Abstract: The invention relates to compounds and methods for inhibiting production and function of tyrosinase in the skin of a mammal. The invention further relates to compounds and methods for inhibiting darkening of the skin of a mammal, as well as lightening the skin of a mammal and reducing pigmentation in the skin of a mammal.
TITLE OF THE INVENTION
Compositions and Methods for Skin Lightening

BACKGROUND OF THE INVENTION
There are two distinct types of skin aging. Aging caused by the genes we inherit is called intrinsic (internal) aging. The other type of aging is known as extrinsic (external) aging and is caused by environmental factors, such as exposure to the sun's rays. Intrinsic aging, also known as chronoaging, is a continuous process that normally begins in our mid-20s. Within the skin, collagen production slows, and elastin, the substance that enables skin to snap back into place, has a bit less spring. Dead skin cells do not shed as quickly and turnover of new skin cells may decrease slightly. While these changes usually begin in our 20s, the signs of intrinsic aging are typically not visible for decades. The signs of intrinsic aging are: fine wrinkles, thin and transparent skin, loss of underlying fat leading to hollowed cheeks and eye sockets as well as noticeable loss of firmness on the hands and neck, and dry skin that may itch. There is a reduction in the cells and blood vessels that supply the skin. There is also a flattening of the dermal-epidermal junction, which results in weaker mechanical resistance at this junction. As a consequence, older people are more susceptible to blister formation in cases of mechanical trauma or disease processes (Oikarinen et al., 1990, Photodermatal. Photoimmunol. Photomed., 7:3-4). Genes control how quickly the normal aging process unfolds.

A number of extrinsic, or external, factors act together with the normal aging process to prematurely age our skin. Most premature aging is caused by sun exposure. Other external factors that prematurely age our skin are repetitive facial expressions, gravity, sleeping positions, and smoking.

Without protection from the sun's rays, just a few minutes of exposure each day over the years can cause noticeable changes to the skin. Freckles, age spots, vascular changes (diffuse erythema, ecchymoses, and telangiectasias, spider veins), rough and leathery skin, fine wrinkles that disappear when stretched, loose skin, a blotchy complexion, actinic keratoses (thick wart-like, rough, reddish patches of skin), pigmentedary changes (lentigines, freckles, and areas of hypo- and hyper-pigmentation), development of seborrheic keratosis, actinic keratosis, comedones, and cysts, elastosis, atrophy, and skin cancer can all be traced to sun
exposure. In addition, protein glycation is enhanced in photoaged skin (Jeanmaire C. et al., Br J Dermatol. 2001 July; 145(1): 10-8).

"Photoaging" is the term dermatologists use to describe this type of aging caused by exposure to the sun's rays. The amount of photoaging that develops depends on: 1) a person's skin color and 2) their history of long-term or intense sun exposure. People with fair skin who have a history of sun exposure develop more signs of photoaging than those with dark skin. In the darkest skin, the signs of photoaging are usually limited to fine wrinkles and a mottled complexion.

Photoaging occurs over a period of years. With repeated exposure to the sun, the skin loses the ability to repair itself, and the damage accumulates. Scientific studies have shown that repeated ultraviolet (UV) exposure breaks down collagen and impairs the synthesis of new collagen. The sun also attacks our elastin. Sun-weakened skin ceases to spring back much earlier than skin protected from UV rays. Skin also becomes loose, wrinkled, and leathery much earlier with unprotected exposure to sunlight. While it may seem that the signs of photoaging appear overnight, they actually lie invisible beneath the surface of the skin for years. UV photography enables us to see the damage accumulating beneath the surface of the skin years before the signs of photoaging appear.

As skin ages, there is an increase in oxidative stress, an increase in inflammation, a decrease in collagen levels, overexpression of the enzyme MMP, an increase in protein glycation, and an increase in mitochondrial decay. Additional aging processes include the intrinsic rate of proton leakage across the inner mitochondrial membrane, decreased membrane fluidity, and decreased levels and function of cardiolipin. The mitochondria, which create the energy the cells need by converting dietary and other cellular fuels into ATP, are adversely affected by these aging processes. It has been shown that oxidants generated by mitochondria are the major source of the oxidative lesions in the mitochondria that accumulate with age. (Ames B N, et al., Biochim Biophys Acta. 1995 May 24; 1271(l):165-70). As the skin ages, the mitochondria become severely impaired, and this leads to both a decrease in ATP production and greater oxidative damage.

The enzyme tyrosinase is essential for the conversion of the amino acid tyrosine into melanin in the skin. Hyperpigmentation can result when too much
melanin is produced. Ingredients such as arbutin, kojic acid, and thymol inhibit or suppress tyrosinase, preventing the appearance of additional pigmentation.

Normal skin color is formed by melanin, a natural pigment that also determines hair and eye color. In the skin, the enzyme tyrosinase is involved in the biochemical pathway responsible for the conversion of the amino acid tyrosine into melanin. Hyperpigmentation occurs when too much melanin is produced and forms deposits in the skin. The cells that make pigment are called melanocytes. They are located at the bottom of the epidermis. Melanocytes produce melanosomes, which are passed onto other cells of the epidermis and make their way up to the top layer of skin. Synthesis of melanin occurs exclusively in melanosomes. When too much melanin is produced, deposits are formed and hyperpigmentation appears in the skin.

Tyrosinase is a copper-containing monooxygenase catalyzing the 
hydroxylation of monophenols to the corresponding catechols (monophenolase or cresolase activity), and the oxidation of monophenols to the corresponding o-quinones (diphenolase or catecholase activity). These functions of tyrosinase play an important role in the formation of melanin pigments during melanogenesis. Melanin production is principally responsible for skin color and plays an important role in prevention of sun-induced skin injury. However, abnormal accumulation of melanin products in skin is responsible for hyperpigmentations including melasma, melasma, chloasma, freckles, and senile lentigines, which could be a serious aesthetic problem (Jeon et al. (2005) Bull. Korean Chem. Soc., Vol. 26: 1135-1 137).

Recently, a global market demand has developed for skin whitening agents. Several tyrosinase inhibitors have been used in the cosmetic industry as skin-whitening agents. Unfortunately, many of these inhibitors have not yet been demonstrated to be clinically efficacious when critically analyzed in carefully controlled studies. Thus, many efforts are still devoted to screening recognized and putative skin-whitening agents. A correct assay method is essential for the development of effective inhibitors of tyrosinase. Until recently, the screening and evaluation methods of these skin-whitening agents have adopted the DOPA oxidation test by mushroom tyrosinase because of its commercial availability, but the test does not utilize human tyrosinase. Moreover, a comparative study on the inhibitory effects of skin-whitening agents by human tyrosinase and mushroom tyrosinase has not yet been done.
Natural plant glycosides are extracts shown to interfere in the process that causes melanin to appear in the skin, preventing further accumulation of excess melanin and discoloration. While hyperpigmentation is typically not a medically harmful condition, except in instances of certain skin cancers, it is a common clinical condition for which many people seek remedies because they view it as aesthetically displeasing. Hyperpigmentation affects people of all skin colors and races, and tends to increase with age. For example, almost all African-American infants become darker shortly after birth. Freckles, small, flat, tan-to-black spots anywhere on the body, become more permanent during the first or second decade of life and increase in number with sun exposure. Often hereditary, freckles can darken with sun exposure and fade with less sun exposure. Solar lentigines, known colloquially as age spots or liver spots, are small, mottled or darkened patches of skin which appear in older adults, particularly on the face, the backs of hands, and arms in individuals who have been exposed to the sun.

Hyperpigmentation also results from inflammation or other skin insults. For example, skin diseases such as acne or shingles may leave darkened spots. Scars from skin injury or surgery also may become hyperpigmented. Cosmetic procedures— including laser resurfacing, laser hair removal, chemical peels and dermabrasion — also may leave the affected area darker than the normal skin color. All these conditions maybe categorized as post-inflammatory hyperpigmentation.

In addition to hyperpigmentation, many women suffer from melasma, also known as chloasma, a hormone-induced mask-like skin condition that often results from birth control pills and/or pregnancy. Melasma appears most often as blotchy brown spots on the cheeks, forehead and temples of the face, but also on the abdomen and other areas.

There are currently numerous compositions on the market for the prevention and/or treatment of aging skin for both chronoaging and photoaging. Hydroquinone is a skin lightener available without a prescription in concentrations up to 2 percent, and can be prescribed in concentrations up to 4 percent. However, hydroquinone can be very irritating at high concentration, can cause ochronosis with prolonged use, and is tumorigenic in rats (Maeda and Fukada, J Pharmacol Exp Ther, 1996).
Arbutin is a plant glycoside and skin lightener found in a natural plant, Uva-Ursi (which also has antioxidant properties). Arbutin is a natural hydroquinone molecule attached to a sugar molecule (CeH₆O₆), which makes it water soluble. Arbutin helps prevent additional brown spots from occurring by stopping the production of melanin. Specifically, arbutin works by suppressing tyrosinase, the enzyme that converts tyrosine into melanin in skin. Uva-Ursi also contains three strong antioxidants: ferulic acid, caffeic acid, and chlorogenic acid. These antioxidants neutralize oxygen free radicals that can damage skin. The three acids also act as intermediary acids which produce three flavonoids — myricetin, quercetin and rutin — which help protect skin.

Kojic acid is a skin lightener produced from fungus. Discovered in Japan in 1989, it has been used with excellent results to lighten skin and reduce brown spots. Like arbutin, it blocks the formation of melanin pigment in skin cells.

Thyme is an herb plant indigenous to the Mediterranean region. Thyme extract contains thymol, which has strong antiseptic and antioxidant properties, and helps to prevent future oxidative breakdown of cells. Thymol stops the production of melanin by inhibiting tyrosine conversion from tyrosine to 3, 4-Dihydroxyphenylalanine (Dopa), which is the first step in the biochemical path to melanin. Importantly, thymol does not damage the melanocytes, in which melanin synthesis occurs.

The bitter part of the cucumber plant which contains curcubitacin (forms A, B, C and D). Traditionally, cucumber slices have been used to remove dark circles from the area underneath the eyes.

Despite the availability of these skin lighteners, there still remains a need for an effective topical anti-aging formulation that can be used, without a prescription, to treat the effects of hyperpigmentation, unwanted pigmentation, other related skin conditions, and skin damage. The present invention addresses and meets these needs.

SUMMARY OF THE INVENTION

One embodiment of the present invention comprises a method of inhibiting tyrosinase activity in the skin of a mammal, the method comprising administering to a mammal a composition comprising meglumine. In one aspect, the
composition further comprises a pharmaceutically acceptable carrier. In another aspect, the composition further comprises at least one depigmenting agent. In yet another aspect, the depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine. In another aspect, the composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen. In still another aspect, the mammal is a human.

Another embodiment of the invention comprises a method of inhibiting catechol oxidase activity, the method comprising administering to a mammal a composition comprising meglumine. In one aspect, the composition further comprises a pharmaceutically acceptable carrier. In another aspect, the composition further comprises at least one depigmenting agent. In yet another aspect, the depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine. In another aspect, the composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen. In still another aspect, the mammal is a human.

Still another embodiment of the invention comprises a method of lightening the skin of a mammal, the method comprising administering to a mammal a composition comprising meglumine. In one aspect, the composition further comprises a pharmaceutically acceptable carrier. In another aspect, the composition further comprises at least one depigmenting agent. In yet another aspect the depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine. In another aspect, the composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen. In still another aspect, the mammal is a human.

Yet another embodiment of the invention comprises a method of reducing pigmentation in the skin of a mammal, the method comprising administering to a mammal a composition comprising meglumine. In one aspect, the composition
further comprises a pharmaceutically acceptable carrier. In another aspect, the composition further comprises at least one depigmenting agent. In yet another aspect, the depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine. In another aspect, the composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen. In still another aspect, the mammal is a human.

Still another embodiment of the invention comprises a method of preventing darkening of the skin of a mammal, said method comprising administering to said mammal a composition comprising meglumine. In one aspect, the composition further comprises a pharmaceutically acceptable carrier. In another aspect, the composition further comprises at least one depigmenting agent. In yet another aspect, the depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine. In another aspect, the composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen. In still another aspect, the mammal is a human.

Still another embodiment of the invention comprises a method of preventing melanin accumulation in the skin of a mammal, said method comprising administering to said mammal a composition comprising meglumine. In one aspect, the composition further comprises a pharmaceutically acceptable carrier. In another aspect, the composition further comprises at least one depigmenting agent. In yet another aspect, the depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine. In another aspect, the composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen. In still another aspect, the mammal is a human.

Yet another embodiment of the invention comprises a method of preventing fruit browning, said method comprising applying to said fruit a composition comprising meglumine.
Yet another embodiment of the invention comprises a method of preventing vegetable browning, said method comprising applying to said vegetable a composition comprising meglumine.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of preferred embodiments of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown.

In the drawings:

Figure 1 is an image illustrating the prevention of apple browning according to the present invention. The apple half on the left (A) was treated with water. The apple half on the right (B) was treated with 10% meglumine-HCl.

Figure 2 is an image illustrating the prevention of browning in a potato according to the present invention. The potato half on the left (A) was treated with water. The potato half on the right (B) was treated with 10% meglumine-HCl.

Figure 3 is an image illustrating the inhibition of catechol oxidase according to the present invention. The lanes are described in the text. Lanes 2 and 3, which contain meglumine and meglumine-HCl, illustrate that meglumine prevents the conversion of catechol to benzoquinone by catechol oxidase.

Figure 4 is an image illustrating the inhibition of tyrosinase. The wells are described in the text. In wells 3A and B, which contain meglumine-HCl, the conversion of catechol to benzoquinone by tyrosine, is prevented.

Figure 5 is an image illustrating the titration of meglumine-HCl inhibition of tyrosinase. The wells are described in the text. As the concentration of meglumine is increased from 0.5 mM to 100 mM the extent of inhibition at 20 minutes is increased.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates generally to compositions and methods of treating deleterious and/or undesirable conditions that involve inhibiting the activity
of at least one enzyme responsible for pigmentation or coloring of the skin within the skin tissue of a mammal. This is because it has now been discovered, as described in greater detail elsewhere herein, that meglumine can inhibit the activity of catechol oxidase, as well as tyrosinase and other tyrosinase-like enzymes.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described herein.

As used herein, each of the following terms has the meaning associated with it in this section.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

As used herein, "alleviating a disease or disorder symptom," means reducing the severity of the symptom.

As used herein, "amino acids" are represented by the full name thereof, by the three-letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

<table>
<thead>
<tr>
<th>Full Name</th>
<th>Three-Letter Code</th>
<th>One-Letter Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic Acid</td>
<td>Asp</td>
<td>D</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>G1u</td>
<td>E</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>H</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>N</td>
</tr>
<tr>
<td>Glutamine</td>
<td>GIn</td>
<td>Q</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
</tr>
</tbody>
</table>
The term "biological sample," as used herein, refers to samples obtained from a living organism, including skin, hair, tissue, blood, plasma, cells, sweat and urine.

The term "clearance," as used herein, refers to the physiological process of removing a compound or molecule, such as by diffusion, exfoliation, removal via the bloodstream, and excretion in urine, or via other sweat or other fluid.

A "compound," as used herein, refers to any type of substance or agent that is commonly considered a drug, or a candidate for use as a drug, as well as combinations and mixtures of the above, or modified versions or derivatives of the compound.

A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. As used herein, normal aging is included as a disease.

A "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

An "effective amount" or "therapeutically effective amount" of a compound is that amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered, or gives the appearance of providing a therapeutic effect as in a cosmetic.
As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the peptide of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material can describe one or more methods of alleviating the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention can, for example, be affixed to a container which contains the identified compound invention or be shipped together with a container which contains the identified compound. Alternatively, the instructional material can be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

The term "peptide" typically refers to short polypeptides. As used herein, the term "pharmaceutically-acceptable carrier" means a chemical composition with which an appropriate compound or derivative can be combined and which, following the combination, can be used to administer the appropriate compound to a subject.

As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

"Polypeptide" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. A "polynucleotide" means a single strand or parallel and anti-parallel strands of a nucleic acid. Thus, a polynucleotide may be either a single-stranded or a double-stranded nucleic acid.

A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

The term "protein" typically refers to large polypeptides.
The term "skin," as used herein, refers to the commonly used definition of skin, e.g., the epidermis and dermis, and the cells, glands, mucosa and connective tissue which comprise the skin.

The term "standard," as used herein, refers to something used for comparison. For example, it can be a known standard agent or compound which is administered and used for comparing results when administering a test compound, or it can be a standard parameter or function which is measured to obtain a control value when measuring an effect of an agent or compound on a parameter or function. "Standard" can also refer to an "internal standard", such as an agent or compound which is added at known amounts to a sample and which is useful in determining such things as purification or recovery rates when a sample is processed or subjected to purification or extraction procedures before a marker of interest is measured. Internal standards are often but are not limited to, a purified marker of interest which has been labeled, such as with a radioactive isotope, allowing it to be distinguished from an endogenous substance in a sample.

"Synthetic peptides or polypeptides" mean a non-naturally occurring peptide or polypeptide. Synthetic peptides or polypeptides can be synthesized, for example, using an automated polypeptide synthesizer. Those of skill in the art know of various solid phase peptide synthesis methods.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

By "transdermal" delivery is intended both transdermal (or "percutaneous") and transmucosal administration, i.e., delivery by passage of a drug through the skin or mucosal tissue and into the bloodstream. Transdermal also refers to the skin as a portal for the administration of drugs or compounds by topical application of the drug or compound thereto.

The term "topical application", as used herein, refers to administration to a surface, such as the skin. This term is used interchangeably with "cutaneous application".

The term to "treat," as used herein, means reducing the frequency with which symptoms are experienced by a patient or subject or administering an agent or compound to reduce the frequency with which symptoms are experienced.
As used herein, "treating a disease or disorder" means reducing the frequency with which a symptom of the disease or disorder is experienced by a patient. Disease and disorder are used interchangeably herein.

1. Compositions

The invention is concerned with compounds and compositions, as well as the use of compounds and compositions as skin lightening agents. In one embodiment, the compounds and compositions of the invention may be used for reducing overall skin pigmentation. In another embodiment, the compounds and compositions of the invention may be used for the reduction of discrete areas of hyperpigmentation, such as freckles, melasma, and chloasma, as well as for reducing inflammation-induced pigmentation.

The invention comprises an inhibitor composition comprising meglumine, or salts thereof, wherein the composition inhibits the activity of tyrosinase. In one embodiment, the inhibitor composition comprises meglumine, or salts thereof. In another embodiment, the inhibitor composition comprises an analogue, modification, derivative or adduct of meglumine. In another embodiment, the inhibitor composition comprises l-deoxy-l-(methylamino)-D-xylitol (DYN30). In still another embodiment, the composition consists of meglumine. In still another embodiment, the inhibitor composition consists of l-deoxy-l-(methylamino)-D-xylitol (DYN30).

In another embodiment of the invention, an inhibitor composition comprising meglumine is provided, wherein the composition inhibits the activity of catechol oxidase. In one embodiment, the inhibitor composition comprises meglumine, or salts thereof. In another embodiment, the inhibitor composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the inhibitor composition comprises l-deoxy-l-(methylamino)-D-xylitol (DYN30). In still another embodiment, the composition consists of meglumine. In still another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the inhibitor composition consists of l-deoxy-l-(methylamino)-D-xylitol (DYN30).
In yet another embodiment of the invention, an inhibitor composition is provided, wherein the composition inhibits the accumulation of melanin in the skin of a mammal. In one embodiment, the inhibitor composition comprises meglumine, or salts thereof. In another embodiment, the inhibitor composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the inhibitor composition comprises 1-deoxy-L-(methylamino)-D-xylitol (DYN30). In still another embodiment, the composition consists of meglumine. In still another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the inhibitor composition consists 1-deoxy-L-(methylamino)-D-xylitol (DYN30).

In one embodiment of the invention, an inhibitor composition is a depigmenting agent. In another embodiment, meglumine, or salts thereof, is a depigmenting agent. In yet another embodiment, an analogue, modification, derivative or adduct of meglumine is a depigmenting agent. In another embodiment, the inhibitor composition comprises 1-deoxy-L-(methylamino)-D-xylitol (DYN30). In still another embodiment, the composition consists of meglumine. In still another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the inhibitor composition consists 1-deoxy-L-(methylamino)-D-xylitol (DYN30).

In another embodiment of the invention, depigmenting agents may be added as an additional agent in the present invention. Depigmenting agents include tyrosinase inhibitors such hydroquinone and its derivatives (e.g., hydroquinone monomethyl ether, hydroquinone monoethyl ether, arbutin), glucosamine and N-acetyl glucosamine, and derivatives thereof, soy and derivatives thereof, retinoids such as retinol; Kojic acid and its derivatives (e.g., kojic dipalmitate); tranexamic acid; vitamins such as niacin, vitamin C and its derivatives; azelaic acid; phytic acid, licorice; mulberry extracts; extracts from rumex species such as rumex crispus extract; chamomile extracts; green tea extracts; lactic acid, pearl extract, Tricholoma matsutake extract, magnesium-ascorbyl-phosphate, edelweiss extract, sedum acre extract, arbutine, ergothione, phyllantus emblica extract, α-MSH antagonists such as Undecylenoyl phenylalanine, germanium, and GABA and songyi mushroom. Bowman-Birk Inhibitors are described in U.S. Pat. No. 6,750,229 (e.g., inhibitors derived from the leguminosae, solanaceae, gramineae or cucurbitaceae family).
Dermalight™ and Clariskin™ from Silab are also depigmenting agents that can be used. Kinetin (N6 furfuryladenine) is a 6-(R-amino) purine cytokinin and is described in U.S. Pat. Nos. 5,602,139, 5,164,394, and 5,021,422. It has been shown to have anti-aging effects on the skin of dogs as well as the depigmenting effects without adverse effects. (Kimura T, Doi K., Rejuvenation Res. 2004 Spring; 7(l):32-9).

Compositions of the invention are those suitable for the application to human skin according to the method of the present invention, which optionally include a further skin benefit agent. Suitable additional skin benefit agents include anti-aging, wrinkle-reducing, skin whitening, anti-acne, and sebum reduction agents. Examples of these include alpha-hydroxy acids, beta-hydroxy acids, polyhydroxy acids, betulinic acid, hyaluronic acid, hydroquinone, t-butyl hydroquinone, Vitamin B derivatives, Vitamin C derivatives; allantoin, a placenta extract; dioic acids, retinoids, and resorcinol derivatives.

An acceptable vehicle may act as a dilutant, dispersant or carrier for the skin benefit ingredients in the composition, so as to facilitate their distribution when the composition is applied to the skin.

The vehicle may be aqueous, anhydrous or an emulsion. Preferably, the compositions are aqueous or an emulsion, especially water-in-oil or oil-in-water emulsion, preferably oil in water emulsion. Water when present will be in amounts which may range from 5 to 99%, preferably from 20 to 70%, optimally between 40 and 70% by weight.

Other acceptable carriers which are useful include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. Examples of these and other pharmaceutically acceptable carriers are described in Remington's Pharmaceutical Sciences (1991, Mack Publication Co., New Jersey).

Besides water, relatively volatile solvents may also serve as carriers within compositions of the present invention. Most preferred are monohydric C1-C3 alkanols. These include ethyl alcohol, methyl alcohol and isopropyl alcohol. The amount of monohydric alkanol may range from 1 to 70%, preferably from 10 to 50%, optimally between 15 to 40% by weight.
Emollient materials may also serve as acceptable carriers. These may be in the form of silicone oils and synthetic esters. Amounts of the emollients may range anywhere from 0.1 to 50%, preferably between 1 and 20% by weight.

Silicone oils may be divided into the volatile and non-volatile variety. The term "volatile" as used herein refers to those materials which have a measurable vapor pressure at ambient temperature. Volatile silicone oils are preferably chosen from cyclic or linear polydimethylsiloxanes containing from 3 to 9, preferably from 4 to 5, silicon atoms. Linear volatile silicone materials generally have viscosities less than about 5 centistokes at 25°C, while cyclic materials typically have viscosities of less than about 10 centistokes. Nonvolatile silicone oils useful as an emollient material include polyalkyl siloxanes, polyalkylaryl siloxanes and polyether siloxane copolymers. The essentially non-volatile polyalkyl siloxanes useful herein include, for example, polydimethyl siloxanes with viscosities of from about 5 to about 25 million centistokes at 25°C. Among the preferred non-volatile Emollients useful in the present compositions are the polydimethyl siloxanes having viscosities from about 10 to about 400 centistokes at 25°C.

Among the ester emollients are: (1) Alkenyl or alkyl esters of fatty acids having 10 to 20 carbon atoms. Examples thereof include isooarachidyl neopentanoate, isononyl isonanonoate, oleyl myristate, oleyl stearate, and oleyl oleate. (2) Ether-esters such as fatty acid esters of ethoxylated fatty alcohols. (3) Polyhydric alcohol esters. Ethylene glycol mono and di-fatty acid esters, diethylene glycol mono- and di-fatty acid esters, polyethylene glycol (200-6000) mono- and di-fatty acid esters, propylene glycol mono- and di-fatty acid esters, polypropylene glycol 2000 monooleate, polypropylene glycol-2000 monostearate, ethoxylated propylene glycol monostearate, glyceryl mono- and di-fatty acid esters, polyglycerol poly-fatty esters, ethoxylated glyceryl mono-stearate, 1,3-butylene glycol monostearate, 1,3-butylene glycol distearate, polyoxyethylene polyol fatty acid ester, sorbitan fatty acid esters, and polyoxyethylene sorbitan fatty acid esters are satisfactory polyhydric alcohol esters. (4) Wax esters such as beeswax, spermaceti, myristyl myristate, stearyl stearate and arachidyl behenate. (5) Sterols esters, of which cholesterol fatty acid esters are examples.

Fatty acids having from 10 to 30 carbon atoms may also be included as cosmetically acceptable carriers for compositions of this invention. Illustrative of this
category are pelargonic, lauric, myristic, palmitic, stearic, isostearic, hydroxystearic, oleic, linoleic, ricinoleic, arachidic, behenic and erucic acids.

Humectants of the polyhydric alcohol-type may also be employed as cosmetically acceptable carriers in compositions of this invention. The humectant aids in increasing the effectiveness of the emollient, reduces scaling, stimulates removal of built-up scale and improves skin feel. Typical polyhydric alcohols include glycerol, polyalkylene glycols and more preferably alkylene polyols and their derivatives, including propylene glycol, dipropylene glycol, polypropylene glycol, polyethylene glycol and derivatives thereof, sorbitol, hydroxypropyl sorbitol, hexylene glycol, 1,3-butylene glycol, 1,2,6-hexanetriol, ethoxylated glycerol, propoxylated glycerol and mixtures thereof. For best results the humectant is preferably propylene glycol or sodium hyaluronate. The amount of humectant may range anywhere from 0.5 to 30%, preferably between 1 and 15% by weight of the composition.

Thickeners may also be utilized as part of the compositions according to the present invention. Typical thickeners include crosslinked acrylates (e.g. Carbopol 982), hydrophobically-modified acrylates (e.g. Carbopol 1382), cellulosic derivatives and natural gums. Among useful cellulosic derivatives are sodium carboxymethylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, ethyl cellulose and hydroxymethyl cellulose. Natural gums suitable for the present invention include guar, xanthan, sclerotium, carrageenan, pectin and combinations of these gums. Amounts of the thickener may range from 0.0001 to 5%, usually from 0.001 to 1%, optimally from 0.01 to 0.5% by weight.

Collectively the water, solvents, silicones, esters, fatty acids, humectants and/or thickeners will constitute an acceptable carrier in amounts from 1 to 99.9%, preferably from 80 to 99% by weight.

An oil or oily material may be present, together with an emulsifier to provide either a water-in-oil emulsion or an oil-in-water emulsion, depending largely on the average hydrophilic-lipophilic balance (HLB) of the emulsifier employed.

Surfactants may also be present in compositions of the present invention. Total concentration of the surfactant will range from 0.1 to 40%, preferably from 1 to 20%, optimally from 1 to 5% by weight of the composition. The surfactant may be selected from the group consisting of anionic, nonionic, cationic and amphoteric actives. Particularly preferred nonionic surfactants are those with a C10
C₂₀ fatty alcohol or acid hydrophobe condensed with from 2 to 100 moles of ethylene oxide or propylene oxide per mole of hydrophobe; C₂₋₁₅ₐlkal phenols condensed with from 2 to 20 moles of alkylene oxide; mono- and di-fatty acid esters of ethylene glycol; fatty acid monoglyceride; sorbitan, mono- and di- C₈ C₂₀ fatty acids; block copolymers (ethylene oxide/propylene oxide); and polyoxyethylene sorbitan as well as combinations thereof. Alkyl polyglycosides and saccharide fatty amides (e.g. methyl gluconamides) are also suitable nonionic surfactants.

Preferred anionic surfactants include soap, alkyl ether sulfate and sulfonates, alkyl sulfates and sulfonates, alkylbenzene sulfonates, alkyl and dialkyl sulfosuccinates, C₈ C₂₀ acyl isethionates, acyl glutamates, C₈ C₂₀ alkyl ether phosphates and combinations thereof.

Other adjunct minor components may also be incorporated into the compositions of the present invention. These ingredients may include coloring agents and/or pigments; opacifiers, perfumes, other thickeners, plasticizers; calamine; antioxidants; chelating agents; as well as additional sunscreens, such as organic sunscreens. Amounts of these other adjunct minor components may range anywhere from 0.001% up to 20% by weight of the composition. For use as sunscreen, metal oxides may be used alone or in mixture and/or in combination with organic sunscreens. Examples of organic sunscreens include but are not limited to

Benzophenone-1 (UVINUL 400; BASF Chemical Co.), Benzophenone-2 (UVINUL D-50; BASF Chemical Co.), Benzophenone-3 (UVINUL M-40; BASF Chemical Co.), Benzophenone-4 (UVINUL MS-40; BASF Chemical Co.), Benzophenone-6 (UVINUL D-49; BASF Chemical Co.), Benzophenone-8 (SPECRA-SORB UV-24; American Cyanamide), Benzophenone-12 (UVINUL 408; BASF Chemical Co.),

Benzophenone-12 (UVINUL HYDRO; Bernel Chemical), Ethyl dihydroxypropyl- PABA (AMERSCREEN P; Amerchol Corp.), Glyceryl PABA (NIPA G.M.P.A.; Nipa Labs.), Homosalate (KEMESTER HMS; Hunko Chemical), Methyl anthranilate (SUNAROME UVA; Felton Worldwide) Octocrylene (UVINUL N-539; BASF Chemical Co.), Octyl dimethyl PABA (AMERSCOL; Amerchol Corp.), Octyl methoxycinnamate (PARSOL MCX; Bernel Chemical), Octyl salicylate

(SUNAROME WMO; Felton Worldwide), PABA (PABA; National Starch), 2-Phenylbenzimidazole-5-sulphonic acid (EUSOLEX 232; EM Industries), TEA salicylate (SUNAROME W, Felton Worldwide), 3-(4-methylbenzylidene)-camphor
(EUSOLEX 6300; EM Industries), 4-Isopropyl dibenzoyl methane (EUSOLEX 8020; EM Industries) Butyl methoxy dibenzoyl methane, (PARSOL 1789; Givaudan Corp.), Etocrylene (UVINUL N-35; BASF Chemical Co.).

The amount of the organic sunscreens in the composition is preferably in the range of about 0.1 wt % to about 10 wt %, more preferably about 1 wt % to 5 wt %.

As will be understood by the skilled artisan, when armed with the disclosure set forth herein, a composition useful in the present invention can include one active ingredient. Alternatively, a composition useful in the present invention can include at least two active ingredients. In one aspect, multiple active ingredients may be active in an additive manner. In another aspect, multiple active ingredients may be active in a synergistic manner. That is, the multiple active ingredients in a composition of the invention may provide a therapeutic effect that is greater than the addition of the therapeutic effects provided by each of the active ingredients alone.

**Pharmaceutical Compositions and Methods for Administration**

The invention relates to the administration of an identified compound in a pharmaceutical or cosmetic composition to practice the methods of the invention, the composition comprising the compound or an appropriate derivative or analogue of the compound and a pharmaceutically-acceptable carrier. For example, a chemical composition with which an appropriate inhibitor of at least one of tyrosinase, catechol oxidase, a tyrosinase-like enzyme, and a catechol oxidase-like enzyme, is used to administer the appropriate compound to an animal. The invention should be construed to include the use of one, or simultaneous use of more than one, inhibitor of tyrosinase, catechol oxidase, a tyrosinase-like enzyme, and a catechol oxidase-like enzyme. When more than one inhibitor is used, they can be administered together or they can be administered separately.

In one embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In another embodiment, the pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 g/kg/day.
The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides.

The compositions of the invention may be administered via numerous routes, including, but not limited to, oral, parenteral, topical, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the compound such as heparin sulfate, or a biological equivalent thereof, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer compounds according to the methods of the invention.

Compounds which are identified using any of the methods described herein may be formulated and administered to a mammal for treatment of skin aging and skin-related conditions described herein.
The invention encompasses the preparation and use of pharmaceutical compositions comprising a compound useful for treatment of various skin related conditions described herein, including skin aging and photoaging. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise at least one active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

An obstacle for topical administration of pharmaceuticals is the stratum corneum layer of the epidermis. The stratum corneum is a highly resistant layer comprised of protein, cholesterol, sphingolipids, free fatty acids and various other lipids, and includes cornified and living cells. One factor that limits the penetration rate (flux) of a compound through the stratum corneum is the amount of the active substance which can be loaded or applied onto the skin surface. The greater the amount of active substance which is applied per unit of area of the skin, the greater the concentration gradient between the skin surface and the lower layers of the skin, and in turn the greater the diffusion force of the active substance through the skin. Therefore, a formulation containing a greater concentration of the active substance is more likely to result in penetration of the active substance through the skin, and more of it, and at a more consistent rate, than a formulation having a lesser concentration, all other things being equal.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts.
Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.
Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Enhancers of permeation may be used. These materials increase the rate of penetration of drugs across the skin. Typical enhancers in the art include ethanol, glycerol monolaurate, PGML (polyethylene glycol monolaurate), dimethylsulfoxide, and the like. Other enhancers include oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone.

One acceptable vehicle for topical delivery of some of the compositions of the invention may contain liposomes. The composition of the liposomes and their use are known in the art (for example, see Constanza, U.S. Patent No. 6,323,219).

The source of active compound to be formulated will generally depend upon the particular form of the compound. Small organic molecules and peptidyl or oligo fragments can be chemically synthesized and provided in a pure form suitable for pharmaceutical/cosmetic usage. Products of natural extracts can be purified according to techniques known in the art. Recombinant sources of compounds are also available to those of ordinary skill in the art.

In alternative embodiments, the topically active pharmaceutical or cosmetic composition may be optionally combined with other ingredients such as moisturizers, cosmetic adjuvants, anti-oxidants, chelating agents, bleaching agents, skin lighteners, tyrosinase inhibitors and other known depigmentation agents, surfactants, foaming agents, conditioners, humectants, wetting agents, emulsifying agents, fragrances, viscosifiers, buffering agents, preservatives, sunscreens and the like. In another embodiment, a permeation or penetration enhancer is included in the composition and is effective in improving the percutaneous penetration of the active ingredient into and through the stratum corneum with respect to a composition lacking
the permeation enhancer. Various permeation enhancers, including oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone, are known to those of skill in the art. In another aspect, the composition may further comprise a hydrotropic agent, which functions to increase disorder in the structure of the stratum corneum, and thus allows increased transport across the stratum corneum. Various hydrotropic agents such as isopropyl alcohol, propylene glycol, or sodium xylene sulfonate, are known to those of skill in the art. The compositions of this invention may also contain active amounts of retinoids (i.e., compounds that bind to any members of the family of retinoid receptors), including, for example, tretinoin, retinol, esters of tretinoin and/or retinol and the like.

The topically active pharmaceutical or cosmetic composition should be applied in an amount effective to affect desired changes. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where a change is desired. An active compound should be present in the amount of from about 0.0001% to about 15% by weight volume of the composition. More preferable, it should be present in an amount from about 0.0005% to about 5% of the composition; most preferably, it should be present in an amount of from about 0.001% to about 1% of the composition. Such compounds may be synthetically-or naturally-derived.

Liquid derivatives and natural extracts made directly from biological sources may be employed in the compositions of this invention in a concentration (w/v) from about 1 to about 99%. Fractions of natural extracts and protease inhibitors may have a different preferred range, from about 0.01% to about 20% and, more preferably, from about 1% to about 10% of the composition. Of course, mixtures of the active agents of this invention may be combined and used together in the same formulation, or in serial applications of different formulations.

The composition of the invention may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of an aqueous gel because of repeated patient use when it is exposed to contaminants in the environment from, for example, exposure to air or the patient's skin, including contact with the fingers used for applying a composition of the invention such as a therapeutic gel or cream. Examples of preservatives useful
in accordance with the invention included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. A particularly preferred preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

The composition preferably includes an antioxidant and a chelating agent which inhibit the degradation of the compound for use in the invention in the aqueous gel formulation. Preferred antioxidants for some compounds are BHT, BHA, alphatocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition which may be detrimental to the shelf life of the formulation. While BHT and disodium edetate are the particularly preferred antioxidant and chelating agent respectively for some compounds, other suitable and equivalent antioxidants and chelating agents may be substituted therefor as would be known to those skilled in the art.

Controlled-release preparations may also be used and the methods for the use of such preparations are known to those of skill in the art.

In some cases, the dosage forms to be used can be provided as slow or controlled-release of one or more active ingredients therein using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled-release formulations known to those of ordinary skill in the art, including those described herein, can be readily selected for use with the pharmaceutical compositions of the invention. Thus, single unit dosage forms suitable for oral administration, such as tablets, capsules, gelcaps, and caplets that are adapted for controlled-release are encompassed by the present invention.

All controlled-release pharmaceutical products have a common goal of improving drug therapy over that achieved by their non-controlled counterparts.
Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include extended activity of the drug, reduced dosage frequency, and increased patient compliance. In addition, controlled-release formulations can be used to affect the time of onset of action or other characteristics, such as blood level of the drug, and thus can affect the occurrence of side effects.

Most controlled-release formulations are designed to initially release an amount of drug that promptly produces the desired therapeutic effect, and gradually and continually release of other amounts of drug to maintain this level of therapeutic effect over an extended period of time. In order to maintain this constant level of drug in the body, the drug must be released from the dosage form at a rate that will replace the amount of drug being metabolized and excreted from the body.

Controlled-release of an active ingredient can be stimulated by various inducers, for example pH, temperature, enzymes, water, or other physiological conditions or compounds. The term "controlled-release component" in the context of the present invention is defined herein as a compound or compounds, including, but not limited to, polymers, polymer matrices, gels, permeable membranes, liposomes, or microspheres or a combination thereof that facilitates the controlled-release of the active ingredient.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water, and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents
include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin, and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para- hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water, and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further
comprise one or more emulsifying agents such as naturally occurring gums such as
gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or
lecithin phosphatide, esters or partial esters derived from combinations of fatty acids
and hexitol anhydrides such as sorbitan monooleate, and condensation products of
such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate.
These emulsions may also contain additional ingredients including, for example,
sweetening or flavoring agents.

As used herein, an "oily" liquid is one which comprises a carbon-
containing liquid molecule and which exhibits a less polar character than water.

A formulation of a pharmaceutical composition of the invention
suitable for oral administration may be prepared, packaged, or sold in the form of a
discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a
cachet, a troche, or a lozenge, each containing a predetermined amount of the active
ingredient. Other formulations suitable for oral administration include, but are not
limited to, a powdered or granular formulation, an aqueous or oily suspension, an
aqueous or oily solution, a paste, a gel, a toothpaste, a mouthwash, a coating, an oral
rinse, or an emulsion. The terms oral rinse and mouthwash are used interchangeably
herein.

A pharmaceutical composition of the invention may be prepared,
packaged, or sold in a formulation suitable for oral or buccal administration. Such a
formulation may comprise, but is not limited to, a gel, a liquid, a suspension, a paste,
a toothpaste, a mouthwash or oral rinse, and a coating. For example, an oral rinse of
the invention may comprise a compound of the invention at about 1.4 %,
chlorhexidine gluconate (0.12%), ethanol (11.2%), sodium saccharin (0.15%), FD&C
Blue No. 1 (0.001%), peppermint oil (0.5%), glycerine (10.0%), Tween 60 (0.3%),
and water to 100%. In another embodiment, a toothpaste of the invention may
comprise a compound of the invention at about 5.5%, sorbitol, 70% in water (25.0%),
sodium saccharin (0.15%), sodium lauryl sulfate (1.75%), carbolopol 934, 6%
dispersion in (15%), oil of spearmint (1.0%), sodium hydroxide, 50% in water
(0.76%), dibasic calcium phosphate dihydrate (45%), and water to 100%. The
examples of formulations described herein are not exhaustive and it is understood that
the invention includes additional modifications of these and other formulations not
described herein, but which are known to those of skill in the art.
A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture. Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate. Known surface-active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide for pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients.
including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e., about 20°C) and which is liquid at the rectal temperature of the subject (i.e., about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e., such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to,
subcutaneous, intraperitoneal, intramuscular, intrastemal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer system. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such
formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed. (1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA), which is incorporated herein by reference.

Typically, dosages of the compound of the invention which may be administered to an animal, preferably a human, will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration.

The compound can be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.
II. Methods

The invention features methods of lightening the skin of a mammal. In one embodiment, a method of lightening the skin of a mammal includes the administration of a composition to a mammal, wherein the composition comprises meglumine, or salts thereof. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the composition consists of meglumine. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists l-deoxy-l-(methylamino)-D-xylitol (DYN30).

The invention also features methods of reducing pigmentation in the skin of a mammal. In one embodiment, a method for reducing pigmentation in the skin of a mammal includes the administration of a composition to a mammal, wherein the composition comprises meglumine, or salts thereof. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the composition consists of meglumine. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists l-deoxy-l-(methylamino)-D-xylitol (DYN30).

The invention additionally features methods of preventing darkening of the skin of a mammal. In one embodiment, a method for preventing darkening of the skin of a mammal includes the administration of a composition to a mammal, wherein the composition comprises meglumine, or salts thereof. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the composition consists of meglumine. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists l-deoxy-l-(methylamino)-D-xylitol (DYN30).
The invention also features a method of inhibiting tyrosinase in the skin of a mammal. In one embodiment, a method of inhibiting tyrosinase in the skin of a mammal includes the administration of a composition to a mammal, wherein the composition comprises meglumine, or salts thereof. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the composition consists of meglumine. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists 1-deoxy-l-(methylamino)-D-xylitol (DYN30).

In another aspect, the invention features a method of inhibiting catechol oxidase activity. In one embodiment, a method for inhibiting catechol oxidase activity in the skin of a mammal includes the administration of a composition to a mammal, wherein the composition comprises meglumine, or salts thereof. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the composition consists of meglumine. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists 1-deoxy-l-(methylamino)-D-xylitol (DYN30).

In still another aspect, the invention features a method of inhibiting the accumulation of melanin in the skin of a mammal. In one embodiment, a method of inhibiting melanin accumulating in the skin of a mammal comprises administration of a composition to a mammal, wherein the composition comprises meglumine, or salts thereof. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine. In yet another embodiment, the composition consists of meglumine. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists 1-deoxy-l-(methylamino)-D-xylitol (DYN30).
III. Kits

The invention encompasses various kits relating to skin lightening in an organism, preferably a mammal, even more preferably a human. The kit comprises a cosmetically acceptable or pharmaceutically acceptable composition comprising at least one skin lightening agent. In one embodiment, a skin lightening agent is meglumine, or salts thereof. In additional embodiments, the kits of the present invention comprise a composition comprising meglumine, or salts thereof, wherein the composition inhibits the activity of catechol oxidase. In another embodiment, the composition comprises an analogue, modification, derivative or adduct of meglumine.

In yet another embodiment, the composition consists of meglumine, or salts thereof. In another embodiment, the composition consists of an analogue, modification, derivative or adduct of meglumine. In another embodiment, the composition comprises 1-deoxy-l-(methylamino)-D-xylitol (DYN30). In yet another embodiment, the inhibitor composition consists 1-deoxy-l-(methylamino)-D-xylitol (DYN30).

In one aspect of the invention, the kit comprises a composition that is a depigmenting agent. In another aspect, meglumine, or salts thereof, is a depigmenting agent. In yet another aspect, an analogue, modification, derivative or adduct of meglumine is a depigmenting agent. In another embodiment, 1-deoxy-l-(methylamino)-D-xylitol (DYN30) is a depigmenting agent.

In another aspect, depigmenting agents may be added as an additional agent in the present invention. Depigmenting agents include tyrosinase inhibitors such as hydroquinone and its derivatives (e.g., hydroquinone monomethyl ether, hydroquinone monoethyl ether, arbutin), soy and derivatives thereof, retinoids such as retinol; Kojic acid and its derivatives (e.g., kojic dipalmitate); tranexamic acid; vitamins such as niacin, vitamin C and its derivatives; azelaic acid; phytic acid, licorice; mulberry extracts; extracts from rumex species such as rumex crispus extract; chamomile extracts; green tea extracts; lactic acid, pearl extract, Tricholoma matsutake extract, magnesium-ascorbyl-phosphate, edelweiss extract, sedum acre extract, arbutine, ergothione, phyllantus emblica extract, α-MSH antagonists such as Undecylenoyl phenylalanine, germanium, and GABA and songyi mushroom.

Bowman-Birk Inhibitors are described in U.S. Pat. No. 6,750,229 (e.g., inhibitors derived from the leguminosae, solanaceae, gramineae or cucurbitaceae family). Dermalight™ and Clariskin™ from Silab are also depigmenting agents that can be
used. Kinetin (N6 furfuryladenine) is a 6-(R-amino) purine cytokinin and is described in U.S. Pat. Nos. 5,602,139, 5,164,394, and 5,021,422. It has been shown to have anti-aging effects.

The kit further comprises an optional applicator and instructional material for the use thereof to be used in accordance with the teachings provided herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Methods

Transdermal Drug Delivery

There are several advantages to delivering compounds, including drugs or other therapeutic agents, into the body through the skin, a process called transdermal drug delivery. Transdermal drug delivery offers an attractive alternative to injections and oral medications. It provides the capacity for multi day therapy with a single application thereby improving patient compliance. Such delivery would extend the activity of drugs having short half-life through the reservoir of drug present in the delivery system and its controlled release characteristics. Transdermal drug delivery avoids gastrointestinal tract difficulties during absorption caused by enzymes or drug interactions with food. Not only that, it avoids first pass i.e. the initial passage of a drug substance through the systemic and portal circulation. However, applications of transdermal drug delivery are limited to only a few drugs as

Transdermal transport of solutes is largely controlled by stratum corneum lipid bilayers. Solute transport in stratum corneum lipid bilayers, like in other lipid bilayer systems, is highly anisotropic and size-dependent. Specifically, lipid bilayers exhibit strong structural heterogeneity that results in spatial variations in solute partition and diffusion coefficients. As a result, molecules are believed to diffuse across skin following a tortuous pathway within either the tail-group (for hydrophobic molecules) or head-group (for hydrophilic molecules) regions, in which transport between bilayers can occur at bilayer-bilayer interfaces or other sites of structural disorganization [Marrink, SJ. and Berendsen, HJ. Permeation Process of Small Molecules across Lipid Membranes Studied by Molecular Dynamics Simulations. 1996. J. Phys. Chem. 100(41): p.16729 -16738].

A few drugs will penetrate the skin effectively. Nicotine, estrogen, scopolamine, fentanyl, and nitroglycerine are among the few drugs that can be successfully delivered transdermally from patches simply because they are relatively small and potent at small doses of 0.1 mg to 15 mg/day [Kanikkannan, N. et al. Structure-activity relationship of chemical penetration enhancers in transdermal drug delivery. 2000. Curr Med Chem 7(6): p.593-608]. Many other drugs can be delivered only when an additional enhancement system is provided to "force" them to pass through the skin. Among several methods of transdermal drug delivery are electroporation, sonophoresis, iontophoresis, permeation enhancers (cyclodextrins), and liposomes.

Compounds of this invention can be administered via topical use of any of these transdermal delivery methods.

Liposomes

Liposomes are microscopic, fluid-filled pouches whose walls are made of layers of phospholipids identical to those that make up the cell membranes. They are well known and their structures and properties have been thoroughly researched. Essentially, they are small uni- or multi-lamellar lipid/water structures with diameters in the nanometer to micron range. Liposomes can be formed from a variety of natural phospholipids, such as cholesterol, stearylamine, or phosphatidylcholines. They can
be formulated to incorporate a wide range of materials as a payload either in the water or in the lipid compartments.

Liposomes are extremely versatile and are variable due to their composition. They can be used to deliver vaccines, proteins (enzymes), nucleotides, plasmids, drugs, or cosmetics to the body. Liposomes can be used as carriers for lipophilic drugs like the anti-tumor and the anti-viral derivatives of AZT [Kamps, J.A. et al. Preparation and characterization of conjugates of (modified) human serum albumin and liposomes: drug carriers with an intrinsic anti-HIV activity. 1996. Biochim Biophys Acta 1278(2): p.183-90]. Insulin can also be delivered via liposomes [Muramatsu, K. et al. The relationship between the rigidity of the liposomal membrane and the absorption of insulin after nasal administration of liposomes modified with an enhancer containing insulin in rabbits. 1999. Drug Dev Ind Pharm 25(10): p.1099-105]. For medical uses as drug carriers, the liposomes can also be injected intravenously and when they are modified with lipids, their surfaces become more hydrophilic and hence the circulation time in the bloodstream can be increased significantly. Such so-called "stealth" liposomes are especially being used as carriers for hydrophilic (water soluble) anti cancer drugs like doxorubicin. Toxantrone and others are especially effective in treating diseases that affect the phagocytes of the immune system because they tend to accumulate in the phagocytes, which recognize them as foreign invaders [Rentsch, K.M. et al. Determination of mitoxantrone in mouse whole blood and different tissues by high-performance liquid chromatography. 1996. J Chromatogr B Biomed Appl 679(1-2): p.185-92]. They have also been used experimentally to carry normal genes into a cell to replace defective, disease-causing genes [Guo, W. and Lee, RJ. Efficient gene delivery using anionic liposome-complexed polyplexes (LPDII). 2000. Biosci Rep 20(5): p.419-32].

Liposomes are also sometimes used in cosmetics because of their moisturizing qualities. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble.

**Sonophoresis**

Sonophoresis or phonophoresis has been widely used in sports medicine since the sixties. Controlled studies in humans in vivo have demonstrated absence or mild effects of the technique with the parameters currently used (frequency...
1-3 MHz, intensity 1-2 W/cm(2), duration 5-10 mins, continuous or pulse mode). However, it was demonstrated in 1995 that administration of macromolecules with conserved biological activity was feasible in animals in vivo using low frequency ultrasound. This led to new research into this method of transdermal administration [Machet, L. and Boucaud, A. Phonophoresis: efficiency, mechanisms and skin tolerance. 2002. Int J Pharm 243(1-2): p.1-15].

In this method, a short application of ultrasound is used to permeabilize skin for a prolonged period of time. The enhancement induced by ultrasound is particularly significant at low-frequencies (f<100 kHz). During this period, ultrasonically permeabilized skin may be utilized for drug delivery. In addition, a sample of interstitial fluid or its components may be extracted through permeabilized skin for diagnostic applications. Detailed studies on drug delivery have been performed using insulin and mannitol as model drugs. Studies on diagnostics were performed using glucose as a model analyte [Mitragotri, S. and Kost, J. Low-frequency sonophoresis: a noninvasive method of drug delivery and diagnostics. 2000. Biotechnol Prog 16(3): p.488-92].

In vitro, in vivo, as well as clinical studies have also demonstrated the successful effect of low-frequency ultrasound on transdermal drug delivery and glucose extraction. Mechanistic insights gained through a number of investigations have also been reviewed [Mitragotri, S. and Kost, J. Low-frequency sonophoresis: a review. 2004. Adv Drug Deliv Rev 56(5): p.589-601].

At the School of Pharmacy, Faculty of Sciences, University of Geneva, a study was done to shed light on the mechanism(s) by which low-frequency ultrasound (20 KHz) enhances the permeability of the skin. The physical effects on the barrier and the transport pathway, in particular, were examined. The amount of lipid removed from the intercellular domains of the stratum corneum following sonophoresis was determined by infrared spectroscopy. Transport of the fluorescent probes nile red and calcein, under the influence of ultrasound, was evaluated by laser-scanning confocal microscopy. The results were compared with the appropriate passive control data and with data obtained from experiments in which the skin was exposed simply to the thermal effects induced by ultrasound treatment. A significant fraction of the intercellular lipids of the stratum corneum (approximately 30%) which are principally responsible for skin barrier function, were removed during the
application of low-frequency sonophoresis. Although the confocal images from the nile red experiments were not particularly informative, ultrasound clearly and significantly (again, relative to the corresponding controls) facilitated transport of the hydrophilic calcein via discrete permeabilized regions, whereas other areas of the barrier were apparently unaffected. Lipid removal from the stratum corneum is implicated as a factor contributing the observed permeation enhancement effects of low-frequency ultrasound [Alvarez-Roman, R. et al. Skin permeability enhancement by low frequency sonophoresis: lipid extraction and transport pathways. 2003. J Pharm Sci 92(6): p.1138-46].

The impact of low-frequency sonophoresis appears to be much more important than that of high-frequency sonophoresis, with significant increases in transport into and from the skin following its application. Although the mechanism of action remains incompletely defined, cavitation and thermal processes are strongly implicated [Merino, G. et al. Ultrasound-enhanced transdermal transport. 2003. J Pharm Sci 92(6): p.1125-37].

In another study, application of low-frequency ultrasound was shown to increase skin permeability, thereby facilitating delivery of macromolecules (low-frequency sonophoresis. The study sought to determine a theoretical description of transdermal transport of hydrophilic permeants induced by low-frequency sonophoresis. Parameters such as pore size distribution, absolute porosity, and dependence of effective tortuosity on solute characteristics were investigated. Pig skin was exposed to low-frequency ultrasound at 58 kHz to achieve different skin resistivities. Transdermal delivery of four permeants [mannitol, luteinizing hormone releasing hormone (LHRH), inulin, dextran] in the presence and absence of ultrasound was measured. The porous pathway model was modified to incorporate the permeant characteristics into the model and to achieve a detailed understanding of the pathways responsible for hydrophilic permeant delivery. The slopes of the log kp(p) versus log R graphs for individual solutes changed with solute molecular area, suggesting that the permeability-resistivity correlation for each permeant is related to its size. The tortuosity that a permeant experiences within the skin also depends on its size, where larger molecules experience a less tortuous path. With the modified porous pathway model, the effective tortuosities and skin porosity were calculated independently. The results of this study showed that low-frequency sonophoresis

In vitro experiments with full thickness pig skin to measure enhancements of skin conductivity and drug permeability have been performed and ultrasound was applied to pretreat the skin using a sonicator operating at a frequency of either 20 or 40 kHz. Pitting of aluminum foil was also noted to measure cavitation, which is the principal mechanism of low-frequency sonophoresis. The skin conductivity enhancement was found to be inversely proportional to the distance of the horn from the skin. As the intensity increased, skin conductivity enhancement also increased up to a certain threshold, and then dropped off. The intensities (I(max)) at which maximum enhancement occur are about 14 W/cm2 for 20 kHz and 17 W/cm2 for 40 kHz. These findings may be useful in optimizing low-frequency sonophoresis. Overall, the dependence of transport on ultrasound parameters is similar to that of aluminum foil pitting. Hence, these results support the role of cavitation in low-frequency sonophoresis [Terahara, T. et al. Dependence of low-frequency sonophoresis on ultrasound parameters; distance of the horn and intensity. 2002. Int J Pharm 235(1-2): p.35-42].

Enhancement of drug transport via low frequency sonophoresis is thought to be mediated through cavitation, the formation and collapse of gaseous bubbles. It has been hypothesized that the efficacy of low-frequency sonophoresis can be significantly enhanced by provision of nuclei for cavitation. In a particular study, two porous resins, Diaion HP20 and Diaion HP2MG (2MG), were used as cavitation nuclei. The effect of these resins on cavitation using pitting of aluminum foil was measured. 2MG showed a higher efficacy in enhancing cavitation compared with Diaion HP20. 2MG was also effective in enhancing transdermal mannitol transport. These results confirmed that the addition of cavitation nuclei such as porous resins further increases the effect of low-frequency ultrasound on skin permeability [Terahara, T. et al. Porous resins as a cavitation enhancer for low-frequency sonophoresis. 2002. J Pharm Sci 91(3): p.753-9].
Electroporation

Electroporation is the transitory structural perturbation of lipid bilayer membranes due to the application of very short (< 1 sec) high voltage pulses. Its application to the skin has been shown to increase transdermal drug delivery by several orders of magnitude. Moreover, electroporation used alone or in combination with other enhancement methods, expands the range of drugs (small to macromolecules, lipophilic or hydrophilic, charged or neutral molecules), which can be delivered transdermally. Molecular transport through transiently permeabilized skin by electroporation results mainly from enhanced diffusion and electrophoresis.

The efficacy of transport depends on the electrical parameters and the physicochemical properties of drugs. The in vivo application of high voltage pulses is well tolerated but muscle contractions are usually induced. The electrode and patch design is an important issue to reduce the discomfort of the electrical treatment in humans [Denet, A.R. et al. Skin electroporation for transdermal and topical delivery. 2004. Adv Drug Deliv Rev 56(5): p.659-74].

Iontophoresis

Iontophoresis or ElectroMotive Drug Administration (EMDA) is a very effective method of delivering drugs to the affected site that is commonly used in many countries including the USA. Instead of injecting the drug (usually a steroid) directly into the inflamed, iontophoresis spreads a high concentration of drug evenly through the tissue applying a low density electrical current for times ranging from minutes to hours that attracts the ions in the molecules of the drug and drives them through the skin to be absorbed by the inflamed tissue.

Transdermal iontophoretic delivery of hydrocortisone solubilized in an aqueous solution of hydroxypropyl-beta-cyclodextrin (HP-beta-CyD) has been investigated and compared with chemical enhancement of co-solvent formulations [Chang, S.L. and Banga, A.K. Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solutions. 1998. J Pharm Pharmacol 50(6): p.635-40]. The passive permeation of hydrocortisone through human cadaver skin was higher when delivered from propylene glycol than when delivered after solubilization in an aqueous solution of HP-beta-CyD. However, the iontophoretic delivery of the 1% hydrocortisone-9% HP-beta-CyD solution was higher than the amount delivered passively by the 1% hydrocortisone-propylene glycol formulation, even if oleic acid was used as a
chemical enhancer. Iontophoretic delivery of 1% hydrocortisone with 3% or 15% HP-beta-CyD was lower than that of the 9% HP-beta-CyD solution. These data suggest that free hydrocortisone rather than complexes is predominantly delivered iontophotically through the skin and the HP-beta-CyD complex serves as a carrier to replenish depletion of hydrocortisone. HP-beta-CyD prevents hydrocortisone from forming a skin reservoir. Iontophoresis provides better enhancement of transdermal delivery of hydrocortisone than the chemical approach when just sufficient HP-beta-CyD is added to solubilize the hydrocortisone [Chang, S.L. and Banga, A.K. Transdermal iontophoretic delivery of hydrocortisone from cyclodextrin solutions. 1998. J Pharm Pharmacol 50(6): p.635-40].

Penetration Enhancers

Another long-standing approach for improving transdermal drug delivery uses penetration enhancers (also called sorption promoters or accelerants), which penetrate into skin to reversibly decrease the barrier resistance. Numerous compounds have been evaluated for penetration enhancing activity, including sulphoxides (such as dimethylsulphoxide, DMSO), Azones (e.g. laurocapram), pyrrolidones (for example 2-pyrrolidone, 2P), alcohols and alkanols (ethanol, or decanol), glycols (for example propylene glycol, PG, a common excipient in topically applied dosage forms), surfactants (also common in dosage forms) and terpenes.

Many potential sites and modes of action have been identified for skin penetration enhancers. The intercellular lipid matrix in which the accelerants may disrupt the packing motif, the intracellular keratin domains or through increasing drug partitioning into the tissue by acting as a solvent for the permeant within the membrane. Further potential mechanisms of action, for example with the enhancers acting on desmosomal connections between corneocytes or altering metabolic activity within the skin, or exerting an influence on the thermodynamic activity/solubility of the drug in its vehicle are also feasible [Williams, A.C. and Barry, B.W. Penetration enhancers. 2004. Adv Drug Deliv Rev 56(5): p.603-18].

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic central cavity. Cyclodextrins are able to form water-soluble inclusion complexes with many lipophilic water-insoluble drugs. In aqueous solutions, drug molecules located in the central cavity are in a dynamic equilibrium with free drug molecules. Furthermore, lipophilic molecules in the
aqueous complexation media will compete with each other for a space in the cavity. Due to their size and hydrophilicity only insignificant amounts of cyclodextrins and drug/cyclodextrin complexes are able to penetrate into lipophilic biological barriers, such as intact skin. In general, cyclodextrins enhance topical drug delivery by increasing the drug availability at the barrier surface. At the surface the drug molecules partition from the cyclodextrin cavity into the lipophilic barrier. Thus, drug delivery from aqueous cyclodextrin solutions is both diffusion controlled and membrane controlled. It appears that cyclodextrins can only enhance topical drug delivery in the presence of water [Loftsson, T. and Masson, M. Cyclodextrins in topical drug formulations: theory and practice. 2001. Int J Pharm 225(1-2): p.15-30].

It is well known that cyclodextrins can enhance the permeation of poorly soluble drugs through biological membranes. However, the permeability will decrease if cyclodextrin is added in excess of the concentration needed to solvate the drug. The effect of cyclodextrins cannot be explained as solely due to increased solubility of the drug in the aqueous donor phase nor can it be explained by assuming that cyclodextrins act as classical permeation enhancers, i.e. by decreasing the barrier function of the lipophilic membrane. Researches have modeled the effect of cyclodextrins in terms of mixed barrier consisting of both diffusion and membrane controlled diffusion, where the diffusion of the drug in the aqueous diffusion layer is significantly slower than in the bulk of the donor. This diffusion model is described by simple mathematical equation where the properties of the system are expressed in terms of two constants P(M)/Kd and M 1/2. Data for the permeation of hydrocortisone through hairless mouse skin in the presence of various cyclodextrins, and cyclodextrin polymer mixtures, were fitted to obtain values for these two constants. The rise in flux with increased cyclodextrin complex concentration and fall with excess cyclodextrin was accurately predicted. Data for the permeation of drugs through semi-permeable cellophane membrane could also be fitted to the equation. It was concluded that cyclodextrins act as permeation enhancers carrying the drug through the aqueous barrier, from the bulk solution towards the lipophilic surface of biological membranes, where the drug molecules partition from the complex into the lipophilic membrane [Masson, M. et al. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. 1999. J Control Release 59(1): p.107-18].

Tyrosinase assay
Many various tyrosinase assays can be used in accordance with the present invention. One example of such an assay is set forth by Jeon et al. (2005) Bull. Korean Chem. Soc, Vol. 26: 1135-1 137). Briefly, the assay can be tested and/or standardized using mushroom tyrosinase, L-3,4-dihydroxyphenylalanine(L-dopa), and kojic acid were obtained (Sigma, St. Louis, MO). Human tyrosinase can be obtained by expression of a cDNA in E. coli, as described in the art. Recombinant human tyrosinase can be expressed in E. coli under the control of the tac promoter and purified by a DEAE-Sephacel column and an immobilized metal affinity column. The purified recombinant human tyrosinase should appear as a single protein band corresponding to a molecular weight of 66 kDa on SDS-PAGE, in agreement with the reported size of the human tyrosinase. Purified recombinant human tyrosinase is then used for the measurement of inhibitory effects.

L-Dopa oxidation of tyrosinases is assayed according to the methods known in the art. The inhibitory effects on the activity of tyrosinase are measured by pre-incubating the enzyme with each inhibitor for 2 min and initiating the reaction by addition of 3 mM L-dopa at 37 °C. The concentration of inhibitor giving 50% inhibition (IC50) is determined from a plot of residual activity against inhibitor concentration.

Example 1: Meglumine prevents normal browning of apple by inhibiting catechol oxidase activity

An apple (Red Delicious) was purchased and cut into two pieces. One half of the apple was dipped for 10 seconds skin side up into water and the other half dipped the same way into 10% meglumine-HCl. The pieces were left to sit at room temperature for over 2 hours. The photo in Figure 1 was taken at 2 hours. The half on the left (A) was dipped in water while the half on the right (B) was dipped in 10% meglumine-HCl. These data demonstrate that meglumine prevented the normal browning of the apple. The browning is due to the enzyme catechol oxidase, which is similar to the enzyme tyrosinase, an enzyme essential for melanin production in skin. Catechol oxidase is routinely used in the literature as a substitute for tyrosinase.

Example 2: Meglumine prevents normal browning of potato by inhibiting catechol oxidase activity
A potato was purchased and cut into two pieces. One half of the potato was dipped for 10 seconds skin side up into water and the other half dipped the same into 10% meglumine-HCl. The pieces were left to sit at room temperature for over 2 hours. The picture illustrated in Figure 2 was taken at 2 hours. The half on the left (A) was dipped in water while the half on the right (B) was dipped in 10% meglumine-HCl. These data demonstrate that meglumine prevented the normal browning of the potato. The browning is due to the enzyme catechol oxidase, which is similar to the enzyme tyrosinase, an enzyme essential for melanin production in skin. Catechol oxidase is routinely used in the literature as a substitute for tyrosinase.

Example 3: Meglumine inhibits catechol oxidase

A protein extract containing catechol oxidase was made by homogenizing 5 grams of a ripe but not soft banana in 10 ml of 50 mM potassium phosphate Buffer, pH 7.2 in a 15 ml dounce homogenizer. The homogenized banana extract transferred to an oak ridge centrifuge tube and centrifuged in a Beckman JA20 rotor for 15 minutes at 12,000 rpm. The extract supernatant was diluted with 50mM potassium phosphate buffer tol/10 before use. Catechol (Fisher #AC158981000) was prepared at 10 mM in water. All other compounds were dissolved in water to 1M.

Catechol oxidase converts catechol (clear) to benzoquinone (yellow). The ability of several compounds to prevent this conversion was tested as described in the table below (Table 1). The final volume for the assay was approximately 2.0 ml.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Tube Number</th>
<th>Final Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10 mM Catechol</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Banana Extract (1/10 dil)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1 M Meglumine</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>1 M Meglumine-HCl</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>1 M Arginine</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>1 M Sorbitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 M N-acetyl-glucosamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Reactants minus banana extract were mixed at room temperature. Banana extract was added and the reaction incubated at room temperature. Figure 3 shows the reaction at 10 minutes. Lane 1 is the control reaction without inhibitor; note the yellow color. Lanes 2 and 3 clearly demonstrate that meglumine or meglumine-HCl inhibits the activity of catechol oxidase since those tubes are clear. Arginine is not an inhibitor (lane 4) and does not appear to enhance the inhibition of catechol oxidase by meglumine. The sugars sorbitol (lane 7) and n-acetyl-glucosamine (lane 8) do not inhibit catechol oxidase.

Example 4: Meglumine inhibits tyrosinase

The enzyme tyrosinase is a crucial enzyme in the melanin synthesis pathway in mammalian skin. It converts L-dopa plus tyrosine into dopachrome. It also converts catechol (clear) to benzoquinone (yellow). The ability of several compounds to prevent this conversion was tested as described in Table 2. Mushroom Tyrosinase (Sigma T3824-25KU) was diluted in 1 ml of 50 mM Potassium Phosphate Buffer, pH 7.2, 10% glycerol to a concentration of 25 Kunits/ml. This was then diluted 1000-fold in 50 mM Potassium Phosphate Buffer, pH 7.2 before use in the reactions.

<table>
<thead>
<tr>
<th>Well Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>Final Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 units/ml Tyrosinase</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>2.5 u/ml</td>
<td></td>
</tr>
<tr>
<td>10 mM Catechol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>250 uM</td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>25</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Meglumine-HCl</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Arginine</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Kojik Acid</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Ascorbic Acid</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Sorbitol</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM Xylitol</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mM</td>
<td>20</td>
<td>10 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reactants minus tyrosinase were mixed at room temperature. Tyrosinase was added and the reaction incubated at room temperature. All reactions were run in duplicate. Figure 4 shows the reaction at 2 minutes. Wells IA and B are negative control reactions (no catechol or inhibitor). Wells 2A and B are positive controls; note the yellow color. Meglumine-HCl clearly inhibits tyrosinase (wells 3A and B). Tyrosinase also appears to have been inhibited by kojic acid (wells 5A and B), ascorbic acid (wells 6A and B), hydroquinone (wells 10A and B), and N-phenyl-thiourea (wells 11A and B). Both arginine (wells 4A and B) and glucosamine (wells 9A and B) create a red color of unknown origin. Neither sorbitol (wells 7A and B) nor xylitol (wells 8A and B) inhibit tyrosinase.

Example 5: Meglumine inhibition of tyrosinase is concentration dependent

The concentration-dependence of tyrosinase inhibition by Meglumine-HCl was determined. The reagents were as above and the ability of meglumine to inhibit tyrosinase was determined at 0.5, 1, 5, 10, 50, and 100 mM as detailed in table 3.

Table 3

<table>
<thead>
<tr>
<th>Well Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Final Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 units/ml Tyrosinase</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>2.5 u/ml</td>
</tr>
<tr>
<td>10 mM Catechol</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>250 uM</td>
</tr>
<tr>
<td>H2O</td>
<td>25</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10 mM Meglumine-HCl</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>100mM Meglumine-HCl</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
Reactants minus tyrosinase were mixed at room temperature in a 96-well polystyrene plate. Tyrosinase was added and the reaction incubated at room temperature for 20 minutes. Figure 5 shows the reaction at 20 minutes. As the concentration of meglumine is increased from 0.5 mM (well 3) to 100 mM (well 8), the extent of inhibition at 20 minutes in increased. This effect is characteristic of enzyme inhibition.

Example 6: Effect of Meglumine on Tyrosinase Activity

Tyrosinase activity was measured as follows. A 500 microL mixture was prepared containing 50mM potassium phosphate buffer pH 7.6; 600 microM L-DOPA or 3 mM tyrosine substrate; 250, 500 or 1000 microM meglumine hydrochloride, 5 mM kojic acid, 5mM hydroquinone or 100mM N-acetylglucosamine; and 12.5 U mushroom tyrosinase enzyme (Sigma). A blank reaction was also set up without tyrosinase enzyme. The mixture was incubated for 48 hr at 37°C.

After the incubation period, melanin was precipitated and extracted using a variation of a procedure described by D.S. Kim et al. (J. Cell Science 116:1699; 2003). Meglumine-HCL was added in a 0.5ml aliquot to a final concentration of 500mM in 50mM potassium phosphate buffer pH 7.6, the mixture was vortexed and then spun at 7500 x g for 20 min. The supernatant was removed, and 250 microL of a solution containing 1M NaOH and 10% DMSO was added to the pellet. The precipitate was vortexed, and the suspension placed at 100 degrees C for 1 hr with vortexing every 30 min, and then centrifuged at 7500 x g for 5 min. An aliquot of 200 microL of the supernatant was placed into a 96-well polystyrene multiwell plate and the absorbance read at 415 nm using a multi-well plate reader.

The percentage values represent (A415 of test material-blank /A415 of control-blank) x 100 (Table 4).
The results show that the amount of melanin formed (indicated by $A_{415}$ value) by tyrosinase was decreased in the presence of meglumine with both L-DOPA and tyrosine as substrates. The amount of melanin was also decreased in the presence of other tyrosinase inhibitors kojic acid, hydroquinone and N-acetyl glucosamine, but the decrease was substrate dependent for kojic acid and N-acetyl glucosamine.

Example 7: Effect of Meglumine on Melanin Formation from L-DOPA

The tyrosinase enzyme produces dopaquione from L-DOPA, which then spontaneously forms dopachrome which is the precursor to melanin or an insoluble, dark colored precipitate. The effect of meglumine on tyrosinase activity and the appearance of melanin was measured. A 190 microL assay mixture containing 50mM potassium phosphate buffer pH 7.6, 600 microM L-dopa and 10 microL of either meglumine hydrochloride, kojic acid or ascorbic acid (final concentrations of 200mM, 5mM and 5mM respectively) was prepared. Ten microL of tyrosinase enzyme was added to a final concentration of 12.5 U/ml, and the mixture incubated for 72 hr at 37°C. An additional reaction was also done in the absence of tyrosinase.

The reaction above was repeated except that the meglumine hydrochloride, kojic acid or ascorbic acid was added 2 hr after the start of the reaction by the addition of tyrosinase.

After the incubation period, melanin was precipitated and extracted using a variation of a procedure described by D.S. Kim et al. (J. Cell Science 116:1699; 2003). After the 72 hr incubation period, 200 microL of 50% glycerol in 50mM potassium phosphate buffer pH 7.6 was added, the mixture was vortexed and spun at 7500 x g for 20 min. The supernatant was removed, and 250 microL of a
solution containing 1M NaOH and 10% DMSO was added to the pellet. The precipitate was vortexed, and the suspension placed at 100 degrees C for 1 hr with vortexing every 30 min, and then centrifuged at 7500 x g for 5 min. An aliquot of 200 microL of the supernatant was placed into a 96-well polystyrene multiwell plate and the absorbance read at 415 nm.

All 'test' A_{415} values had the background (10% DMSO in 1M NaOH) value subtracted. The percentage values represent (A_{415} of test material /A_{415} of 'control') x 100 (Table 5).

<table>
<thead>
<tr>
<th></th>
<th>% of Control A_{415}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Tyrosinase</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Meglumine-HCl</td>
<td>16</td>
</tr>
<tr>
<td>Kojic Acid</td>
<td>4</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>110</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>2</td>
</tr>
</tbody>
</table>

The results show that the amount of melanin formed (indicated by A_{415} value) in both the presence and absence of tyrosinase was decreased in the presence of meglumine. The amount of melanin formed with tyrosinase was also decreased in the presence of other tyrosinase inhibitors kojic acid, hydroquinone and ascorbic acid.

Meglumine also decreased the amount of melanin when added after the formation of dopaquinone from L-DOPA by tyrosinase.

Example 8: Effect of topical cream with meglumine on skin pigmentation

Two preparations of skin cream were tested for their ability to change the pigment of freckled skin. One preparation contains meglumine and liposomes and the other does not (placebo). The placebo (base cream) was purchased from FMI (Allentown, PA) and contained polawax, jojoba oil, glycerine, germaben II, Crodofos SG, NaOH, Lipex 205, Dimethicone DC 200, Trolamine NF, Carbopol Ultrez 10, Ucare Polymer LK, Juniper Blend and Purified Deionized Water. The other preparation (Test Cream) combined 82 g of the base cream from FMI, with 8 g meglumine hydrochloride, and 10 g of a mixture containing 8 g of Natipide II
(American Lecithin Company) and 2 g of meglumine hydrochloride (prepared by stirring together) to give a homogeneous emulsion.

This was a double-blind, placebo controlled trial. Sixteen people between 18-65 years of age with a Fitzpatrick Skin Type of I-III and showing at least moderate pigmentation on the lateral aspects of the upper arms participated in this test. Each panelist was given the active and placebo products (one for each arm) to use on the designated lateral aspect of the upper arm according to a randomization schedule. Panelists will treat the lateral aspect of the upper arm by applying the cream twice daily for 3 weeks.

During the study, panelists were instructed as follows:

- Do not use any moisturizers or topical medication (applied to the skin) on the upper outer arms.
- Avoid sunburn, tanning or other direct sun exposure.
- Not to use a tanning bed
- Do not scrub the arm test sites, do not wash arms with loofahs or body bath sponges or apply any other products to test sites.

Panelists were given the test and placebo cream products (one for each arm) according to a randomization schedule. The product instructions are as follows:

Apply to the designated arm twice daily, once in the morning and again in the evening. Always apply the product designated for the left arm to the left arm and the product designated for the right arm to the right arm.

Skin erythema was measured instrumentally using reflectance techniques based on the standardized tristimulus system recommended by CIE (Commission International de L'Eclairage) using a Minolta CR-200b Chromameter which has an 8mm measuring area using the illuminant conditions of D_65 which most closely approximates normal daylight conditions. This is a hand held device which is gently placed against the surface to be color characterized. When triggered, a pulsed xenon light source flashes and this light is reflected off the surface and measured back into the device. Within the device, there are 6 silicon photocells that are filtered to detect primary stimulus values for red, green and blue wavelengths of light. For color readings, the values are translated into the L*a*b* coordinates whose spacing correlates closely with color changes perceived by the human eye. This is an
internationally recognized convention for numerically expressing color differences established by the C.I.E. The L* value represents the density value from black to white. The a* and b* values represent the color axes ranging from green to red and from blue to yellow, respectively. [Babulak, S.W., Rhein, L.D., Scala, D.D., Simion, A.F. and Grove, G.L., Quantitation of Erythema in a Soap Chamber Test Using the Minolta Chroma (Reflectance) Meter: Comparison of Instrumental Results with Visual Assessments, J. Soc. Cosmet. Chem. 37:475-479, 1986.]

Ten sets of L*, a* and b* readings will be taken for each of the test sites at the beginning of the study and after 3 weeks.

Using the L*, a* and b* readings, calculations of delta E and delta C were also made using the following formulas:

\[ \text{Delta E} = \sqrt{((\text{delta L*})^2 + (\text{delta a*})^2 + (\text{delta b*})^2)} \]

\[ \text{Delta C} = \sqrt{((\text{delta a*})^2 + (\text{delta b*})^2)} \]

Delta E is used to describe the distance between two colors and Delta C is the chroma.

<table>
<thead>
<tr>
<th></th>
<th>Placebo Cream</th>
<th>Test Cream</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta C</td>
<td>1.00 ± 0.57</td>
<td>1.24 ± 0.72</td>
<td>0.0171</td>
</tr>
<tr>
<td>Delta E</td>
<td>1.36 ± 0.91</td>
<td>1.74 ± 1.11</td>
<td>0.0142</td>
</tr>
</tbody>
</table>

Calculation of the Delta E and Delta C values, which are linear changes in color space demonstrate that the Test Cream results in a significantly (p<0.05) greater change than the placebo cream (Table 6). As will be understood based on the disclosure set forth herein, other non-meglumine compounds may be useful according to the present invention. Such compounds include, but are not
limited to, sorbitol lysine, mannitol lysine, galacticol lysine, n-octyl-glucitol, morpholinofructose, morpholinosorbitol, morpholinomannitol.

Example 9: Effect of Meglumine Analog on Melanin Formation

The effect of an analog of meglumine on melanin formation was tested. The hydrochloride salts of this compound were made using a generic procedure. For example, 1-deoxy-l-(methylamino)-D-xylitol (DYN30) was made using D-xylose. D-xylose (2 mmol) was dissolved in 10 ml of methanol. Five ml of 2M methylamine in methanol was added followed by 1ml of a slurry of activated Raney nickel. This was shaken under 28 psi of hydrogen for six hours. The catalyst was removed by filtration and the filtrate evaporated to remove excess methylamine. The residue was dissolved in 2 ml of water and the pH adjusted to 7 with IN HCl. The solution was concentrated to about 1 ml and 10 ml of isopropanol added. The resulting precipitate was collected by filtration and dried.

A 190 microL assay mixture containing 50mM potassium phosphate buffer pH 7.6, 600 microM L-dopa and either 100mM meglumine hydrochloride or 100mM DYN30 was prepared in duplicate in a polystyrene 96-well plate. To half of the wells, ten microL of mushroom tyrosinase enzyme was added to a final concentration of 12.5 U/ml, and the mixture incubated for 72 hr at 37°C. The other half of the wells received 10 microL of 50mM potassium phosphate buffer pH 7.6.

After the 72 hr incubation period, the plate was photographed (Figure 6). The results show that the amount of melanin (dark colored precipitate) formed both in the presence (+Tase) or absence of tyrosinase (No Tase) was decreased in the presence of meglumine, and the presence of meglumine analogue, DYN30.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.
CLAIMS

What is claimed:

1. A method of inhibiting tyrosinase activity in the skin of a mammal, said method comprising administering to said mammal a composition comprising meglumine.

2. The method of claim 1, wherein said composition further comprises a pharmaceutically acceptable carrier.

3. The method of claim 1, wherein said composition further comprises at least one depigmenting agent.

4. The method of claim 3, wherein said depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine, or a combination thereof.

5. The method of claim 1, wherein said composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen, or a combination thereof.

6. The method of claim 1, wherein said mammal is a human.


8. The method of claim 7, wherein said composition further comprises a pharmaceutically acceptable carrier.
9. The method of claim 7, wherein said composition further comprises at least one depigmenting agent.

10. The method of claim 9, wherein said depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine, or a combination thereof.

11. The method of claim 7, wherein said composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen, or a combination thereof.

12. The method of claim 7, wherein said mammal is a human.


14. The method of claim 13, wherein said composition further comprises a pharmaceutically acceptable carrier.

15. The method of claim 13, wherein said composition further comprises at least one depigmenting agent.

16. The method of claim 15, wherein said depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine, or a combination thereof.

17. The method of claim 13, wherein said composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen, or a combination thereof.
18. The method of claim 13, wherein said mammal is a human.


21. The method of claim 20, wherein said composition further comprises a pharmaceutically acceptable carrier.

22. The method of claim 20, wherein said composition further comprises at least one depigmenting agent.

23. The method of claim 22, wherein said depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine, or a combination thereof.

24. The method of claim 20, wherein said composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen, or a combination thereof.

25. The method of claim 20, wherein said mammal is a human.

27. A method of preventing darkening of the skin of a mammal, said
method comprising administering to said mammal a composition comprising
meglumine.

28. The method of claim 27, wherein said composition further
comprises a pharmaceutically acceptable carrier.

29. The method of claim 27, wherein said composition further
comprises at least one depigmenting agent.

30. The method of claim 29, wherein said depigmenting agent is
selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-
acetyl glucosamine, or a combination thereof.

31. The method of claim 27, wherein said composition further
comprises at least one additional active ingredient selected from the list consisting of
an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin
lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent,
and a sunscreen, or a combination thereof.

32. The method of claim 27, wherein said mammal is a human.

33. A method of inhibiting melanin accumulation in the skin of a
mammal, said method comprising administering to said mammal a composition
comprising meglumine.

34. A method of inhibiting melanin accumulation in the skin of a
mammal, said method comprising administering to said mammal a composition
comprising l-deoxy-l-(methylamino)-D-xylitol (DYN30).

35. The method of claim 33, wherein said composition further
comprises a pharmaceutically acceptable carrier.
36. The method of claim 33, wherein said composition further comprises at least one depigmenting agent.

37. The method of claim 36, wherein said depigmenting agent is selected from the list consisting of kojic acid, hydroquinone, glucosamine, and N-acetyl glucosamine, or a combination thereof.

38. The method of claim 33, wherein said composition further comprises at least one additional active ingredient selected from the list consisting of an anti-aging agent, a wrinkle-reducing agent, a skin whitening agent, a skin lightening agent, a skin bleaching agent, an anti-acne agent, a sebum-reduction agent, and a sunscreen, or a combination thereof.

39. The method of claim 33, wherein said mammal is a human.

40. A method of preventing fruit browning, said method comprising applying to said fruit a composition comprising meglumine.

41. A method of preventing vegetable browning, said method comprising applying to said vegetable a composition comprising meglumine.