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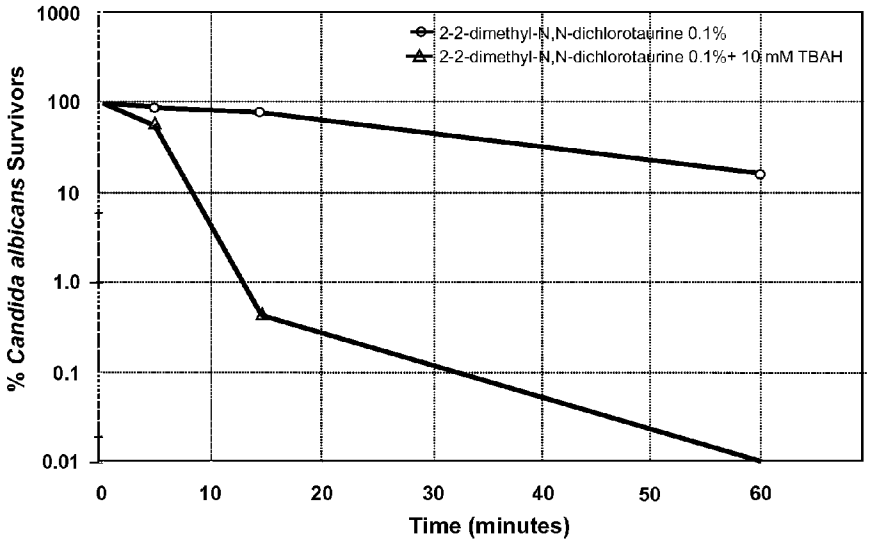
(54) Title: N-HALOGENATED AMINO ACID FORMULATIONS

FIGURE 1/2



(57) Abstract: The present invention relates to a method for treating a tissue infection comprising contacting the infected tissue with a pharmaceutically effective amount of a formulation comprising a N-halogenated amino acid and a phase transfer agent. This specification also describes a formulation having antimicrobial activity comprising a N-halogenated amino acid and a phase transfer agent.

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N-HALOGENATED AMINO ACID FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority under 35 U.S.C. §119 to U.S. Provisional Patent Application No. 60/915,291 filed May 1, 2007, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods for improving the antimicrobial properties of N-halogenated amino acid compounds and formulations. The present invention further relates to N-halogenated amino acid-containing formulations with improved antimicrobial properties.

BACKGROUND OF THE INVENTION

It is generally desirable to use the minimum quantity of an antimicrobial compound necessary to achieve desired effects. This is because undesirable side-effects are more probable when higher concentrations of an antimicrobial are used at a delivery site through the use of, for example, high concentration formulations, more frequent dosing, or longer-duration treatment. Unfortunately, while the use of lower concentrations of antimicrobial compounds generally helps to reduce the potential for undesirable effects, this practice increases the risk that the compounds may not achieve the required level of antimicrobial effect. Also, microbial resistance can develop quickly if antimicrobial compounds are not used at a sufficient concentration. Therefore, inventions that improve the antimicrobial activity of antimicrobial compounds are desirable as they allow for decreased concentrations of such compounds to be used at a delivery site, reducing the incidence and risk of undesired side effects and microbial resistance.

N-halogenated amino acid compounds are known to have desirable antimicrobial properties including antibacterial, anti-infective, antifungal, and/or antiviral properties. Many such N-halogenated amino acid compounds are disclosed in U.S. Patent Application Publication Nos. 2005/0065115 and 2006/0247209, the entire contents of which are incorporated by reference herein.

The combination of one N-halogenated amino acid, N-chlorotaurine, and an amine such as ammonium chloride has been shown in the literature to have greater antimicrobial activity than N-chlorotaurine by itself. Gottardi et al., Hyg Med., Vol. 21:597-605, 1996. This effect appears to be caused by any unsubstituted primary or secondary amine, due in certain cases to the formation of chloroamine compounds by transhalogenation of the N-chlorotaurine. However, N-chlorotaurine itself is not stable in combination with ammonium chloride. Also, the increased antimicrobial activity of the N-chlorotaurine and ammonium chloride combination is not derived from the N-chlorotaurine moiety itself, but from the formation of an additional chemical moiety possessing antimicrobial properties. Combinations of N-chlorotaurine and ammonia or any primary or secondary amine thus do not possess the necessary stability and shelf life required for a marketable product.

To cite one of many applications, the use of formulations having antimicrobial properties is important for the treatment of ophthalmic infections such as conjunctivitis. Conjunctivitis can be caused by various kinds of microbes, with most cases being due to bacteria and/or viruses. Unfortunately, conjunctivitis symptoms are not specific to the etiology of the infectious agent and significant testing may be required to determine the causative agent or microbe. Viral conjunctivitis, often caused by adenovirus, is highly contagious yet has no currently known efficacious treatment that provides other than symptom relief. Care must be taken in selecting appropriate agents for treating conjunctivitis, given the sensitive tissues affected by the infection. In view of the above-recited difficulties in treatment, formulations for treating conjunctivitis are needed that have broad-spectrum antimicrobial properties capable of treating bacteria, viruses, fungi, etc., a benign toxicological profile, and/or characteristics that prevent the transmission of contagious infectious agents.

Microbial resistance to conventional antimicrobial treatment is an ongoing concern to medical professionals. Until the problem of resistance is overcome, a steady supply of new treatments and therapies for treating microbial infections is required in order to blunt the effect of microbe mutations that render conventional therapies less effective or, in certain cases, ineffective.

BRIEF SUMMARY OF THE INVENTION

The present invention relates to methods for enhancing the antimicrobial activities of N-halogenated amino acid compounds. The present inventors have discovered that the antimicrobial activity of N-halogenated amino acid compounds can be enhanced by formulating the N-halogenated amino acid with a phase transfer agent. Phase transfer agents include, but are not limited to, quaternary amine compounds such as tetrabutylammonium hydroxide (TBAH) and phosphonium salts such as tetrabutylphosphonium chloride (TBPC). Phase transfer agents include compounds that form ion pairs with N-halogenated amino acids.

The present invention further relates to N-halogenated amino acid-containing formulations with improved antimicrobial characteristics. These formulations comprise a N-halogenated amino acid such as, for example, 2,2-dimethyl-N,N-dichlorotaurine and a phase transfer agent such as a quaternary amine. The formulations of the present invention have excellent antimicrobial activity, and allow the use of low concentrations of the N-halogenated amino acid compounds by increasing their efficacy.

While not desiring to be bound by theory, it is believed that some phase transfer agents, such as quaternary amine compounds, form ion pairs with N-halogenated amino acid compounds. Alone, N-halogenated amino acid compounds are very polar and poorly penetrate lipophilic tissues. Ion pairs formed with such ion pairing agents as quaternary amines are believed to increase the antimicrobial efficacy of the N-halogenated amino acid compounds. Ion pairing may improve the penetration of the N-halogenated amino acid compounds through lipophilic tissues. Other phase transfer agents may improve the apparent permeability of N-halogenated amino acids by mechanisms other than ion pair formation, also resulting in improved antimicrobial properties.

Previous observations noted that ammonium chloride can enhance the activity of N-chlorotaurine, likely due to the formation of chloroamine compounds resulting from decomposition of the N-chlorotaurine. In these cases, the anti-infective activities are not derived from N-chlorotaurine alone, but from a reaction product or from the contribution of a reaction product's anti-infective activity. In contrast, certain embodiments of the present invention enhance the activity of N-halogenated

amino acid compounds by the formation of ion pairs with phase transfer agents, and do not cause degradation of the N-halogenated amino acid and its salts.

An embodiment of the present invention is a formulation having antimicrobial activity that comprises a N-halogenated amino acid and a phase transfer agent.

Another embodiment of the present invention is a method for improving the antimicrobial activity of a formulation comprising a N-halogenated amino acid. The method comprises adding a phase transfer agent to the N-halogenated amino acid formulation.

The foregoing brief summary broadly describes the features and technical advantages of certain embodiments of the present invention. Additional features and technical advantages will be described in the detailed description of the invention that follows. Novel features which are believed to be characteristic of the invention will be better understood from the detailed description of the invention when considered in connection with any accompanying figures. However, figures provided herein are intended to help illustrate the invention or assist with developing an understanding of the invention, and are not intended to be definitions of the invention's scope.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete understanding of the present invention and the advantages thereof may be acquired by referring to the following description, taken in conjunction with the accompanying drawings and wherein:

FIGURE 1 is a graph showing the antimicrobial activity enhancement of an N-halogenated amino acid, 2,2-dimethyl-N,N-dichlorotaurine, when tetrabutyl-ammonium hydroxide (TBAH) is added; and

FIGURE 2 is a graph illustrating the results of a partitioning experiment using the N-halogenated amino acid, 2,2-dimethyl-N,N-dichlorotaurine, in combination with variable concentrations of TBAH.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

As used herein, the term "antimicrobial" refers to an ability to kill or inhibit the growth of microbes (to include, without limitation, bacterial, viruses, yeast, fungi, spores, protozoa, parasites, etc.), or to attenuate or eradicate a microbial infection.

As used herein, the term "ion pairing agent" refers to any compound that forms an ion pair with an N-halogenated amino acid in solution.

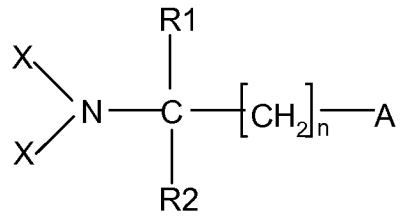
As used herein, the term "phase transfer agent" refers to any compound that increases the solubility of an N-halogenated amino acid in organic solution. Phase transfer agents include, but are not limited to, ion pairing agents. Phase transfer agents increase the apparent permeability of N-halogenated amino acids when formulated together in solution.

As used herein, the term "subject" refers to either a human or to non-human domesticated or non-domesticated animals (such as primates, mammals, vertebrates, invertebrates, etc.). The terms "subject" and "patient" may be used interchangeably herein.

As used herein, the terms "treatment", "treating", and the like mean obtaining a desired pharmacologic and/or physiologic effect. The desired effect may be, without limitation, prevention of a disease or infection in certain usage and/or may be therapeutic in terms of a partial or complete cure for a disease or infection and/or adverse effect attributable to the disease or infection.

II. Methods and Formulations

The N-halogenated amino acids of the present invention have the following general formula:



where X is one or more halogens and R1 and R2 are any of the nonpolar, uncharged polar, and charged polar amino acid and amino acid derivative side chains known to those of skill in the art. A represents an acid such as a carboxylic, sulfonic, phosphoric, boric or other acid known to those of skill in the art. There may be one or more carbon atoms between the amine and acid, and each carbon may contain one or more R substituents.

The preferred N-halogenated amino acids of the present invention have the following structure: haloamino-stabilizer-linker-acid, where (a) the "haloamino" is either N-halogen or N,N-dihalogen (e.g., -NHCl or $-NCl_2$); (b) the "stabilizer" comprises sidechains attached to the carbon next to the haloamino group (e.g., hydrogen, $-CH_3$, lower alkyl, the group -COOH or a C_{3-6} cycloalkyl ring); (3) the "linker" is either alkyl or cycloalkyl; and (d) the "acid" is one of the following: -COOH, $-SO_3H$, $-P(=O)(OH)_2$, $-B(OH)_2$ or hydrogen, and all the pharmaceutically acceptable salts of these acids generally known to those skilled in the art, including but not limited to sodium, potassium, calcium, etc.

The most preferred N-halogenated amino acids are 2,2-dimethyl-N,N-dichlorotaurine, analogs of 2,2-dimethyl-N,N-dichlorotaurine formed by replacement of the sulfonic acid group with carboxylic acid, phosphoric acid, borate, etc., 2,2-di alkyl-N,N-dichlorotaurine, and 2,2-R-N,N-dichlorotaurine, where R is an aliphatic or aromatic side chain. Methyl groups of N-halogenated amino acids may be replaced with alkyl, aryl, benzyl, or other hydrocarbon cyclic or non-cyclic groups.

Generally, the phase transfer agents of the present invention have a basic structure with a head group and lipophilic alkyl chains or aryl substituents. The majority of these phase transfer agents are made from natural building blocks such as fatty acids and alcohols. The lipophilic alkyl and aryl substituents together normally contain a total of about 4-8 carbons to about 30 carbons. The most preferred total number of carbons of the alkyl and aryl substituents is from about 15 to 20 carbons.

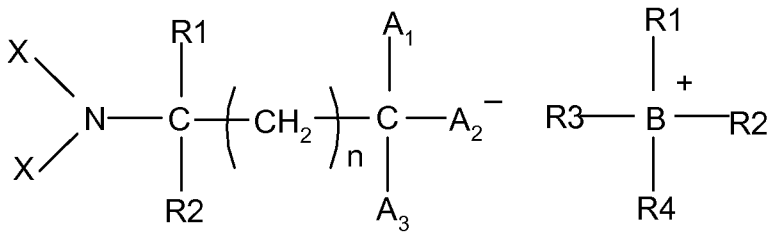
The preferred phase transfer agents of the present invention are quaternary amine compounds and include, but are not limited to tetrabutylammonium hydroxide (TBAH), tetrapropylammonium hydroxide (TPAH), tetrabutylphosphonium chloride (TBPC), hexadecyltrimethylammonium hydroxide, dodecyltriethylammonium hydroxide, and combinations thereof. Also included are the various salts of quaternary amine compounds known to those skilled in the art. These include but are not limited to chloride, bromide, sulfate, phosphate, and acetate.

Other phase transfer agents that may be used in embodiments of the present invention include benzalkonium chloride (BAC) and its homologues and analogs of varying carbon chain lengths. Such BAC-like compounds include, but are not limited to, benzalkonium chloride, benthonium chloride, cetalkonium chloride, cetrimonium bromide, cetylpyridinium chloride, stearalkonium chloride, and the homologues and analogs of these compounds, including various chain lengths of the lipophilic moiety. A BAC homologue with a 4 to 10 carbon lipophilic chain may form ion pairs with 2,2-dimethyl-N,N-dichlorotaurine in aqueous solution with an increased partition into the lipophilic phase. These BAC homologues and analogs are of particular interest as they may possess lower microbiologic activity and may be less irritating to biologic tissues, such as corneal and conjunctival tissues. Preferred BAC homologues and analogs have a 10 carbon lipophilic chain.

Further phase transfer agents that may be used in embodiments of the present invention include, but are not limited to, phospholipid cholines such as dimyristoylphosphatidylcholine (DMPC).

Phosphonium ion phase transfer agents include but are not limited to tetraalkylphosphonium salts of various alkyl chain lengths from one to 22 carbons, including unsaturated and aromatic alkyl substituents known to those skilled in the art. Salts include but are not limited to chloride, bromide, sulfate, phosphate, borate, and acetate. Examples of such phosphonium ion salts are tetrabutylphosphonium chloride (TBPC) and benzyldecyldimethylphosphonium chloride.

Preferred combinations of N-halogenated amino acids and phase transfer agents form ion pairs of the following general structure:



where for the negatively charged portion of the ion pair:

X is chlorine, bromine and/or iodine;

R1 is hydrogen or alkyl, C1-C6;

R2 is hydrogen or alkyl, C1-C6;

R1 and R2 together with the carbon atom to which they attach form a C3-C6 cycloalkyl ring;

n is 0 or an integer from 1-6;

A_1 is hydrogen or alkyl;

A_2 is COO^-, SO_3^-, PO_3^-, or other acid;

A_3 is hydrogen or alkyl;

and where for the positively charged portion of the ion pair:

B is nitrogen or phosphorous; and

R1 to R4 are each selected from alkyl esters, alcohols, hydroxyls, ketones, acids, sulfur-containing and aromatic esters, hydroxyls, ketones, and sulfur-containing acids, and R1 to R4 may not be hydrogen. Further, R1 to R4 should have a carbon atom directly connecting to the nitrogen atom forming a positive charge. This positive charge forms an ion pair with the negatively charged acid moiety of the N-halogenated amino acid.

III. Applications

The invention is particularly directed toward treating mammalian and human subjects having or at risk of having a microbial tissue infection. Microbial tissue infections that may be treated or prevented in accord with the method of the present invention are referred to in J. P. Sanford et al., "The Sanford Guide to Antimicrobial Therapy 2007" 37th Edition (Antimicrobial Therapy, Inc.). Particular microbial tissue infections that may be treatable by embodiments of the present invention include those infections caused by bacteria, viruses, protozoa, fungi, yeast, spores, and parasites. The present invention is also particularly directed to antimicrobial formulations for and methods of treating ophthalmic, otic, dermal, upper respiratory, lung/lower respiratory, esophageal, and nasal/sinus infections.

Certain embodiments of the present invention are particularly useful for treating ophthalmic tissue infections. Examples of ophthalmic conditions that may be treated using formulations and methods of the present invention include conjunctivitis, keratitis, blepharitis, dacyrocystitis, hordeolum and corneal ulcers. The methods and formulations of the invention may also be used prophylactically in various ophthalmic surgical procedures that create a risk of infection.

Otic and nasal/sinus tissue infections may also be treated by embodiments of the present invention. Examples of otic conditions that may be treated with formulations and methods of the present invention include otitis externa and otitis media, including those situations where the tympanic membrane has ruptured or tympanostomy tubes have been implanted. Examples of nasal/sinus conditions that may be treated with formulations and methods of the present invention include rhinitis, sinusitis, nasal carriage and situations where the nasal or sinus tissues are affected by surgery. Examples of respiratory infections and infectious agents include pneumonia, influenza, bronchitis, respiratory syncytial virus, etc.

Embodiments of the present invention may be used for disinfecting surfaces, particularly in healthcare-related structures such as hospitals, veterinary clinics, dental and medical offices, and for applications such as the sterilization of surgical instruments such as scalpels, electronic instrumentation, etc. Surgical instruments can be coated with certain formulations of the invention to provide for a sterile coating prior to surgery. Certain embodiments of the present invention may be used for the disinfection of public areas such as schools, public transportation facilities, restaurants, hotels and laundries and for the disinfection of household surfaces such as toilets, basins, and kitchen areas.

Certain formulations described herein may be used to disinfect and/or clean contact lenses in accordance with processes known to those skilled in the art and described in additional detail in co-pending U.S. Provisional Patent Application No. 60/970,634 entitled "N-HALOGENATED AMINO ACID FORMULATIONS AND METHODS FOR CLEANING AND DISINFECTION," herein incorporated by reference in its entirety. More specifically, contact lenses are removed from a patient's eyes and then immersed in such formulations for a time sufficient to disinfect the lenses. Disinfection and/or cleaning typically requires soaking the lenses in the formulation for approximately 4 to 6 hours.

Other embodiments of the present invention may also be used in disinfection or treatment solutions for skin and body tissue surfaces of a subject, providing antimicrobial activity against bacteria, fungi, viruses, protozoa, etc. Such treatment may be prophylactic or may be used to treat infected body tissue or wounds having one or more varieties of infectious agents present. These embodiments may also be used for treating the dermatological diseases caused by bacteria, fungi, viruses, protozoa, etc. Such embodiments may comprise formulations having one or more N-halogenated amino acids and phase transfer agents in a vehicle suitable for topical use. Disinfectant solutions for the skin are especially useful to disinfect hands, particularly in healthcare and unhygienic settings. Disinfection may also be useful in surgical settings, both for healthcare providers and to provide a clean field on a surgical subject.

Certain embodiments of the present invention may be used for treating onychomycosis. Onychomycosis refers to the invasion of a nail plate by a fungus. The infection may be due to a dermatophyte, yeast, or nondermatophyte mold. The term "tinea unguium" is used specifically to describe invasive dermatophytic onychomycosis. Implicated dermatophytes include, but are not limited to: *Epidermophyton floccosum*, *Microsporum audouinii*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Trichophyton tonsurans*. Additional fungi that may cause onychomycosis include, but are not limited to, *Acremonium* spp., *Aspergillus* spp., *Candida* spp., *Fusarium oxysporum*, *Scopulariopsis brevicaulis*, *Onychocola canadensis*, and *Scytalidium dimidiatum*.

Embodiments of the present invention may also be used prophylactically to prevent infection of a tissue by an infectious agent. In such embodiments, a tissue at risk of infection is contacted with a formulation of the present invention.

IV. Pharmaceutics and Formulations

A. Dosage

The phrase "pharmaceutically effective amount" is an art-recognized term, and refers to an amount of an agent that, when incorporated into a pharmaceutical formulation of the present invention, produces some desired effect at a reasonable benefit/risk ratio applicable to any medical treatment. The effective amount may vary depending on such factors as the disease or infectious agent being treated, the particular formulation being administered, or the severity of the disease or infectious agent.

The phrase "pharmaceutically acceptable" is art-recognized and refers to formulations, polymers and other materials and/or dosage forms which are suitable for use in contact with the tissues of a subject without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio as determined by one of ordinary skill in the art.

In particular embodiments, a formulation is administered once a day. However, the formulations of the present invention may also be formulated for administration at any frequency of administration, including once a week, once every 5 days, once every 3 days, once every 2 days, twice a day, three times a day, four times a day, five times a day, six times a day, eight times a day, every hour, or any greater frequency. Such dosing frequency is also maintained for a varying duration of time depending on the therapeutic regimen. The duration of a particular therapeutic regimen may vary from one-time dosing to a regimen that extends for months or years. One of ordinary skill in the art would be familiar with determining a therapeutic regimen for a specific indication. Factors involved in this determination include the disease to be treated, particular characteristics of the subject, and the particular antimicrobial formulation.

B. Formulations

In addition to the N-halogenated amino acid and a phase transfer agent, the formulations of the present invention optionally comprise one or more excipients. Excipients commonly used in pharmaceutical formulations include, but are not limited to, tonicity agents, preservatives, chelating agents, buffering agents, surfactants and antioxidants. Other excipients comprise solubilizing agents, stabilizing agents, comfort-enhancing agents, polymers, emollients, pH-adjusting

agents and/or lubricants. Any of a variety of excipients may be used in formulations of the present invention including water, mixtures of water and water-miscible solvents, such as C1-C7-alkanols, vegetable oils or mineral oils comprising from 0.5 to 5% non-toxic water-soluble polymers, natural products, such as alginates, pectins, tragacanth, karaya gum, xanthan gum, carrageenin, agar and acacia, starch derivatives, such as starch acetate and hydroxypropyl starch, and also other synthetic products such as polyvinyl alcohol, polyvinylpyrrolidone, polyvinyl methyl ether, polyethylene oxide, preferably cross-linked polyacrylic acid and mixtures of these products. The concentration of the excipient is, typically, from 1 to 100,000 times the concentration of the N-halogenated amino acid and the phase transfer agent. In preferred embodiments, excipients are selected on the basis of their inertness towards the N-halogenated amino acid and the phase transfer agent.

Suitable tonicity-adjusting agents include, but are not limited to, mannitol, sodium chloride, glycerin, sorbitol and the like. Suitable buffering agents include, but are not limited to, phosphates, borates, acetates and the like. Suitable surfactants include, but are not limited to, ionic and nonionic surfactants, though nonionic surfactants are preferred, RLM 100, POE 20 cetylstearyl ethers such as Procol® CS20 and poloxamers such as Pluronic® F68. Suitable antioxidants include, but are not limited to, sulfites, ascorbates, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

The formulations set forth herein may comprise one or more preservatives. Examples of such preservatives include p-hydroxybenzoic acid ester, alkyl-mercury salts of thiosalicylic acid, such as thiomersal, phenylmercuric nitrate, phenylmercuric acetate, phenylmercuric borate, sodium perborate, sodium chlorite, parabens such as methylparaben or propylparaben, alcohols such as chlorobutanol, benzyl alcohol or phenyl ethanol, guanidine derivatives such as polyhexamethylene biguanide, sodium perborate, or sorbic acid. In certain embodiments, the formulation may be self-preserved that no preservation agent is required.

For use in sinus and respiratory infection applications, formulations may be used that are suitable for aerosol formation using nebulizers or other such devices well known to those of skill in the art.

Some formulations of the present invention are ophthalmically suitable for application to a subject's eyes. For ophthalmic administration, the formulation may

be a solution, a suspension, a gel, or an ointment. In preferred aspects, formulations that include the N-halogenated amino acid and the phase transfer agent will be formulated for topical application to the eye in aqueous solution in the form of drops. The term "aqueous" typically denotes an aqueous formulation wherein the excipient is >50%, more preferably >75% and in particular >90% by weight water. These drops may be delivered from a single dose ampoule which may preferably be sterile and thus render bacteriostatic components of the formulation unnecessary. Alternatively, the drops may be delivered from a multi-dose bottle which may preferably comprise a device which extracts any preservative from the formulation as it is delivered, such devices being known in the art.

In other aspects, components of the invention may be delivered to the eye as a concentrated gel or a similar vehicle, or as dissolvable inserts that are placed beneath the eyelids. In yet other aspects, components of the invention may be delivered to the eye as ointment, water-in-oil and oil-in-water emulsions.

For topical formulations to the eye, the formulations are preferably isotonic, or slightly hypotonic in order to combat any hypertonicity of tears caused by evaporation and/or disease. This may require a tonicity agent to bring the osmolality of the formulation to a level at or near 210-320 milliosmoles per kilogram (mOsm/kg). The pH of the solution may be in an ophthalmic acceptable range of 3.0 to 8.0. The formulations of the present invention generally have an osmolality in the range of 220-320 mOsm/kg, and preferably have an osmolality in the range of 235-300 mOsm/kg. The ophthalmic formulations will generally be formulated as sterile aqueous solutions.

In certain embodiments, the N-halogenated amino acid and the phase transfer agent are formulated in a formulation that comprises one or more tear substitutes. A variety of tear substitutes are known in the art and include, but are not limited to: monomeric polyols, such as, glycerol, propylene glycol, and ethylene glycol; polymeric polyols such as polyethylene glycol; cellulose esters such hydroxypropylmethyl cellulose, carboxy methylcellulose sodium and hydroxy propylcellulose; dextrans such as dextran 70; vinyl polymers, such as polyvinyl alcohol; and carbomers, such as carbomer 934P, carbomer 941, carbomer 940 and carbomer 974P. Such formulations of the present invention may be used with contact lenses or other ophthalmic products.

In some embodiments, the formulations set forth herein have a viscosity of 0.5-100 cps, preferably 0.5-50 cps, and most preferably 1-20 cps. This relatively low viscosity insures that the product is comfortable, does not cause blurring, and is easily processed during manufacturing, transfer and filling operations.

The N-halogenated amino acids and phase transfer agents described herein may be included in various types of formulations having activities in addition to antimicrobial activity. Examples of such formulations include: ophthalmic pharmaceutical formulations (such as ocular lubricating products and artificial tears), astringents, topical disinfectants (alone or in combination with other antimicrobial agents such as, for example, betadine, etc.) and so on.

To effectively treat various microbial infections and to minimize side-effects, the antimicrobial activity of a formulation should be maximized so that a minimum amount of active ingredient is used. The activity of the antimicrobial formulations of the present invention is the result of the antimicrobial agent itself; the formulation components other than the N-halogenated amino acid and the phase transfer agent (in certain embodiments) normally cause little effect. The amount of the phase transfer agent required to enhance the antimicrobial activity of the N-halogenated amino acid in particular formulations can be determined by persons skilled in the art. The concentration required to enhance the antimicrobial activity of formulations while retaining acceptable safety and toxicity properties is referred to herein as "an effective amount". In most embodiments an effective amount of phase transfer agent is usually the same concentration in molarity as the N-halogenated amino acid concentration since ion pairs are formed in a one-to-one ratio. However, for safety and toxicological reasons, an effective amount can be altered higher or lower than the concentration which forms a one-to–one molar ratio. In certain embodiments, the effective amount of a phase transfer agent is calculated relative to the N-halogenated amino acid on a molar basis, ranging from 1:10 to 10:1, with a 1:1 ratio of phase transfer agent to N-halogenated amino acid being preferred.

It is also contemplated that the concentrations of the ingredients comprising the formulations of the present invention can vary. In preferred embodiments, the N-halogenated amino acid is present in ophthalmic formulations at a concentration of about 0.1% to 0.25% w/v. A person of ordinary skill in the art would understand that the concentrations can vary depending on the addition, substitution, and/or subtraction of ingredients in a given formulation.

Preferred formulations are prepared using a buffering system that maintains the formulation at a pH of about 3 to a pH of about 8.0. Topical formulations (particularly topical ophthalmic formulations, as noted above) are preferred which have a physiological pH matching the tissue to which the formulation will be applied or dispensed.

In certain embodiments of the present invention, a formulation can be administered in a two-part system. For instance, the N-halogenated amino acid can be present in one part of the formulation and the phase transfer agent separated in a separate container or different portion of the same container until a user is ready to administer the formulation. At the instant of administration or before, the two parts may be mixed by a user. In a preferred two-part system, a phase transfer agent is present in solution form and an N-halogenated amino acid is present in solid form. The two-part system may be useful in cases where one or more components of the formulation have stability problems when combined. Also, a two-part system may be utilized as part of a nasal/sinus spray dispensing system in certain embodiments.

C. Route of Administration

In the methods set forth herein, administration to a subject of a pharmaceutically effective amount of a formulation that includes an N-halogenated amino acid and a phase transfer agent may be by any method known to those of ordinary skill in the art.

For example, the formulation may be administered locally, topically, intradermally, intralesionally, intranasally, subcutaneously, orally, by inhalation, by injection, by localized perfusion bathing target cells directly, via a catheter, or via lavage.

In particular embodiments, the formulation is administered topically to an ocular surface. Regarding ophthalmic administration, it is contemplated that all local routes to the eye may be used, including topical, subconjunctival, periocular, retrobulbar, subtenon, intraocular, subretinal, posterior juxtascleral, and suprachoroidal administration.

Various otic administration techniques are also contemplated. In particular embodiments, the formulation may be delivered directly to the ear canal (for example: topical otic drops or ointments; slow release devices in the ear or implanted adjacent

to the ear). Local administration routes include otic intramuscular, intratympanic cavity and intracochlear injection routes for the formulations. It is further contemplated that certain formulations of the invention may be formulated in intraotic inserts or implant devices. For instance, delivery of the formulations can be accomplished by endoscopic assisted (including laser-assisted endoscopy to make the incision into the tympanic membrane) injection into the tympanic cavity as set forth, for example, in Tsue et al., Amer. J. Otolaryngology, Vol. 16(3):158-164, 1995; Silverstein et al., Ear, Nose & Throat Journal, Vol. 76:674-678, 1997; Silverstein et al., Otolaryngol Head Neck Surg., Vol. 120:649-655, 1999. Local administration can also be achieved by injection through the tympanic membrane using a fine (EMG recording) needle, through use of an indwelling catheter placed through a myringotomy incision, and injection or infusion through the Eustachian tube by means of a small tubal catheter. Furthermore, the formulations can be administered to the inner ear by placement of gelfoam or similar absorbent and adherent product soaked with the formulations against the window membrane of the middle/inner ear or adjacent structure with due discretion and caution by a skilled clinician.

Administration of the formulations described herein for the treatment of sinus tissue infection, nasal infection, upper respiratory infection, lung/lower respiratory infection, esophageal infection, and the various combinations can be via a number of methods known to those of skill in the art. Preferred administration for lower respiratory infections will be via aerosol formation by use of a nebulizer or other similar device. Formulations for the treatment of sinus infections can be administered in droplet form (often otic formulations can be used for the treatment of sinus infections) or by aerosol formation. Esophageal infections may be treated by administration of a liquid or aerosol formulation.

Other modes of administration of the formulations of the present invention are via skin patches, intrapulmonary, intranasally, via liposomes formulated in an optimal manner, and via slow release depot formulations. Various devices can be used to deliver the formulations to the affected ear compartment; for example, via catheter or as exemplified in U.S. Patent No. 5,476,446 which provides a multi-functional apparatus specifically designed for use in treating and/or diagnosing the inner ear of the human subject. Also see U.S. Patent No. 6,653,279 for other devices for this purpose.

V. Examples

The following examples are presented to further illustrate selected embodiments of the present invention.

Examples 1-4 below were prepared according to embodiments of the present invention.

EXAMPLE 1

Ingredient	% w/v
Sodium 2,2-dimethyl-N,N-dichlorotaurine	0.1
Benzyldecyldimethylammonium Chloride (C10 BAC)	0.125
Sodium Acetate Trihydrate	0.07
Sodium Chloride	0.8
Hydrochloric Acid	q.s. pH 4
Sodium Hydroxide	q.s. pH 4
Purified Water	q.s. 100%

EXAMPLE 2

Ingredient	% w/v
Sodium 2,2-dimethyl-N,N-dichlorotaurine	0.1
Tetrabutylammonium Hydroxide (TBAH)	0.11
Sodium Acetate Trihydrate	0.07
Sodium Chloride	0.8
Hydrochloric Acid	q.s. pH 4
Sodium Hydroxide	q.s. pH 4
Purified Water	q.s. 100%

EXAMPLE 3

Ingredient	% w/v
Sodium 2,2-dimethyl-N,N-dichlorotaurine	0.1
1,3-Diisopropylimidazolium Chloride	0.076
Sodium Acetate Trihydrate	0.07
Sodium Chloride	0.8
Hydrochloric Acid	q.s. pH 4
Sodium Hydroxide	q.s. pH 4
Purified Water	q.s. 100%

EXAMPLE 4

Ingredient	% w/v
Sodium 2,2-dimethyl-N,N-dichlorotaurine	0.1
Tetrabutylphosphonium Chloride	0.12
Sodium Acetate Trihydrate	0.07
Sodium Chloride	0.8
Hydrochloric Acid	q.s. pH 4
Sodium Hydroxide	q.s. pH 4
Purified Water	q.s. 100%

EXAMPLE 5

The antimicrobial activity of certain formulations described herein was evaluated by a standard microbiological analysis. The results of this evaluation are summarized in Table 1 below. For the evaluation, bacterial and fungal isolates were grown overnight on appropriate agar media as source of fresh cells. A suspension of these fresh cells was prepared in saline at approximately 1 X 10^8 cfu/mL. These suspensions were added directly to the test agents (various solutions of sodium 2,2-dimethyl-N,N-dichlorotaurine and control solutions). The initial concentration of cells in the test agent solutions was approximately 1 X 10^6 cfu/mL. The exposure of microorganisms to the test agent was conducted at room temperature for up to 60 minutes. At selected times, an aliquot was withdrawn and diluted into phosphate buffered saline at 4^0C. Viability was determined following serial dilution and filtration onto Milliflex cassettes.

TABLE 1

Product	Sampling Time	# Colonies	Correction Factor	Dilution Factor	Viable Cells/ml	% Survivors
C. albicans Control (H_2O)	0	44	1.11	10000	488400	100.00
	5	59	1.11	10000	654900	134.09
	15	59	1.11	10000	654900	134.09
	60	51	1.11	10000	566100	115.91
2,2-dimethyl-N,N-dichlorotaurine 0.1% pH 4.0 No Buffer	0	52	1.11	10000	577200	100.000
	5	39	1.11	10000	432900	75.000
	15	59	1.11	10000	654900	113.462
	60	30	1.11	10000	333000	57.692
2,2-dimethyl-N,N-dichlorotaurine 0.001% pH 4.0 No Buffer	0	52	1.11	10000	577200	100.000
	5	65	1.11	10000	721500	125.000
	15	166	1.11	1000	184260	31.923
	60	86	1.11	100	9546	1.654
Vehicle for 2,2-dimethyl-N,N-dichlorotaurine 0.1% pH 4.0 No Buffer	0	52	1.11	10000	577200	100.000
	5	58	1.11	10000	643800	111.538
	15	58	1.11	10000	643800	111.538
	60	59	1.11	10000	654900	113.462
2,2-dimethyl-N,N-dichlorotaurine 0.1% pH 4.0 w/ acetate	0	56	1.11	10000	621600	100.0000
	5	51	1.11	10000	566100	91.0714
	15	50	1.11	10000	555000	89.2857
	60	110	1.11	1000	122100	19.6429
2,2-dimethyl-N,N-dichlorotaurine 0.001% pH 4.0 w/ acetate	0	56	1.11	10000	621600	100.0000
	5	40	1.11	10000	444000	71.4286
	15	71	1.11	1000	78810	12.6786
	60	22	1.11	100	2442	0.3929

Product	Sampling Time	# Colonies	Correction Factor	Dilution Factor	Viable Cells/ml	% Survivors
Vehicle for 2,2-dimethyl-N,N-dichlorotaurine 0.1% pH 4.0 w/ acetate	0	56	1.11	10000	621600	100.0000
	5	36	1.11	10000	399600	64.2857
	15	54	1.11	10000	599400	96.4286
	60	58	1.11	10000	643800	103.5714
2,2-dimethyl-N,N-dichlorotaurine 0.1% pH4 acetate+0.26%TBAH	0	11	1.11	10000	122100	100.00
	5	58	1.11	1000	64380	52.73
	15	75	1.11	10	832.5	0.68
	60	0	1.11	1	0	0.00
2,2-dimethyl-N,N-dichlorotaurine 0.001% pH4 acetate+0.26%TBAH diluted in ph4 acetate	0	11	1.11	10000	122100	100.00
	5	13	1.11	10000	144300	118.18
	15	42	1.11	1000	46620	38.18
	60	16	1.11	10	177.6	0.15
Vehicle for 2,2-dimethyl-N,N-dichlorotaurine 0.1% pH4 acetate+0.26%TBAH	0	11	1.11	10000	122100	100.00
	5	17	1.11	10000	188700	154.55
	15	22	1.11	10000	244200	200.00
	60	25	1.11	10000	277500	227.27
2,2-dimethyl-N,N-dichlorotaurine 0.1% pH 4.0 w/ acetate +0.11%TMAC	0	19	1.11	10000	210900	100.00
	5	18	1.11	10000	199800	94.74
	15	74	1.11	1000	82140	38.95
	60	145	1.11	10	1609.5	0.76

Product	Sampling Time	# Colonies	Correction Factor	Dilution Factor	Viable Cells/ml	% Survivors
2,2-dimethyl-N,N-dichlorotaurine 0.001% pH 4.0 w/ acetate +0.11%TMAC diluted in ph4 acetate	0	19	1.11	10000	210900	100.00
	5	18	1.11	10000	199800	94.74
	15	16	1.11	1000	17760	8.42
	60	80	1.11	1	88.8	0.04
Vehicle for 2,2-dimethyl-N,N-dichlorotaurine 0.1% pH 4.0 w/ acetate +0.11%TMAC	0	19	1.11	10000	210900	100.00
	5	16	1.11	10000	177600	84.21
	15	14	1.11	10000	155400	73.68
	60	12	1.11	10000	133200	63.16

The anti-infective activity of the N-halogenated amino acid 2,2-dimethyl-N,N-dichlorotaurine, as measured by the percentage of survivors of *C. albicans,* was dramatically improved when the formulation contained phase transfer agents such as tetrabutylammonium hydroxide (TBAH) and tetramethylammonium chloride (TMAC). As shown above in Table 1, the percentage of *C. albicans* survivors in the 0.1% 2,2-dimethyl-N,N-dichlorotaurine formulated with acetate buffer in pH 4 was 89% after 15 minutes exposure. The test formulation comprising a quaternary amine phase transfer agent dramatically reduced the survivor percentage. The percentage survivors of 0.1% 2,2-dimethyl-N,N-dichlorotaurine in acetate buffer at pH 4 containing 10 millimolar concentrations of quaternary amines after 15 minutes exposure were <1% and 39% for, TBAH and TMAC, respectively. All of the above formulations show improved antimicrobial activity relative to control, with some variation between the different quaternary amines.

FIGURE 1 graphically illustrates one such anti-infective experiment. The graph clearly shows that the antimicrobial activity of an N-halogenated amino acid, 2,2-dimethyl-N,N-dichlorotaurine was dramatically increased when 10 mM TBAH phase transfer agent is added.

EXAMPLE 6 - Partitioning Experiment

Example 6 provides evidence of ion pairing taking place between a N-halogenated amino acid and a phase transfer agent, and the resulting changes in partitioning behavior. The partitioning experiment can be used to evaluate a compound's apparent lipophilicity and the potential improvement in antimicrobial activity when used on tissue.

Aqueous solutions were prepared containing 0.1% (4mM) sodium 2,2-dimethyl-N,N-dichlorotaurine, tetrabutylammonium hydroxide (TBAH) at 0 mM, 1 mM, 4 mM, or 10 mM, 5 mM sodium acetate, sodium chloride to adjust osmolality to isotonic, and sodium hydroxide and/or hydrochloric acid to adjust pH to 4.

These aqueous solutions were assayed for 2,2-dimethyl-N,N-dichlorotaurine by reverse phase high pressure liquid chromatography. Each solution was then combined with an equal volume of dichloromethane, mixed on a rocker overnight, and the aqueous phase reassayed. The percent loss of 2,2-dimethyl-N,N-dichlorotaurine from the aqueous phase and theoretical percent of 2,2-dimethyl-N,N-dichlorotaurine partitioning to the dichloromethane were calculated and plotted versus the concentration of TBAH.

TABLE 2

TBAH Conc	2,2-dimethyl-N,N-dichlorotaurine in aqueous Before Partitioning		2,2-dimethyl-N,N-dichlorotaurine in aqueous After Partitioning		Calculated 2,2-dimethyl-N,N-dichlorotaurine % in CH_2Cl_2 After Partitioning	
mM	mM	% 2,2-dimethyl-N,N-dichlorotaurine	% 2,2-dimethyl-N,N-dichlorotaurine	% in Aqueous	Theory % in Dichloromethane	Log P
0	4	0.10072	0.09675	96.1	3.9	-1.39
1	4	0.10083	0.07672	76.1	23.9	-0.50
4	4	0.10083	0.03573	35.4	64.6	0.26
10	4	0.10086	0.01198	11.9	88.1	0.87

Data obtained from the experiment is summarized in Table 2. The theoretical percentage of 2,2-dimethyl-N,N-dichlorotaurine in dichloromethane phase is calculated as 100 percent minus the percent remaining in aqueous phase. FIGURE 2 graphically illustrates the results of the above-described partitioning experiment. When 4 mM 2,2-dimethyl-N,N-dichlorotaurine in aqueous solution is combined with varying concentrations of TBAH, the quantity of 2,2-dimethyl-N,N-dichlorotaurine found in the aqueous solution decreases. Without added TBAH, almost all the 2,2-dimethyl-N,N-dichlorotaurine is found in the aqueous fraction. However, with 10 mM TBAH, most of the 2,2-dimethyl-N,N-dichlorotaurine has left the aqueous fraction and partitioned to the dichloromethane. This experiment is evidence of ion pair formation with TBAH phase transfer agent, which increases the apparent lipophilicity of the 2,2-dimethyl-N,N-dichlorotaurine.

The present invention and its embodiments have been described in detail. However, the scope of the present invention is not intended to be limited to the particular embodiments of any process, manufacture, composition of matter, compounds, means, methods, and/or steps described in the specification. Various modifications, substitutions, and variations can be made to the disclosed material without departing from the spirit and/or essential characteristics of the present invention. Accordingly, one of ordinary skill in the art will readily appreciate from the disclosure that later modifications, substitutions, and/or variations performing

substantially the same function or achieving substantially the same result as embodiments described herein may be utilized according to such related embodiments of the present invention. Thus, the following claims are intended to encompass within their scope modifications, substitutions, and variations to processes, manufactures, compositions of matter, compounds, means, methods, and/or steps disclosed herein.

CLAIMS

What is claimed is:

1. A method for improving the antimicrobial activity of a formulation comprising a N-halogenated amino acid comprising:

adding a phase transfer agent to said formulation.

2. The method of claim 1 wherein the phase transfer agent is selected from the group consisting of:

quaternary amines, tetrabutylammonium hydroxide (TBAH), tetrapropylammonium hydroxide (TPAH), tetrabutylphosphonium chloride (TBPC), hexadecyltrimethylammonium hydroxide, dodecyltriethylammonium hydroxide, and combinations thereof.

3. The method of claim 1 wherein the N-halogenated amino acid is a chlorotaurine.

4. The method of claim 3 wherein the chlorotaurine is sodium 2,2-dimethyl-N,N-dichlorotaurine.

5. A formulation having antimicrobial activity comprising:
a N-halogenated amino acid and a phase transfer agent.

6. The formulation of claim 5 wherein the phase transfer agent is selected from the group consisting of:

quaternary amines, tetrabutylammonium hydroxide (TBAH), tetrapropylammonium hydroxide (TPAH), tetrabutylphosphonium chloride (TBPC), hexadecyltrimethylammonium hydroxide, dodecyltriethylammonium hydroxide, and combinations thereof.

7. The formulation of claim 5 wherein the N-halogenated amino acid is a chlorotaurine.

8. The formulation of claim 7 wherein the chlorotaurine is sodium 2,2-dimethyl-N,N-dichlorotaurine.

9. A method for treating a tissue infection comprising:

contacting the infected tissue with a pharmaceutically effective amount of a formulation comprising a N-halogenated amino acid and a phase transfer agent.

10. The method of claim 9 wherein the phase transfer agent is selected from the group consisting of:

quaternary amines, tetrabutylammonium hydroxide (TBAH), tetrapropylammonium hydroxide (TPAH), tetrabutylphosphonium chloride (TBPC), hexadecyltrimethylammonium hydroxide, dodecyltriethylammonium hydroxide, and combinations thereof.

11. The method of claim 9 wherein the N-halogenated amino acid is a chlorotaurine.

12. The method of claim 11 wherein the chlorotaurine is sodium 2,2-dimethyl-N,N-dichlorotaurine.

13. The method of claim 9 wherein said infected tissue is ocular, otic, nasal, sinus, or dermal tissue.

14. The method of claim 9 wherein said formulation is a two-part formulation.

15. A method for improving the apparent lipophilicity of a N-halogenated amino acid formulation comprising:

adding a phase transfer agent to said formulation.

16. The method of claim 15 wherein the phase transfer agent is selected from the group consisting of:

quaternary amines, tetrabutylammonium hydroxide (TBAH), tetrapropylammonium hydroxide (TPAH), tetrabutylphosphonium chloride (TBPC), hexadecyltrimethylammonium hydroxide, dodecyltriethylammonium hydroxide, and combinations thereof.

17. The method of claim 15 wherein the N-halogenated amino acid is a chlorotaurine.

18. The method of claim 17 wherein the chlorotaurine is sodium 2,2-dimethyl-N,N-dichlorotaurine.

19. The method of claim 15 wherein said tissue is ocular, otic, nasal, sinus or dermal tissue.

20. The method of claim 15 wherein said formulation is a two-part formulation.

21. A method for disinfecting surfaces comprising:

treating a surface to be disinfected with a formulation comprising a N-halogenated amino acid and a phase transfer agent.

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22. The method of claim 21 wherein the surface to be treated is a surgical instrument.

23. The method of claim 21 wherein said surface is a body tissue.

24. A method for treating respiratory infections comprising:

contacting the site of the respiratory infection with a pharmaceutically effective amount of a formulation comprising a N-halogenated amino acid and a phase transfer agent.

25. The method of claim 24 where the respiratory infection is selected from the group consisting of:

sinus tissue infection, nasal infection, upper respiratory infection, lung/lower respiratory infection, esophageal infection, and combinations thereof.

26. A method for disinfecting and/or cleaning a contact lens comprising:

contacting a contact lens with a formulation comprising a N-halogenated amino acid and a phase transfer agent for a time sufficient to disinfect and/or clean the lens.

27. A method for preventing tissue infection comprising:

contacting a tissue at risk for infection with a pharmaceutically effective amount of a formulation comprising a N-halogenated amino acid and a phase transfer agent.

FIGURE 1/2

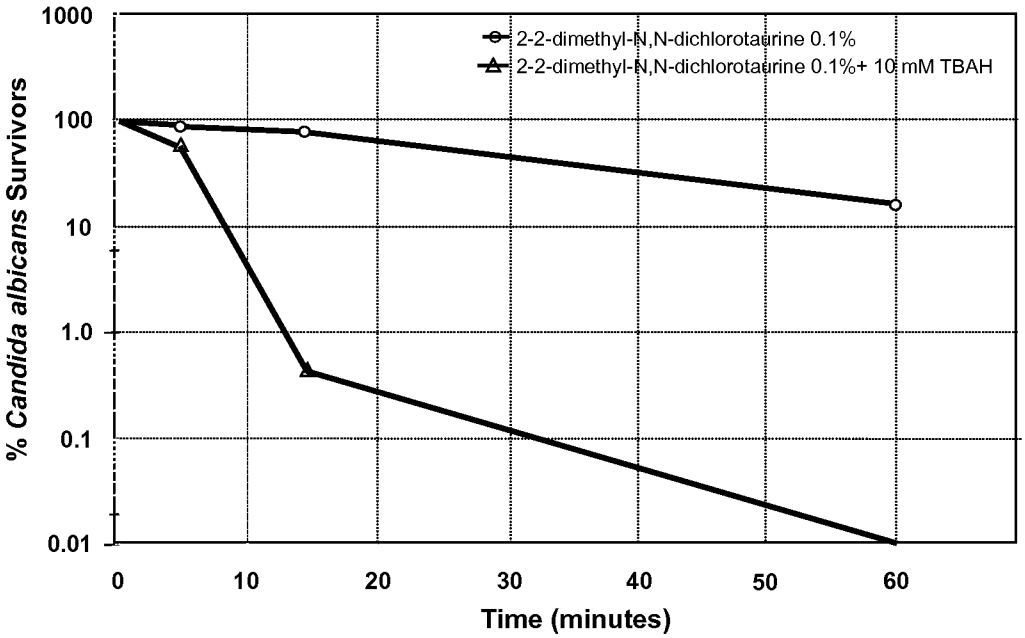
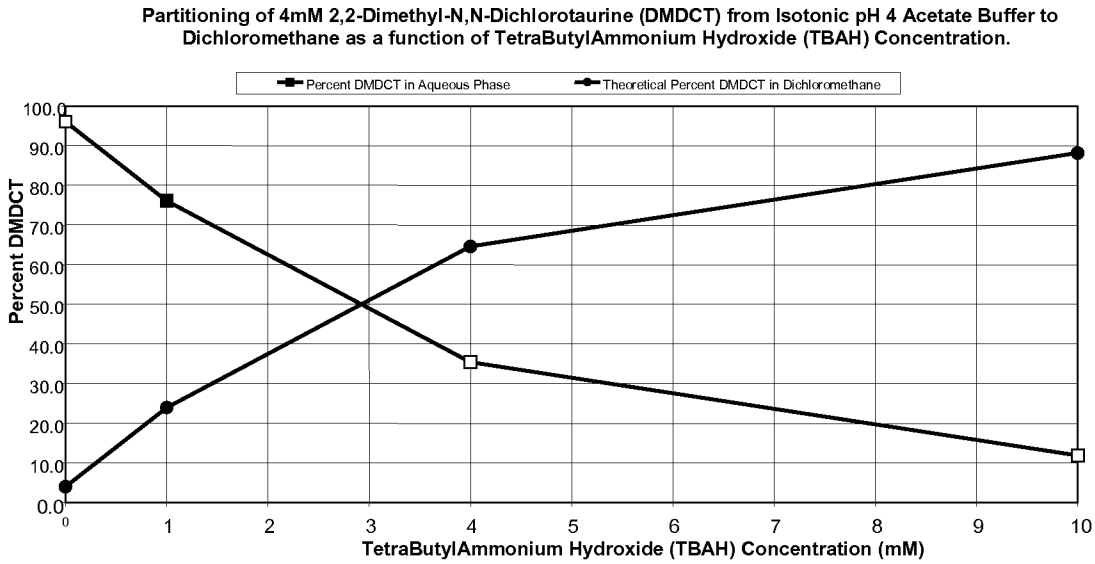

1000
100
10
1.0
0.1
0.01
% Candida albicans Survivors
0
10
20
30
40
50
60
Time (minutes)
2-2-dimethyl-N,N-dichlorotaurine 0.1%
2-2-dimethyl-N,N-dichlorotaurine 0.1%+ 10 mM TBAH

FIGURE 2/2


Partitioning of 4mM 2,2-Dimethyl-N,N-Dichlorotaurine (DMDCT) from Isotonic pH 4 Acetate Buffer to Dichloromethane as a function of TetraButylAmmonium Hydroxide (TBAH) Concentration.
Percent DMDCT in Aqueous Phase
Theoretical Percent DMDCT in Dichloromethane
Percent DMDCT
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
1
2
3
4
5
6
7
8
9
10
TetraButylAmmonium Hydroxide (TBAH) Concentration (mM)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/061942

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K45/06 A61P31/04

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAGL M ET AL: "RAPID KILLING OF MYCOBACTERIUM TERRAE BY N-CHLOROTAURINE IN THE PRESENCE OF AMMONIUM IS CAUSED BY THE REACTION PRODUCT MONOCHLORAMINE" JOURNAL OF PHARMACY AND PHARMACOLOGY, LONDON, vol. 50, no. 11, 1 November 1998 (1998-11-01), pages 1317-1320, XP001027195 ISSN: 0022-3573 *cf. abstract, page 1318, left col., the last three paras. of "results", page 1319, para. on the right-sided col.*	1-27

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2008	28/07/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stoltner, Anton

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/061942

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GOTTARDI ET AL: "N-Chlorotaurine and ammonium chloride: An antiseptic preparation with strong bactericidal activity" INTERNATIONAL JOURNAL OF PHARMACEUTICS, AMSTERDAM, vol. 335, no. 1-2, 28 March 2007 (2007-03-28), pages 32-40, XP022003885 ISSN: 0378-5173 *cf. abstract, page 39, para. 4.7 on the right-sided col., bridging with para. 4.8 "conclusion" on page 40*	1-27
X	NAGL M ET AL: "Interaction of N-chlorotaurine with amino acids and ammonium: Enhancement of bactericidal activity and clinical consequences" AMINO ACIDS, SPRINGER VERLAG, AU, vol. 21, no. 1, 1 January 2001 (2001-01-01), page 77, XP009103282 ISSN: 0939-4451 *cf. page 77, 2nd para. on the left-hand col.*	1-27
Y	NAGL M ET AL: "TOLERANCE OF N-CHLOROTAURINE, A NEW ANTIMICROBIAL AGENT, IN INFECTIOUS CONJUNCTIVITIS-A PHASE II PILOT STUDY" OPHTHALMOLOGICA, KARGER, BASEL, CH, vol. 214, no. 2, 1 March 2000 (2000-03-01), pages 111-114, XP001055765 ISSN: 0030-3755 *cf. abstract and discussion part on page 114*	1-27
Y	GOTTARDI WALDEMAR ET AL: "Chemical properties of N-chlorotaurine sodium, a key compound in the human defence system" ARCHIV DER PHARMAZIE, VCH VERLAGSGESELLSCHAFT MBH, WEINHEIM, DE, vol. 335, no. 9, 1 November 2002 (2002-11-01), pages 411-421, XP009103274 ISSN: 0365-6233 *cf. abstract, page 419, last para. on the right col., bridging with para. 2 of the left col. on page 20*	1-27

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/061942

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TEUCHNER B ET AL: "TOLERANCE AND EFFICACY OF THE NEW ANTIMICROBIAL AGENT N-CHLORTAURINE IN VIRAL KERATOCONJUNCTIVITES" ANNUAL MEETING OF THE ASSOCIATION FOR RESEARCH IN VISION ANDOPHTHALMOLOGY, XX, XX, 15 March 2001 (2001-03-15), page 5578, XP001027296 *cf. abstract*	1-27