5%</td>
<td>Shiny off-white</td>
</tr>
<tr>
<td>1</td>
<td>45°</td>
<td>4.5%</td>
<td>Shiny off-white</td>
</tr>
<tr>
<td>30</td>
<td>25°</td>
<td>4.6%</td>
<td>Shiny off-white</td>
</tr>
<tr>
<td>30</td>
<td>45°</td>
<td>4.5%</td>
<td>Shiny off-white</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>Commercial 4% Hydroquinone Drug</th>
<th>Appearance before 45°, 24h</th>
<th>Appearance after 45°, 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRI-LUMA Cream</td>
<td>Greenish-yellow</td>
<td>Dark green</td>
</tr>
<tr>
<td>LUSTRA</td>
<td>Shiny bright white</td>
<td>Light tan</td>
</tr>
<tr>
<td>LUSTRA AF</td>
<td>Shiny bright white</td>
<td>Dark tan</td>
</tr>
<tr>
<td>EPIQUIN MICRO</td>
<td>Dull white-yellow</td>
<td>Brown</td>
</tr>
<tr>
<td>Formula of Example 2</td>
<td>Shiny off-white</td>
<td>Shiny off-white</td>
</tr>
</tbody>
</table>

### TABLE 5

<table>
<thead>
<tr>
<th>Micrograph</th>
<th>No. of Liposomes</th>
<th>No. of Latex Spheres</th>
<th>Liposome concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>9</td>
<td>3.58 x 10^{12}/ml</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>8</td>
<td>3.45 x 10^{12}/ml</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>10</td>
<td>3.91 x 10^{12}/ml</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>3.65 x 10^{12}/ml</td>
</tr>
</tbody>
</table>

*No. of liposomes x 2.3 x 10^{14}/ml/No. of latex spheres x 100
### TABLE 6

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>Medium + ROXISOMES (1.0 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr post UV</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>6 hr post UV</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

### TABLE 7

<table>
<thead>
<tr>
<th>Subject</th>
<th>2000 Hz (Aβ)</th>
<th>250 Hz (Aδ)</th>
<th>5 Hz (C fibers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Baseline</td>
<td>89.3</td>
<td>16.3</td>
<td>12.3</td>
</tr>
<tr>
<td>1 Post-lotion</td>
<td>98.7</td>
<td>7.7</td>
<td>9.3</td>
</tr>
<tr>
<td>2 Baseline</td>
<td>65.3</td>
<td>12.7</td>
<td>16.3</td>
</tr>
<tr>
<td>2 Post-lotion</td>
<td>81.3</td>
<td>22.3</td>
<td>20.3</td>
</tr>
</tbody>
</table>
What is claimed is:

1. A method for lightening skin color and repairing DNA damage by ultraviolet light comprising applying a topical composition to skin of a person in need of skin lightening wherein the composition comprises (a) one or more skin lightening agents in a combined amount effective to lighten skin and (b) one or more DNA repair agents in a combined amount effective to repair DNA damage by ultraviolet light.

2. A method for reducing skin darkening and repairing DNA damage by ultraviolet light comprising applying a topical composition to skin of a person in need of a reduction in skin darkening wherein the composition comprises (a) one or more skin lightening agents in a combined amount effective to reduce skin darkening and (b) one or more DNA repair agents in a combined amount effective to repair DNA damage by ultraviolet light.

3. The method of Claim 1 or 2 wherein the topical composition further comprises an anti-inflammatory agent.

4. The method of Claim 1 or 2 wherein the topical composition further comprises an anti-nociceptive agent.

5. A method for lightening skin color and repairing DNA damage by ultraviolet light comprising applying a topical composition to skin of a person in need of skin lightening wherein the composition comprises (a) hydroquinone in an amount effective to lighten skin and (b) one or more DNA repair enzymes encapsulated in liposomes in a combined amount effective to repair DNA damage by ultraviolet light.

6. A method for reducing skin darkening and repairing DNA damage by ultraviolet light comprising applying a topical composition to skin of a person in need of a reduction in skin darkening wherein the composition comprises (a) hydroquinone in an amount effective to reduce skin darkening and (b) one or more DNA repair enzymes encapsulated in liposomes in a combined amount effective to repair DNA damage by ultraviolet light.

7. The method of Claim 5 or 6 wherein the topical composition further comprises L-ergothioneine.

8. The method of Claim 5 or 6 wherein the topical composition further comprises a biomimetic of Evodia extract.

9. A method for preparing a skin lightening composition comprising:
(a) preparing an aqueous solution comprising hydroquinone;
(b) adding ergothioneine to the aqueous solution of step (a); and
(c) adding one or more lipid-containing components to the aqueous solution of step (b).

10. The method of Claim 9 wherein the one or more lipid-containing components comprises pH-sensitive liposomes.

11. The method of Claim 9 wherein the ergothioneine is L-ergothioneine.

12. A composition for lightening skin color and repairing DNA damage by ultraviolet light comprising (a) one or more skin lightening agents in a combined amount effective to lighten skin, (b) one or more DNA repair agents in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle.

13. A composition for reducing skin darkening and repairing DNA damage by ultraviolet light comprising (a) one or more skin lightening agents in a combined amount effective to reduce skin darkening, (b) one or more DNA repair agents in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle.

14. The composition of Claim 12 or 13 wherein the composition further comprises an anti-inflammatory agent.

15. The composition of Claim 12 or 13 wherein the composition further comprises an anti-nociceptive agent.

16. A composition for lightening skin color and repairing DNA damage by ultraviolet light comprising (a) hydroquinone in an amount effective to lighten skin, (b) one or more DNA repair enzymes encapsulated in liposomes in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle.

17. A composition for reducing skin darkening and repairing DNA damage by ultraviolet light comprising (a) hydroquinone in an amount effective to reduce skin darkening, (b) one or more DNA repair enzymes encapsulated in liposomes in a combined amount effective to repair DNA damage by ultraviolet light, and (c) a cosmetically or pharmaceutically acceptable vehicle.
18. The composition of Claim 17 wherein the liposomes are pH-sensitive liposomes.

19. The composition of Claim 16, 17, or 18 wherein the composition further comprises L-ergothioneine.

20. The composition of Claim 16, 17, or 18 wherein the composition further comprises a biomimetic of Evodia extract.

21. A composition for topical application to skin comprising:
   (a) hydroquinone,
   (b) ergothioneine, and
   (c) a cosmetically or pharmaceutically acceptable vehicle,
wherein the composition has a pH which is greater than or equal to 6.2 and less than 6.8, said pH being measured in the absence of any ingredients which substantially interfere with the measurement of pH.

22. The composition of Claim 21 wherein the composition does not include a cationic salt of an acidic ascorbyl ester.
Untreated Control

FIG. 1A
Example 2 Formula

FIG. 1B
TRI-LUMA

FIG. 1C