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(54) Titre : COMPOSITIONS DE TOXINES CLOSTRIDIALES NON PROTEIQUES STABILISEES
(54) Title: STABILIZED NON-PROTEIN CLOSTRIDIAL TOXIN COMPOSITIONS

(57) Abrégé/Abstract:
Pharmaceutical compositions that stabilize a Clostridial toxin active ingredient are described. The compositions can be liquid or solid compositions, and comprise a surfactant and an antioxidant. In some embodiments, the compositions comprise a surfactant selected from a poloxamer and a polysorbate; an antioxidant selected from methionine, N-acetyl cysteine, ethylenediaminetetraacetic acid and combinations thereof; and a tonicity agent and/or a lyoprotector selected from, for example, trehalose and sucrose.

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(54) Title: STABILIZED NON-PROTEIN CLOSTRIDIAL TOXIN COMPOSITIONS

(57) Abstract: Pharmaceutical compositions that stabilize a Clostridial toxin active ingredient are described. The compositions can be liquid or solid compositions, and comprise a surfactant and an antioxidant. In some embodiments, the compositions comprise a surfactant selected from a poloxamer and a polysorbate; an antioxidant selected from methionine, N-acetyl cysteine, ethylenediaminetetraacetic acid and combinations thereof; and a tonicity agent and/or a lyoprotector selected from, for example, trehalose and sucrose.

STABILIZED NON-PROTEIN CLOSTRIDIAL TOXIN COMPOSITIONS

FIELD

[0001] The present invention relates to solid and liquid pharmaceutical compositions comprising a clostridial toxin active ingredient and one or more non-protein excipient.

BACKGROUND

[0002] A pharmaceutical composition is a formulation which contains at least one active ingredient (such as a Clostridial toxin) as well as, for example, one or more excipients, buffers, carriers, stabilizers, preservatives and/or bulking agents, and is suitable for administration to a patient to achieve a desired diagnostic result or therapeutic effect. The pharmaceutical compositions disclosed herein have diagnostic, therapeutic and/or research utility.

[0003] For storage stability and convenience of handling, a pharmaceutical composition can be formulated as a lyophilized (i.e. freeze dried) or vacuum dried powder which can be reconstituted with a suitable fluid, such as saline or water, prior to administration to a patient. Alternately, the pharmaceutical composition can be formulated as an aqueous solution or suspension. A pharmaceutical composition can contain a proteinaceous active ingredient. Unfortunately, a protein active ingredient can be very difficult to stabilize (i.e. maintained in a state where loss of biological activity is minimized), thereby resulting in a loss of protein and/or loss of protein activity during the formulation, reconstitution (if required) and storage of the pharmaceutical composition prior to use. Stability problems can arise due to surface adsorption of a protein active ingredient, physical instability, such as, *e.g.*, denaturation or aggregation, or chemical instability, such as, *e.g.*, cross-linking, deamidation, isomerization, oxidation, formation of acidic or basic species, Maillard reaction, and fragmentation. To prevent such instability, various protein-based excipients, such as albumin and gelatin, have been used to stabilize a protein active ingredient present in a pharmaceutical composition.

[0004] Unfortunately, despite their known stabilizing effects, significant drawbacks exist to the use of protein excipients, such as albumin or gelatin, in a pharmaceutical composition. For example albumin and gelatin are expensive and increasingly difficult to obtain. Furthermore, blood products or animal derived products such as albumin and gelatin, when administered to a patient can subject the patient to a potential risk of receiving blood borne pathogens or infectious agents. Thus, it is known that the possibility exists that the presence of an animal-derived protein excipient in a pharmaceutical composition can result in inadvertent incorporation of infectious elements into the pharmaceutical composition. For example, it has been reported that use of human serum albumin

may transmit prions into a pharmaceutical composition. Thus, it is desirable to find suitable non-protein excipients, such as, *e.g.*, stabilizers, cryo-protectants and lyo-protectants, which can be used to stabilize the protein active ingredient present in a pharmaceutical composition.

[0005] The unique characteristics of Clostridial toxins further constrain and hinder the selection of suitable non-protein excipients for a pharmaceutical composition comprising a Clostridial toxin active ingredient. For example, Clostridial toxins are large proteins having an average molecular weight of approximately 150 kDa, and are further complexed with non-toxin associated proteins that increase the size to approximately 300-900-kDa. The size of a Clostridial toxin complex makes it much more fragile and labile than smaller, less complex proteins, thereby compounding the formulation and handling difficulties if Clostridial toxin stability is to be maintained. Hence, the use of non-protein excipients, such as, *e.g.*, stabilizers, cryo-protectants and lyo-protectants must be able to interact with the Clostridial toxin active ingredient in a manner which does not denature, fragment or otherwise inactivate the toxin or cause disassociation of the non-toxin associated proteins present in the toxin complex.

[0006] Another problem associated with a Clostridial toxin active ingredient, is the exceptional safety, precision, and accuracy that is necessary for at all steps of the formulation process. Thus, a non-protein excipient should not itself be toxic or difficult to handle so as to not exacerbate the already extremely stringent requirements.

[0007] Still another difficulty linked with a Clostridial toxin active ingredient, is the incredible low amounts of Clostridial toxin that is used in a pharmaceutical composition. As with enzymes generally, the biological activities of the Clostridial toxins are dependant, at least in part, upon their three dimensional conformation. Thus, a Clostridial toxin is detoxified by heat, various chemicals, surface stretching, and surface drying. Additionally, it is known that dilution of a Clostridial toxin complex obtained by the known culturing, fermentation and purification methods to the much lower concentration used in a pharmaceutical composition results in rapid inactivation of the toxin. The extremely low amount of a Clostridial toxin active ingredient that is used in a pharmaceutical composition, makes this active ingredient very susceptible to adsorption to, *e.g.*, the surfaces of laboratory glassware, vessels, to the vial in which the pharmaceutical composition is reconstituted and to the inside surface of a syringe used to inject the pharmaceutical composition. Such adsorption of a Clostridial toxin active ingredient to surfaces can lead to a loss of active ingredient and to denaturation of the remaining Clostridial toxin active ingredient, both of which reduce the total activity of the active ingredient present in the pharmaceutical composition. Hence, the use of

non-protein excipients, such as, *e.g.*, stabilizers, cryo-protectants and lyo-protectants must be able to act as surface blockers to prevent the adsorption of a Clostridial toxin active ingredient to a surface.

[0008] Yet another problem connected to a Clostridial toxin active ingredient, is the pH-sensitivity associates with complex formation. For example, the 900-kDa BoNT/A complex is known to be soluble in dilute aqueous solutions at pH 3.5-6.8. However, at a pH above about 7 the non-toxic associated proteins dissociate from the 150-kDa neurotoxin, resulting in a loss of toxicity, particularly as the pH rises above pH 8.0. See Edward J. Schantz et al., pp. 44-45, *Preparation and characterization of botulinum toxin type A for human treatment*, in Jankovic, J., et al., Therapy with Botulinum Toxin (Marcel Dekker, Inc., 1994). As the non-toxic associated proteins are believed to preserve or help stabilize the secondary and tertiary structures upon which toxicity is depends, the dissociation of these proteins results in a more unstable Clostridial toxin active ingredient. Thus, non-protein excipients useful to formulate a pharmaceutical composition comprising a Clostridial toxin active ingredient must be able to operate within the confines of a pH level necessary to maintain the activity a Clostridial toxin active ingredient.

[0009] What is needed therefore is a Clostridial toxin pharmaceutical composition wherein a Clostridial toxin active ingredient (such as a botulinum toxin) is stabilized by a non-protein excipient. The present invention relates to solid and liquid Clostridial toxin pharmaceutical compositions with one or more non-protein excipients which functions to stabilize the Clostridial toxin active ingredient present in the solid or liquid pharmaceutical composition.

SUMMARY

[0010] In one aspect, there is provided a pharmaceutical composition comprising a Clostridial toxin active ingredient, a tonicity agent, a surfactant and an antioxidant. In some embodiments, the pharmaceutical compositions comprisies a botulinum toxin. In some embodiments, the pharmaceutical composition comprises trehalose. In some embodiments, the pharmaceutical composition comprises sodium chloride. In some embodiments, the composition comprises a poloxamer and/or a polysorbate. In some embodiments, the composition comprises poloxamer 188 and/or polysorbate 20. In some embodiments, the antioxidant comprises L-methionine, N-Acetyl-cystein, and/or ethylene diamine tetraacetic acid sodium salt (EDTA) or an EDTA analog. In some embodiments, the composition further comprises a buffering agent. In one embodiment, the buffering agent includes histidine buffer. In some embodiments, the composition has a pH of from 5 to 7. In some embodiments, the composition is a liquid formulation. In some embodiments, the composition is a solid formulation.

[0011] In one aspect, the present disclosure provides a liquid pharmaceutical composition comprising a clostridial toxin derivative, trehalose, poloxamer 188 or polysorbate 20, and L-methionine or N-acetyl-cysteine (NAC). In some embodiments, the liquid pharmaceutical composition comprises a botulinum toxin. In some embodiments, the liquid pharmaceutical composition further comprises EDTA or an EDTA analog. In some embodiments, the liquid pharmaceutical composition comprises a histidine buffer. In some embodiments, the pH of the liquid pharmaceutical composition ranges from 5 to 7. In some embodiments, the relative weight amount of L-methionine ranges from about 0.1% to about 0.3%. In some embodiments, the relative weight amount of NAC ranges from about 0.1% to about 0.5%. In some embodiments, the relative weight amount of EDTA ranges from about 0.01% to about 0.05%. In some embodiments, the relative weight amount of trehalose ranges from about 1.0 to about 10%. In some embodiments, the relative weight amount of poloxamer 188 ranges from about 2% to about 5%. In some embodiments, the relative weight amount of polysorbate 20 ranges from about 0.02% to about 0.06%.

[0012] In another aspect, the present disclosure provides a solid pharmaceutical composition comprising a botulinum toxin, trehalose, poloxamer 188 or polysorbate 20, NAC and EDTA or an EDTA analog. In an alternative embodiment, the solid pharmaceutical composition comprises a botulinum toxin, trehalose, poloxamer 188 and L-methionine. In some embodiments, the solid pharmaceutical composition further comprises histidine buffer. In some embodiments, the relative weight amount of L-methionine ranges from about 0.1% to about 0.3%. In some embodiments, the relative weight amount of NAC ranges from about 0.01% to about 0.05%. In some embodiments, the relative weight amount of EDTA ranges from about 0.01% to about 0.05%. In some embodiments, the relative weight amount of trehalose ranges from about 1.0 to about 10%. In some embodiments, the relative weight amount of poloxamer 188 ranges from about 0.5% to about 5%. In some embodiments, the relative weight amount of polysorbate 20 ranges from about 0.02% to about 0.06%.

DESCRIPTION

[0013] Certain compositions of the present invention provide stable liquid or solid pharmaceutical composition comprising a clostridical toxin derivative, a disaccharide, a surfactant and an antioxidant.

[0014] Certain embodiments also provide methods for the treatment of various diseases, disorders, and conditions, including, for example, depression, headache (such as, for example, migraine, tension headache, and the like), pain, hyperhidrosis, muscle spasticity, cervical dystonia, blepherospasm, overactive bladder (neurogenic detrusor overactivity, and idiopathic overactive

bladder), skin conditions including, for example, wrinkles, irregularities, depressions, and the like using the compositions provided according to aspect of the present invention. Embodiments can include various administration techniques, including, for example, injection, such as intramusclular, intracutaneous, subcutaneous, or the like, instillation, intravenous, transdermal, and topical.

Definitions

[0015] As used herein, the words or terms set forth below have the following definitions:

[0016] "About" or "approximately" as used herein means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, (*i.e.*, the limitations of the measurement system). For example, "about" can mean within 1 or more than 1 standard deviations, per practice in the art. Where particular values are described in the application and claims, unless otherwise stated, the term "about" means within an acceptable error range for the particular value.

[0017] "Administration", or "to administer" means the step of giving (*i.e.* administering) a pharmaceutical composition to a subject, or alternatively a subject receiving a pharmaceutical composition. The pharmaceutical compositions disclosed herein can be locally administered by various methods. For example, intramuscular, intradermal, subcutaneous administration, intrathecal administration, intraperitoneal administration, topical (transdermal), instillation, and implantation (for example, of a slow-release device such as polymeric implant or miniosmotic pump) can all be appropriate routes of administration.

[0018] "Alleviating" means a reduction in the occurrence of a pain, of a headache, or of any symptom or cause of a condition or disorder. Thus, alleviating includes some reduction, significant reduction, near total reduction, and total reduction.

[0019] "Animal protein free" means the absence of blood derived, blood pooled and other animal derived products or compounds. "Animal" means a mammal (such as a human), bird, reptile, fish, insect, spider or other animal species. "Animal" excludes microorganisms, such as bacteria. Thus, an animal protein free pharmaceutical composition can include a botulinum neurotoxin. For example, an “animal protein free” pharmaceutical composition means a pharmaceutical composition which is either substantially free or essentially free or entirely free of a serum derived albumin, gelatin and other animal derived proteins, such as immunoglobulins. An example of an animal protein free pharmaceutical composition is a pharmaceutical composition which comprises or which consists of a botulinum toxin (as the active ingredient) and a suitable polysaccharide as a stabilizer or excipient.

[0020] "Biological activity" describes the beneficial or adverse effects of a drug on living matter. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient but can be modified by the other constituents. Biological activity can be assessed as potency or as toxicity by an *in vivo* LD_{50} or ED_{50} assay, or through an *in vitro* assay such as, for example, cell-based potency assays as described in U.S. 20100203559 and U.S. 20100233802.

[0021] "Botulinum toxin" means a neurotoxin produced by Clostridium botulinum, as well as a botulinum toxin (or the light chain or the heavy chain thereof) made recombinantly by a non-Clostridial species. The phrase "botulinum toxin", as used herein, encompasses the botulinum toxin serotypes A, B, C, D, E, F and G, and their subtypes and any other types of subtypes thereof, or any re-engineered proteins, analogs, derivatives, homologs, parts, sub-parts, variants, or versions, in each case, of any of the foregoing. "Botulinum toxin", as used herein, also encompasses a "modified botulinum toxin". Further "botulinum toxin" as used herein also encompasses a botulinum toxin complex, (for example, the 300, 600 and 900kDa complexes), as well as the neurotoxic component of the botulinum toxin (150 kDa) that is unassociated with the complex proteins.

[0022] "Clostridial toxin" refers to any toxin produced by a Clostridial toxin strain that can execute the overall cellular mechanism whereby a Clostridial toxin intoxicates a cell and encompasses the binding of a Clostridial toxin to a low or high affinity Clostridial toxin receptor, the internalization of the toxin/receptor complex, the translocation of the Clostridial toxin light chain into the cytoplasm and the enzymatic modification of a Clostridial toxin substrate. Non-limiting examples of Clostridial toxins include a Botulinum toxin like BoNT/A, a BoNT/B, a BoNT/C_1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G, a Tetanus toxin (TeNT), a Baratii toxin (BaNT), and a Butyricum toxin (BuNT). The BoNT/C_2 cytotoxin and BoNT/C_3 cytotoxin, not being neurotoxins, are excluded from the term "Clostridial toxin." A Clostridial toxin disclosed herein includes, without limitation, naturally occurring Clostridial toxin variants, such as, *e.g.*, Clostridial toxin isoforms and Clostridial toxin subtypes; non-naturally occurring Clostridial toxin variants, such as, *e.g.*, conservative Clostridial toxin variants, non-conservative Clostridial toxin variants, Clostridial toxin chimeric variants and active Clostridial toxin fragments thereof, or any combination thereof. A Clostridial toxin disclosed herein also includes a Clostridial toxin complex. As used herein, the term "Clostridial toxin complex" refers to a complex comprising a Clostridial toxin and non-toxin associated proteins (NAPs), such as, *e.g.*, a Botulinum toxin complex, a Tetanus toxin complex, a Baratii toxin complex, and a Butyricum toxin complex. Non-limiting examples of Clostridial toxin complexes include those produced by a *Clostridium botulinum*, such as, *e.g.*, a 900-kDa BoNT/A complex, a 500-kDa BoNT/A complex, a 300-kDa BoNT/A complex, a 500-kDa BoNT/B complex,

a 500-kDa BoNT/C_1 complex, a 500-kDa BoNT/D complex, a 300-kDa BoNT/D complex, a 300-kDa BoNT/E complex, and a 300-kDa BoNT/F complex.

[0023] "Clostridial toxin active ingredient" refers to a molecule which contains any part of a clostridial toxin that exerts an effect upon or after administration to a subject or patient. As used herein, the term "clostridial toxin active ingredient" encompasses a Clostridial toxin complex comprising the approximately 150-kDa Clostridial toxin and other proteins collectively called non-toxin associated proteins (NAPs), the approximately 150-kDa Clostridial toxin alone, or a modified Clostridial toxin, such as, *e.g.*, a re-targeted Clostridial toxins.

[0024] "Deformity" means a cosmetic, physical or functional irregularity, defect, abnormality, imperfection, malformation, depression, or distortion.

[0025] "Effective amount" as applied to the biologically active ingredient means that amount of the ingredient which is generally sufficient to effect a desired change in the subject. For example, where the desired effect is a reduction in an autoimmune disorder symptom, an effective amount of the ingredient is that amount which causes at least a substantial reduction of the autoimmune disorder symptom, and without resulting in significant toxicity.

[0026] "Effective amount" when used in reference to the amount of an excipient or specific combination of excipients added to a Clostridial toxin composition, refers to the amount of each excipient that is necessary to achieve the desired initial recovered potency of a Clostridial toxin active ingredient. In aspects of this embodiment, an effective amount of an excipient or combination of excipients results in an initial recovered potency of, *e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In other aspects of this embodiment, a therapeutically effective concentration of a Clostridial toxin active ingredient reduces a symptom associated with the aliment being treated by, *e.g.*, at most 10%, at most 20%,at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90% or at most 100%.

[0027] "Heavy chain" means the heavy chain of a botulinum neurotoxin. It has a molecular weight of about 100kDa and can be referred to as the H chain, or as H.

[0028] H_C means a fragment (about 50kDa) derived from the H chain of a botulinum neurotoxin which is approximately equivalent to the carboxyl end segment of the H chain, or the portion corresponding to that fragment in the intact H chain. It is believed to be immunogenic and to contain the portion of the natural or wild type botulinum neurotoxin involved in high affinity, presynaptic binding to motor neurons.

[0029] H_N means a fragment (about 50kDa) derived from the H chain of a botulinum neurotoxin which is approximately equivalent to the amino end segment of the H chain, or the portion corresponding to that fragment in the intact in the H chain. It is believed to contain the portion of the natural or wild type botulinum neurotoxin involved in the translocation of the L chain across an intracellular endosomal membrane.

[0030] "Light chain" means the light chain of a clostridial neurotoxin. It has a molecular weight of about 50kDa, and can be referred to as the L chain, L, or as the proteolytic domain (amino acid sequence) of a botulinum neurotoxin.

[0031] LH_N or L-H_N means a fragment derived from a clostridial neurotoxin that contains the L chain, or a functional fragment thereof coupled to the H_N domain It can be obtained from the intact clostridial neurotoxin by proteolysis, so as to remove or to modify the H_C domain.

[0032] "Implant" means a controlled release (*e.g.*, pulsatile or continuous) composition or drug delivery system. The implant can be, for example, injected, inserted or implanted into a human body.

[0033] "Local administration" means direct administration of a pharmaceutical at or to the vicinity of a site on or within an animal body, at which site a biological effect of the pharmaceutical is desired, such as via, for example, intramuscular or intra- or subdermal injection or topical administration. Local administration excludes systemic routes of administration, such as intravenous or oral administration. Topical administration is a type of local administration in which a pharmaceutical agent is applied to a patient's skin.

[0034] "Modified botulinum toxin" means a botulinum toxin that has had at least one of its amino acids deleted, modified, or replaced, as compared to a native botulinum toxin. Additionally, the modified botulinum toxin can be a recombinantly produced neurotoxin, or a derivative or fragment of a recombinantly made neurotoxin. A modified botulinum toxin retains at least one biological activity of the native botulinum toxin, such as, the ability to bind to a botulinum toxin receptor, or the ability to inhibit neurotransmitter release from a neuron. One example of a modified botulinum toxin is a botulinum toxin that has a light chain from one botulinum toxin serotype (such as serotype A), and a heavy chain from a different botulinum toxin serotype (such as serotype B). Another example of a modified botulinum toxin is a botulinum toxin coupled to a neurotransmitter, such as substance P.

[0035] "Mutation" means a structural modification of a naturally occurring protein or nucleic acid sequence. For example, in the case of nucleic acid mutations, a mutation can be a deletion,

addition or substitution of one or more nucleotides in the DNA sequence. In the case of a protein sequence mutation, the mutation can be a deletion, addition or substitution of one or more amino acids in a protein sequence. For example, a specific amino acid comprising a protein sequence can be substituted for another amino acid, for example, an amino acid selected from a group which includes the amino acids alanine, aspargine, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, tyrosine or any other natural or non-naturally occurring amino acid or chemically modified amino acids. Mutations to a protein sequence can be the result of mutations to DNA sequences that when transcribed, and the resulting mRNA translated, produce the mutated protein sequence. Mutations to a protein sequence can also be created by fusing a peptide sequence containing the desired mutation to a desired protein sequence.

[0036] "Patient" means a human or non-human subject receiving medical or veterinary care. Accordingly, the compositions as disclosed herein can be used in treating any animal, such as, for example, mammals, or the like.

[0037] "Peripherally administering" or "peripheral administration" means subdermal, intradermal, transdermal, or subcutaneous administration, but excludes intramuscular administration. "Peripheral" means in a subdermal location, and excludes visceral sites.

[0038] "Pharmaceutical composition" means a composition comprising an active pharmaceutical ingredient, such as, for example, a clostridial toxin active ingredient such as a botulinum toxin, and at least one additional ingredient, such as, for example, a stabilizer or excipient or the like. A pharmaceutical composition is therefore a formulation which is suitable for diagnostic or therapeutic administration to a subject, such as a human patient. The pharmaceutical composition can be, for example, in a lyophilized or vacuum dried condition, a solution formed after reconstitution of the lyophilized or vacuum dried pharmaceutical composition, or as a solution or solid which does not require reconstitution.

[0039] “Pharmacologically aceeptable excipient” is synonymous with “pharmacological excipient” or “excipient” and refers to any excipient that has substantially no long term or permanent detrimental effect when administered to mammal and encompasses compounds such as, *e.g.*, stabilizing agent, a bulking agent, a cryo-protectant, a lyo-protectant, an additive, a vehicle, a carrier, a diluent, or an auxiliary. An excipient generally is mixed with an active ingredient, or permitted to dilute or enclose the active ingredient and can be a solid, semi-solid, or liquid agent. It is also envisioned that a pharmaceutical composition comprising a Clostridial toxin active ingredient can include one or more pharmaceutically acceptable excipients that facilitate processing of an active

ingredient into pharmaceutically acceptable compositions. Insofar as any pharmacologically acceptable excipient is not incompatible with the Clostridial toxin active ingredient, its use in pharmaceutically acceptable compositions is contemplated. Non-limiting examples of pharmacologically acceptable excipients can be found in, *e.g.*, Pharmaceutical Dosage Forms and Drug Delivery Systems (Howard C. Ansel et al., eds., Lippincott Williams & Wilkins Publishers, 7th ed. 1999); Remington: The Science and Practice of Pharmacy (Alfonso R. Gennaro ed., Lippincott, Williams & Wilkins, 20th ed. 2000); Goodman & Gilman's The Pharmacological Basis of Therapeutics (Joel G. Hardman et al., eds., McGraw-Hill Professional, 10th ed. 2001); and Handbook of Pharmaceutical Excipients (Raymond C. Rowe et al., APhA Publications, 4th edition 2003), each of which is hereby incorporated by reference in its entirety.

[0040] The constituent ingredients of a pharmaceutical composition can be included in a single composition (that is, all the constituent ingredients, except for any required reconstitution fluid, are present at the time of initial compounding of the pharmaceutical composition) or as a two-component system, for example a vacuum-dried composition reconstituted with a reconstitution vehicle which can, for example, contain an ingredient not present in the initial compounding of the pharmaceutical composition. A two-component system can provide several benefits, including that of allowing incorporation of ingredients which are not sufficiently compatible for long-term shelf storage with the first component of the two component system. For example, the reconstitution vehicle may include a preservative which provides sufficient protection against microbial growth for the use period, for example one-week of refrigerated storage, but is not present during the two-year freezer storage period during which time it might degrade the toxin. Other ingredients, which may not be compatible with a botulinum toxin or other ingredients for long periods of time, can be incorporated in this manner; that is, added in a second vehicle (*e.g.* in the reconstitution vehicle) at the approximate time of use. A pharmaceutical composition can also include preservative agents such as benzyl alcohol, benzoic acid, phenol, parabens and sorbic acid. Pharmaceutical compositions can include, for example, excipients, such as surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating 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; antioxidants; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials and other ingredients 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.

[0041] “Tonicity agent” means a low molecular weight excipient which is included in a formulation to provide isotonicity. Disaccharide, such as trehalose or sucrose, polyalcohol, such as sorbitol or mannitol, monosaccharide, such as glucose, and salt, such as sodium chloride, can serve as a tonicity agent.

[0042] "Polysaccharide" means a polymer of more than two saccharide molecule monomers. The monomers can be identical or different.

[0043] “Stabilizing agent”, “stabilization agent” or “stabilizer” means a substance that acts to stabilize a Clostridial toxin active ingredient such that the potency of the pharmaceutical composition is increased relative to an unstabilized composition.

[0044] “Stabilizers” can include excipients, and can include protein and non-protein molecules.

[0045] "Therapeutic formulation" means a formulation can be used to treat and thereby alleviate a disorder or a disease, such as, for example, a disorder or a disease characterized by hyperactivity (*i.e.* spasticity) of a peripheral muscle.

[0046] “TEM” as used herein, is synonymous with “Targeted Exocytosis Modulator” or “retargeted endopeptidase.” Generally, a TEM comprises an enzymatic domain from a Clostridial toxin light chain, a translocation domain from a Clostridial toxin heavy chain, and a targeting domain. The targeting domain of a TEM provides an altered cell targeting capability that targets the molecule to a receptor other than the native Clostridial toxin receptor utilized by a naturally-occurring Clostridial toxin. This re-targeted capability is achieved by replacing the naturally-occurring binding domain of a Clostridial toxin with a targeting domain having a binding activity for a non-Clostridial toxin receptor. Although binding to a non-Clostridial toxin receptor, a TEM undergoes all the other steps of the intoxication process including internalization of the TEM/receptor complex into the cytoplasm, formation of the pore in the vesicle membrane and di-chain molecule, translocation of the enzymatic domain into the cytoplasm, and exerting a proteolytic effect on a component of the SNARE complex of the target cell.

[0047] “Topical administration” excludes systemic administration of the neurotoxin. In other words, and unlike conventional therapeutic transdermal methods, topical administration of botulinum toxin does not result in significant amounts, such as the majority of, the neurotoxin passing into the circulatory system of the patient.

[0048] "Treating" means to alleviate (or to eliminate) at least one symptom of a condition or disorder, such as, for example, wrinkles, spasticity, depression, pain (such as, for example, headache pain), bladder overactivity, or the like, either temporarily or permanently.

[0049] "Variant" means a clostridial neurotoxin, such as wild-type botulinum toxin serotype A, B, C, D, E, F or G, that has been modified by the replacement, modification, addition or deletion of at least one amino acid relative to wild-type botulinum toxin, which is recognized by a target cell, internalized by the target cell, and catalytically cleaves a SNARE (SNAP (Soluble NSF Attachment Protein) Receptor) protein in the target cell.

[0050] An example of a variant neurotoxin component can comprise a variant light chain of a botulinum toxin having one or more amino acids substituted, modified, deleted and/or added. This variant light chain may have the same or better ability to prevent exocytosis, for example, the release of neurotransmitter vesicles. Additionally, the biological effect of a variant may be decreased compared to the parent chemical entity. For example, a variant light chain of a botulinum toxin type A having an amino acid sequence removed may have a shorter biological persistence than that of the parent (or native) botulinum toxin type A light chain.

Pharmaceutical compositions

[0051] Certain embodiments of the present invention include a pharmaceutical composition comprising (or consisting of, or consisting essentially of) a Clostridial toxin active ingredient such as a botulinum toxin, a disaccharide, a surfactant and an antioxidant.

[0052] Aspects of the present pharmaceutical compositions provide, in part, a Clostridial toxin active ingredient. As used herein, the term "Clostridial toxin active ingredient" refers to a therapeutically effective concentration of a Clostridial toxin active ingredient, such as, *e.g.*, a Clostridial toxin complex, a Clostridial toxin, a modified Clostridial toxin, or a re-targeted Clostridial toxin. As used herein, the term "therapeutically effective concentration" is synonymous with "therapeutically effective amount," "effective amount," "effective dose," and "therapeutically effective dose" and refers to the minimum dose of a Clostridial toxin active ingredient necessary to achieve the desired therapeutic effect and includes a dose sufficient to reduce a symptom associated with aliment being treated. In aspects of this embodiment, a therapeutically effective concentration of a Clostridial toxin active ingredient reduces a symptom associated with the aliment being treated by, *e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In other aspects of this embodiment, a therapeutically effective concentration of a Clostridial toxin active ingredient reduces a symptom associated with the aliment being treated by, *e.g.*, at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90% or at most 100%.

[0053] It is envisioned that any amount of Clostridial toxin active ingredient can be added in formulating a Clostridial toxin pharmaceutical compositions disclosed in the present specification,

with the proviso that a therapeutically effective amount of Clostridial toxin active ingredient is recoverable. In aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is at least 0.1 U/ml, at least 1.0 U/ml, at least 10 U/ml, at least 50 U/ml, at least 100 U/ml, at least 200 U/ml, or at least 1000 U/ml. In other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is at most 0.1 U/ml, at most 1.0 U/ml, at most 10 U/ml, at most 50 U/ml, at most 100 U/ml, at most 200 U/ml, or at most 1000 U/ml. In yet other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is from about 0.1 U/ml to about 1000 U/ml, or about 1.0 U/ml to about 1000 U/ml. In still other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is from about 0.001 U/ml to about 100 U/ml, about 0.01 U/ml to about 100 U/ml, about 0.1 U/ml to about 100 U/ml, or about 1.0 U/ml to about 100 U/ml. As used herein, the term "unit" or "U" is refers to the LD_{50} dose, which is defined as the amount of a Clostridial toxin, Clostridial toxin complex or modified Clostridial toxin that killed 50% of the mice injected with the Clostridial toxin, Clostridial toxin complex or modified Clostridial toxin. As used herein, the term "about" when qualifying a value of a stated item, number, percentage, or term refers to a range of plus or minus ten percent of the value of the stated item, percentage, parameter, or term.

[0054] In other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is at least 1.0 pg, at least 10 pg, at least 100 pg, at least 1.0 ng, at least 10 ng, at least 100 ng, at least 1.0 μg, at least 10 μg, at least 100 μg, or at least 1.0 mg. In still other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is at most 1.0 pg, at most 10 pg, at most 100 pg, at most 1.0 ng, at most 10 ng, at most 100 ng, at most 1.0 μg, at most 10 μg, at most 100 μg, or at most 1.0 mg. In still other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is about 1.0 pg to about 10 μg, about 10 pg to about 10 μg, about 100 pg to about 10 μg, about 1.0 ng to about 10 μg, about 10 ng to about 10 μg, or about 100 ng to about 10 μg. In still other aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is about 1.0 pg to about 1.0 μg, about 10 pg to about 1.0 μg, about 100 pg to about 1.0 μg, about 1.0 ng to about 1.0 μg, about 10 ng to about 1.0 μg, or about 100 ng to about 1.0 μg. In further aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is about 1.0 pg to about 5.0 μg, about 10 pg to about 5.0 μg, about 100 pg to about 5.0 μg, about 1.0 ng to about 5.0 μg, about 10 ng to about 5.0 μg, or about 100 ng to about 5.0 μg. In further aspects of this embodiment, the amount of Clostridial toxin active ingredient added to the formulation is about 1.0 pg to about 10 μg, about 10 pg to about 10 μg, about 100 pg to about 10 μg, about 1.0 ng to about 10 μg, about 10 ng to about 10 μg, or about 100 ng to about 10 μg.

[0055] In aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises a BoNT/A, a BoNT/B, a BoNT/C_1, a BoNT/D, a BoNT/E, a BoNT/F, a BoNT/G, a TeNT, a BaNT, or a BuNT. In another embodiment, a Clostridial toxin pharmaceutical composition comprises a Clostridial toxin variant as the Clostridial toxin active ingredient. In aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises naturally-occurring Clostridial toxin variant or a non-naturally-occurring Clostridial toxin variant. In other aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises a BoNT/A variant, a BoNT/B variant, a BoNT/C_1 variant, a BoNT/D variant, a BoNT/E variant, a BoNT/F variant, a BoNT/G variant, a TeNT variant, a BaNT variant, or a BuNT variant, where the variant is either a naturally-occurring variant or a non-naturally-occurring variant.

[0056] Aspects of the present pharmaceutical compositions provide, in part, a Clostridial toxin complex as a Clostridial toxin active ingredient. As used herein, the term "Clostridial toxin complex" refers to a complex comprising a Clostridial toxin and associated NAPs, such as, *e.g.*, a Botulinum toxin complex, a Tetanus toxin complex, a Baratii toxin complex, and a Butyricum toxin complex. Non-limiting examples of Clostridial toxin complexes include those produced by a *Clostridium botulinum*, such as, *e.g.*, a 900-kDa BoNT/A complex, a 500-kDa BoNT/A complex, a 300-kDa BoNT/A complex, a 500-kDa BoNT/B complex, a 500-kDa BoNT/C_1 complex, a 500-kDa BoNT/D complex, a 300-kDa BoNT/D complex, a 300-kDa BoNT/E complex, and a 300-kDa BoNT/F complex. Clostridial toxin complexes can be purified using the methods described in Schantz, *supra*, (1992); Hui Xiang et al., *Animal Product Free System and Process for Purifying a Botulinum Toxin*, U.S. Patent 7,354,740, each of which is hereby incorporated by reference in its entirety. Clostridial toxin complexes can be obtained from, *e.g.*, List Biological Laboratories, Inc. (Campbell, California), the Centre for Applied Microbiology and Research (Porton Down, U.K), Wako (Osaka, Japan), and Sigma Chemicals (St Louis, Missouri).

[0057] Aspects of the present pharmaceutical compositions provide, in part, a non-protein excipient. As used herein, the term "non-protein excipient" refers to any excipient that is not a polypeptide comprising at least fifteen amino acids. It is envisioned that any non-protein excipient is useful in formulating a Clostridial toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is recovered using this non-protein excipient.

[0058] Aspects of the present pharmaceutical compositions provide, in part, a sugar. As used herein, the term "sugar" refers to a compound comprising one to 10 monosaccharide units, *e.g.*, a monosaccharide, a disaccharide, a trisaccharide, and an oligosaccharide comprising four to ten

monosaccharide units. It is envisioned that any sugar is useful in formulating a Clostridial toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is recovered using this sugar. Monosaccharides are polyhydroxy aldehydes or polyhydroxy ketones with three or more carbon atoms, including aldoses, dialdoses, aldoketoses, ketoses and diketoses, as well as cyclic forms, deoxy sugars and amino sugars, and their derivatives, provided that the parent monosaccharide has a (potential) carbonyl group. Monosacchrides include trioses, like glyceraldehyde and dihydroxyacetone; tetroses, like erythrose, erythrulose and threose; pentoses, like arabinose, lyxose, ribose, ribulose, xylose, xylulose; hexoses, like allose, altrose, fructose, fucose,galactose, glucose, gulose, idose, mannose, psicose, rhamnose, sorbose, tagatose, talose and trehalose; heptoses, like sedoheptulose and mannoheptulose; octooses, like octulose and 2-keto-3-deoxy-manno-octonate; nonoses like sialose; and decose. Oligosaccharides are compounds in which at least two monosaccharide units are joined by glycosidic linkages. According to the number of units, they are called disaccharides, trisaccharides, tetrasaccharides, pentasaccharides, hexoaccharides, heptoaccharides, octoaccharides, nonoaccharides, decoaccharides, etc. An oligosaccharide can be unbranched, branched or cyclic. Common disaccharides include, without limitation, sucrose, lactose, maltose, trehalose, cellobiose, gentiobiose, kojibiose, laminaribiose, mannobiose, melibiose, nigerose, rutinose, and xylobiose. Common trisaccharides include, without limitation, raffinose, acarbose, maltotriose, and melezitose. Other non-limiting examples of specific uses of sugar excipients can be found in, *e.g.*, Ansel, *supra*, (1999); Gennaro, *supra*, (2000); Hardman, *supra*, (2001); and Rowe, *supra*, (2003), each of which is hereby incorporated by reference in its entirety

[0059] In an embodiment, a Clostridial toxin pharmaceutical composition comprises a sugar. In aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises a monosaccharide. In other aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, a hexoaccharide, a heptoaccharide, an octoaccharide, a nonoaccharide, or a decoaccharide. In yet other aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises an oligosaccharide comprising two to ten monosaccharide units.

[0060] It is envisioned that any amount of sugar is useful in formulating a Clostridial toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is recovered using this sugar amount. In aspects of this embodiment, the amount of sugar added to the formulation is about 0.1% (w/w), about 0.5% (w/w), about 1.0% (w/w), about 1.5% (w/w), about 2.0% (w/w), about

2.5% (w/w), about 3.0% (w/w), about 3.5% (w/w), about 4.0% (w/w), about 4.5% (w/w), about 5.0% (w/w), about 5.5% (w/w), about 6.0% (w/w), about 6.5% (w/w), about 7.0% (w/w), about 7.5% (w/w), about 8.0% (w/w), about 8.5% (w/w), about 9.0% (w/w), about 9.5% (w/w), about 10% (w/w), about 15% (w/w), about 20% (w/w), about 25% (w/w), about 30% (w/w), or about 35% (w/w). In other aspects of this embodiment, the amount of sugar added to the formulation is at least 0.1% (w/w), at least 0.5% (w/w), at least 1.0% (w/w), at least 1.5% (w/w), at least 2.0% (w/w), at least 2.5% (w/w), at least 3.0% (w/w), at least 3.5% (w/w), at least 4.0% (w/w), at least 4.5% (w/w), at least 5.0% (w/w), at least 5.5% (w/w), at least 6.0% (w/w), at least 6.5% (w/w), at least 7.0% (w/w), at least 7.5% (w/w), at least 8.0% (w/w), at least 8.5% (w/w), at least 9.0% (w/w), at least 9.5% (w/w), at least 10% (w/w), at least 15% (w/w), at least 20% (w/w), at least 25% (w/w), at least 30% (w/w), or at least 35% (w/w). In yet other aspects of this embodiment, the amount of sugar added to the formulation is at most 0.1% (w/w), at most 0.5% (w/w), at most 1.0% (w/w), at most 1.5% (w/w), at most 2.0% (w/w), at most 2.5% (w/w), at most 3.0% (w/w), at most 3.5% (w/w), at most 4.0% (w/w), at most 4.5% (w/w), at most 5.0% (w/w), at most 5.5% (w/w), at most 6.0% (w/w), at most 6.5% (w/w), at most 7.0% (w/w), at most 7.5% (w/w), at most 8.0% (w/w), at most 8.5% (w/w), at most 9.0% (w/w), at most 9.5% (w/w), at most 10% (w/w), at most 15% (w/w), at most 20% (w/w), at most 25% (w/w), at most 30% (w/w), or at most 35% (w/w).

[0061] In an embodiment, the present Clostridial toxin pharmaceutical composition comprises a disaccharide. Common disaccharides include, without limitation, sucrose, lactose, maltose, trehalose, cellobiose, gentiobiose, kojibiose, laminaribiose, mannobiose, melibiose, nigerose, rutinose, and xylobiose. In aspects of this embodiment, the clostridial toxin pharmaceutical composition comprises sucrose. In one specific embodiment, the clostridial toxin pharmaceutical composition comprises trehalose. In aspects of this embodiment, the amount of disaccharide added to the formulation added to the formulation is about 0.1% (w/w), about 0.5% (w/w), about 1.0% (w/w), about 1.5% (w/w), about 2.0% (w/w), about 2.5% (w/w), about 3.0% (w/w), about 3.5% (w/w), about 4.0% (w/w), about 4.5% (w/w), about 5.0% (w/w), about 5.5% (w/w), about 6.0% (w/w), about 6.5% (w/w), about 7.0% (w/w), about 7.5% (w/w), about 8.0% (w/w), about 8.5% (w/w), about 9.0% (w/w), about 9.5% (w/w), about 10% (w/w), about 15% (w/w), about 20% (w/w), about 25% (w/w), about 30% (w/w), or about 35% (w/w).

[0062] Aspects of the present pharmaceutical compositions provide, in part, a surfactant. As used hereon, the term "surfactant" refers to a natural or synthetic amphiphilic compound. A surfactant can be non-ionic, zwitterionic, or ionic. It is envisioned that any surfactant is useful in formulating a Clostridial toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is

recovered using this surfactant amount. Non-limiting examples of surfactants include polysorbates like polysorbate 20 (TWEEN® 20), polysorbate 40 (TWEEN® 40), polysorbate 60 (TWEEN® 60), polysorbate 61 (TWEEN® 61), polysorbate 65 (TWEEN® 65), polysorbate 80 (TWEEN® 80), and polysorbate 81 (TWEEN® 81); poloxamers (polyethylene-polypropylene copolymers), like Poloxamer 124 (PLURONIC® L44), Poloxamer 181 (PLURONIC® L61), Poloxamer 182 (PLURONIC® L62), Poloxamer 184 (PLURONIC® L64), Poloxamer 188 (PLURONIC® F68), Poloxamer 237 (PLURONIC® F87), Poloxamer 338 (PLURONIC® L108), Poloxamer 407 (PLURONIC® F127), polyoxyethyleneglycol dodecyl ethers, like BRIJ® 30, and BRIJ® 35; 2-dodecoxyethanol (LUBROL®-PX); polyoxyethylene octyl phenyl ether (TRITON® X-100); sodium dodecyl sulfate (SDS); solutol HS15; 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS); 3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO); sucrose monolaurate; and sodium cholate. Other non-limiting examples of surfactant excipients can be found in, *e.g.*, Ansel, *supra*, (1999); Gennaro, *supra*, (2000); Hardman, *supra*, (2001); and Rowe, *supra*, (2003), each of which is hereby incorporated by reference in its entirety.

[0063] Thus in an embodiment, a Clostridial toxin pharmaceutical composition comprises a surfactant. In aspects of this embodiment, a Clostridial toxin pharmaceutical composition comprises a polysorbate, a poloxamer, a polyoxyethyleneglycol dodecyl ether, 2-dodecoxyethanol , polyoxyethylene octyl phenyl ether, sodium dodecyl sulfate, 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate, 3-[(3-Cholamidopropyl) dimethylammonio]-2-hydroxy-1-propanesulfonate, sucrose monolaurate; or sodium cholate.

[0064] It is envisioned that any amount of surfactant is useful in formulating a Clostridial toxin pharmaceutical compositions disclosed in the present specification, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is recovered using this surfactant amount. In aspects of this embodiment, the amount of surfactant added to the formulation is about 0.01% (w/w), about 0.02% (w/w), about 0.03% (w/w), about 0.04% (w/w), about 0.05% (w/w), about 0.06% (w/w), about 0.07% (w/w), about 0.08% (w/w), about 0.09% (w/w), about 0.1% (w/w), about 0.5% (w/w), about 1.0% (w/w), about 1.5% (w/w), about 2.0% (w/w), about 2.5% (w/w), about 3.0% (w/w), about 3.5% (w/w), about 4.0% (w/w), about 4.5% (w/w), about 5.0% (w/w), about 5.5% (w/w), about 6.0% (w/w), about 6.5% (w/w), about 7.0% (w/w), about 7.5% (w/w), about 8.0% (w/w), about 8.5% (w/w), about 9.0% (w/w), about 9.5% (w/w), about 10% (w/w), about 15% (w/w), about 20% (w/w), about 25% (w/w), about 30% (w/w), or about 35% (w/w). In other aspects of this embodiment, the amount of surfactant added to the formulation is at least 0.01% (w/w), at least 0.02% (w/w), at least 0.03% (w/w), at least 0.04% (w/w), at least 0.05%

(w/w), at least 0.06% (w/w), at least 0.07% (w/w), at least 0.08% (w/w), at least 0.09% (w/w), at least 0.1% (w/w), at least 0.5% (w/w), at least 1.0% (w/w), at least 1.5% (w/w), at least 2.0% (w/w), at least 2.5% (w/w), at least 3.0% (w/w), at least 3.5% (w/w), at least 4.0% (w/w), at least 4.5% (w/w), at least 5.0% (w/w), at least 5.5% (w/w), at least 6.0% (w/w), at least 6.5% (w/w), at least 7.0% (w/w), at least 7.5% (w/w), at least 8.0% (w/w), at least 8.5% (w/w), at least 9.0% (w/w), at least 9.5% (w/w), at least 10% (w/w), at least 15% (w/w), at least 20% (w/w), at least 25% (w/w), at least 30% (w/w), or at least 35% (w/w). In yet other aspects of this embodiment, the amount of surfactant added to the formulation is at most 0.01% (w/w), at most 0.02% (w/w), at most 0.03% (w/w), at most 0.04% (w/w), at most 0.05% (w/w), at most 0.06% (w/w), at most 0.07% (w/w), at most 0.08% (w/w), at most 0.09% (w/w), at most 0.1% (w/w), at most 0.5% (w/w), at most 1.0% (w/w), at most 1.5% (w/w), at most 2.0% (w/w), at most 2.5% (w/w), at most 3.0% (w/w), at most 3.5% (w/w), at most 4.0% (w/w), at most 4.5% (w/w), at most 5.0% (w/w), at most 5.5% (w/w), at most 6.0% (w/w), at most 6.5% (w/w), at most 7.0% (w/w), at most 7.5% (w/w), at most 8.0% (w/w), at most 8.5% (w/w), at most 9.0% (w/w), at most 9.5% (w/w), at most 10% (w/w), at most 15% (w/w), at most 20% (w/w), at most 25% (w/w), at most 30% (w/w), or at most 35% (w/w).

[0065] In some embodiments, the clostridial toxin pharmaceutical composition comprises a poloxamer. Poloxamers which can be used with the present pharmaceutical composition include Poloxamer 124 (PLURONIC® L44), Poloxamer 181 (PLURONIC® L61), Poloxamer 182 (PLURONIC® L62), Poloxamer 184 (PLURONIC® L64), Poloxamer 188 (PLURONIC® F68), Poloxamer 237 (PLURONIC® F87), Poloxamer 338 (PLURONIC® L108), Poloxamer 407 (PLURONIC® F127). In some embodiments, poloxamer 188 may be more advantageous.

[0066] In some emobdiments, the clostridial toxin pharmaceutical composition comprises a polysorbate. Polysorbates which can be used with the present pharmaceutical composition includes polysorbate 20 (TWEEN® 20), polysorbate 40 (TWEEN® 40), polysorbate 60 (TWEEN® 60), polysorbate 61 (TWEEN® 61), polysorbate 65 (TWEEN® 65), polysorbate 80 (TWEEN® 80), and polysorbate 81 (TWEEN® 81). In some embodiments, polysorbate 20 may be more advantageous than some other polysorbates.

[0067] Aspects of the present pharmaceutical compositions provide, in part, at least an antioxidant. Non-limiting examples of antioxidant include, without limitation, methionine, cysteine, N-acetyl-cysteine (NAC), sodium metabisulfite, sodium thiosulfate, butylated hydroxyanisole, butylated hydroxytoluene, vitamin E and analogs including Trolox C; chelators such as EDTA (ethylene diamine tetraacetic acid sodium salt), DPTA (Diethylenetriaminepentaacetic acid) or DPTA-bisamide, calcium DTPA, and CaNaDPTA-bisamide; or combinations thereof. In aspects of

this embodiment, the amount of antioxidant added to the formulation ranges from about 0.01% (w/w) to about 0.10% (w/w).

[0068] It is further envisioned that a Clostridial toxin pharmaceutical composition disclosed in the present specification can optionally include, without limitation, other pharmaceutically acceptable components (or pharmaceutical components), including, without limitation, buffers, preservatives, tonicity adjusters, salts, antioxidants, osmolality adjusting agents, emulsifying agents, sweetening or flavoring agents, and the like. Various buffers and means for adjusting pH can be used to prepare a pharmaceutical composition disclosed in the present specification, provided that the resulting preparation is pharmaceutically acceptable. Such buffers include, without limitation, acetate buffers, borate buffers, citrate buffers, phosphate buffers, neutral buffered saline, and phosphate buffered saline. It is understood that acids or bases can be used to adjust the pH of a pharmaceutical composition as needed. It is envisioned that any buffered pH level can be useful in formulating a Clostridial toxin pharmaceutical composition, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is recovered using this effective pH level. In an aspect of this embodiment, an effective pH level is at least about pH 5.0, at least about pH 5.5, at least about pH 6.0, at least about pH 6.5, at least about pH 7.0 or at about about pH 7.5. In another aspect of this embodiment, an effective pH level is at most about pH 5.0, at most about pH 5.5, at most about pH 6.0, at most about pH 6.5, at most about pH 7.0 or at most about pH 7.5. In yet another aspect of this embodiment, an effective pH level is about pH 5.0 to about pH 8.0, an effective pH level is about pH 5.0 to about pH 7.0, an effective pH level is about pH 5.0 to about pH 6.0, is about pH 5.5 to about pH 8.0, an effective pH level is about pH 5.5 to about pH 7.0, an effective pH level is about pH 5.5 to about pH 5.0, is about pH 5.5 to about pH 7.5, an effective pH level is about pH 5.5 to about pH pH 6.5.

[0069] The pharmaceutical compositions disclosed herein can have a pH of between about 5 and 8 when reconstituted or upon injection. In certain embodiments the composition will have a pH below 8, such as, for example, 7.9, or 7.8, or 7.7, or 7.6, or 7.5, or 7.4, or 7.3, or 7.2, or 7.1, or 7.0, or 6.9, or 6.8, or 6.7, or 6.6, or 6.5, or 6.4, or 6.3, or 6.2, or 6.1, or 6.0, or 5.9, or 5.8, or 5.7, or 5.6, or 5.5, or 5.4, or 5.3, or 5.2, or 5.1, or the like. In some embodiments, the pH ranges from 5 to 7.

[0070] It is envisioned that any concentration of a buffer can be useful in formulating a Clostridial toxin pharmaceutical composition, with the proviso that a therapeutically effective amount of the Clostridial toxin active ingredient is recovered using this effective concentration of buffer. In aspects of this embodiment, an effective concentration of buffer is at least 0.1 mM, at least 0.2 mM, at least 0.3 mM, at least 0.4 mM, at least 0.5 mM, at least 0.6 mM, at least 0.7 mM, at

least 0.8 mM, or at least 0.9 mM. In other aspects of this embodiment, an effective concentration of buffer is at least 1.0 mM, at least 2.0 mM, at least 3.0 mM, at least 4.0 mM, at least 5.0 mM, at least 6.0 mM, at least 7.0 mM, at least 8.0 mM, or at least 9.0 mM. In yet other aspects of this embodiment, an effective concentration of buffer is at least 10 mM, at least 20 mM, at least 30 mM, at least 40 mM, at least 50 mM, at least 60 mM, at least 70 mM, at least 80 mM, or at least 90 mM. In still other aspects of this embodiment, an effective concentration of buffer is at least 100 mM, at least 200 mM, at least 300 mM, at least 400 mM, at least 500 mM, at least 600 mM, at least 700 mM, at least 800 mM, or at least 900 mM. In further aspects of this embodiment, an effective concentration of buffer is at most 0.1 mM, at most 0.2 mM, at most 0.3 mM, at most 0.4 mM, at most 0.5 mM, at most 0.6 mM, at most 0.7 mM, at most 0.8 mM, or at most 0.9 mM. In still other aspects of this embodiment, an effective concentration of buffer is at most 1.0 mM, at most 2.0 mM, at most 3.0 mM, at most 4.0 mM, at most 5.0 mM, at most 6.0 mM, at most 7.0 mM, at most 8.0 mM, or at most 9.0 mM. In yet other aspects of this embodiment, an effective concentration of buffer is at most 10 mM, at most 20 mM, at most 30 mM, at most 40 mM, at most 50 mM, at most 60 mM, at most 70 mM, at most 80 mM, or at most 90 mM. In still other aspects of this embodiment, an effective concentration of buffer is at most 100 mM, at most 200 mM, at most 300 mM, at most 400 mM, at most 500 mM, at most 600 mM, at most 700 mM, at most 800 mM, or at most 900 mM. In still further aspects of this embodiment, an effective concentration of buffer is about 0.1 mM to about 900 mM, 0.1 mM to about 500 mM, 0.1 mM to about 100 mM, 0.1 mM to about 90 mM, 0.1 mM to about 50 mM, 1.0 mM to about 900 mM, 1.0 mM to about 500 mM, 1.0 mM to about 100 mM, 1.0 mM to about 90 mM, or 1.0 mM to about 50 mM.

[0071] Embodiments of the invention can be practiced with a composition that comprises a plurality of botulinum toxin serotypes, such as botulinum toxin serotypes selected from the group consisting of botulinum toxin serotypes A, B, C_1 D, E, F and G. In certain embodiments, purified botulinum toxins, can be used. In other embodiments, modified botulinum toxins may be used.

[0072] In some embodiments, the Clostridial toxin pharmaceutical composition can be formulated as a lyophilized (i.e. freeze dried) or vacuum dried powder which can be reconstituted with a suitable fluid, such as saline or water, prior to administration to a patient. In alternative embodiments, the pharmaceutical composition can be formulated as an aqueous solution or suspension.

[0073] In some embodiments, the solid Clostridial toxin pharmaceutical composition comprises a botulinum toxin, a tonicity agent, a poloxamer and/or a polysorbate and an antioxidant. In some embodiments, the Clostridial toxin pharmaceutical composition comprises a botulinum toxin. In

some embodiments, the Clostridial toxin pharmaceutical composition comprises trehalose. In some embodiments, the Clostridial toxin pharmaceutical composition comprises poloxamer 188 or polysorbate 20. In some embodiments, the composition comprises EDTA or an EDTA analog. In alternative embodiments, the composition comprises methionine and/or NAC. In aspects of these alternative embodiments, the composition further comprises EDTA or an EDTA analog. In some embodiments, the composition further comprises a buffering agent. In one embodiment, the compositon comprises histidine buffer. In some embodiments, the relative weight amounts of trehalose, poloxamer and methionine are within the following ranges respectively: 1 to 10%; 0.5 to 5% and 0.1 to 0.3%. In some embodiments, the relative weight amounts of trehalose, polysorbate and methionine are within the following ranges respectively: 1 to 10%; 0.02% to 0.06%; and 0.1 to 0.3%. In some embodiments, the relative weight amount of EDTA or an EDTA analog is from about 0.01 to 0.10%. In some embodiments, the relative weight amount of NAC ranges from 0.01 to 0.5%.

[0074] In aspects of these embodiments, the Clostridial toxin pharmaceutical composition is formulated as a solid (i.e lyophilized or vacuum dried) composition. In some embodiment, the solid pharmaceutical composition comprises NAC in a relative weight amount of 0.01 to 0.05%. In some embodiments, the pharmaceutical composition further comprises EDTA or an EDTA analog. In alternative embodiments, the solid pharmaceutical composition comprises methionine and EDTA or an EDTA analog.

[0075] In an alternative aspect of these embodiments, the Clostridial toxin pharmaceutical composition is formulated as a liquid. In some embodiments, the liquid pharmaceutical composition comprises NAC in a relative weight amount of 0.1 to 0.5%. In some embodiment,s the liquid pharmaceucial composition comprises NAC and EDTA or an EDTA analog. In some embodiments, the liquid pharmaceutical composition comprises histidine buffer. In some embodiments, the liquid pharmaceutical composition has a pH from 5 to 7.

Methods of treatment

[0076] In embodiments, the invention provides methods of treating diseases, disorders, conditions, and the like, comprising the step of administering a pharmaceutical formulation of the invention to a subject in need thereof in an amount sufficient to produce improved patient function. In certain embodiments, the diseases are of a neuromuscular nature, such as, for example, those diseases that affect muscles and nerve control thereof, such as, for example, overactive bladder, and the like. Certain embodiments relate to the treatment of pain, such as, for example, treatment of headache pain, or back pain, or muscle pain, or the like. In certain embodiments, methods of the

invention encompass the treatment of psychological disorders, including, for example, depression, anxiety, and the like.

[0077] Compositions and methods of the invention can be useful for the treatment, reduction of symptoms, and/or prevention of, for example, achalasia, anal fissure, anismus, blepharospasm, cerebral palsy, cervical dystonia, cervicogenic headache, hemifacial spasm, dyshidrotic eczema, dysphagia, dysphonia, esophageal dysmotility, esophageal muscular ring, esotropia (infantile), eyelift, facial myokemia, gait disturbances (idiopathic toe-walking), generalized dystonia, hemifacial spasm, hyperfunctional facial lines (glabellar, forehead, crows' feet, down-turned angles of the mouth), hyperhidrosis, incontinence (idiopathic or neurogenic), medication overuse headache, migraine headache, myoclonus, muscle mass or activity reduction, involving, for example, the masseter or the like, myofascial pain syndrome, obstructive urinary symptoms, pancreas divisum pancreatitis, Parkinson's disease, puborectalis syndrome, reduction of surgical scar tension, salivary hypersecretion, sialocele, sixth nerve palsy, spasticity, speech/voice disorders, strabismus, surgery adjunct (ophthalmic), tardive dyskinesia, temporomandibular joint disorders, tension headache, thoracic outlet syndrome, torsion dystonia, torticolis, Tourette's syndrome, tremor, whiplash-associated neck pain, pain, itching, inflammation, allergy, cancer and benign tumors, fever, obesity, infectious diseases, viral and bacterial, hypertension, cardiac arrhythmias, vasospasm, atherosclerosis, endothelial hyperplasia, venous thrombosis, varicose veins, apthous stomatitis, hypersalivation, temporomandibular joint syndrome, hyperhidrosis, bromhidrosis, acne, rosacea, hyperpigmention, hypertrophic scars, keloids, calluses and corns, skin wrinkling, excessive sebum production, psoriasis, dermatitis, allergic rhinitis, nasal congestion, post nasal drip, sneezing, ear wax, serous and suppurative otitis media, tonsil and adenoid hypertrophy, tinnitus, dizziness, vertigo, hoarseness, cough, sleep apnea, snoring, glaucoma, conjunctivitis, uveitis, strabismus, Grave's disease, excessive hair growth, hair loss, asthma, bronchitis, emphysema, mucus production, pleuritis, coagulation disorders, myeloproliferative disorders, disorders involving eosinophils, neutrophils, macrophages and lymphocytes, immune tolerance and transplantation, autoimmune disorders, dysphagia, acid reflux, hiatal hernia, gastritis and hyperacidity, diarrhea and constipation, hemorrhoids, urinary incontinence, prostatic hypertrophy, erectile dysfunction, priapism and Peyronie's disease, epididymitis, contraception, menstrual cramps, preventing premature delivery, endometriosis and fibroids, arthritis, osteoarthritis, rheumatoid, bursitis, tendonitis, tenosynovitis, fibromyalgia, seizure disorders, spasticity, headache, and neuralgias.

[0078] In certain embodiments, patients are limited to a maximum of 360U of botulinum toxin administered over any 90-day period.

Treatment of nerve / muscle conditions

[0079] In an embodiment, the neuromuscular disease is hyperhidrosis. A subject suffering from hyperhidrosis, for example, receives about 59U per axilla, or about 58U per axilla, or about 57U per axilla, or about 56U per axilla, or about 55U per axilla, or about 54U per axilla, or about 53U per axilla, or about 52U per axilla, or about 51U per axilla, or about 50U per axilla, or about 49U per axilla, or about 48U per axilla, or about 47U per axilla, or about 46U per axilla, or about 45U per axilla, or about 44U per axilla, or about 43U per axilla, or about 42U per axilla, or about 41U per axilla, or about 40U per axilla, or about 39U per axilla, or about 38U per axilla, or about 37U per axilla, or about 36U per axilla, or less, per treatment of a pharmaceutical formulation of the present invention. In an embodiment, 50U total are injected intradermally into 10-15 sites spaced approximately 1-2cm apart.

[0080] In an embodiment, the neuromuscular disease is hemifacial spasm. A subject suffering from hemifacial spasm, for example receives between about 1.5 to 15U per treatment of a of the pharmaceutical formulation of the present invention. In a further example, the subject receives between about 1.5 to 3U, 1.5 to 5U, 1.5 to 7U, 1.5 to 10U, 1.5 to 12U, 1.5 to 15U, 5 to 10U, 5 to 15U, or 10 to 15U per treatment are administered to a patient with hemifacial spasm. In a still further example, the subject receives about 1.5U, about 2U, about 2.5U, about 3U, about 3.5U, about 4U, about 4.5U about 5U, about 5.5U, about 6U, about 6.5U, about 7U, about 7.5U, about 8U, about 8.5U, about 9U, about 9.5U, about 10U, about 10.5U, about 11U, about 11.5U, about 12U, about 12.5U, about 13U, about 13.5U, about 14U, about 14.5U, or about 15U per treatment are administered to a patient with hemifacial spasm. Dosages greater than 15U per treatment may also be administered to patients with hemifacial spasm to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0081] In an embodiment, the neuromuscular disease is cervical dystonia. A subject suffering from cervical dystonia, for example, receives between about 15 to 300U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 35 to 250U, 65 to 200U, 85 to 175U, 105 to 160U, or 125 to 145U are administered to a patient with cervical dystonia. In an embodiment, dosages to the sternocleidomastoid muscle is limited to 100U or less. Dosages greater than 300U per treatment may also be administered to patients with cervical dystonia to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0082] In an embodiment, the neuromuscular disease is blepharospasm. A subject suffering from blepharospasm, for example, receives between about 1.25 to 2.5U of a pharmaceutical

formulation of the present invention injected into the medial and lateral pretarsal orbicularis oculi of the upper lid and into the lateral pretarsal orbicularis oculi of the lower lid. In a further example, the subject receives about 1.5U, about 1.6U, about 1.7U, about 1.8U, about 1.9U, about 2.0U, about 2.1U, about 2.2U, about 2.3U, about 2.4U, about 2.5U, or more, per injection site. A treatment session can comprise multiple treatments.

[0083] In an embodiment, the neuromuscular disease is strabismus. A subject suffering from strabismus, for example, receives between about 1.25 to 2.5U per injection site of a pharmaceutical formulation of the present invention. In a further example, the subject recieves about 1.5U, , about 1.6U, about 1.7U, about 1.8U, about 1.9U, about 2.0U, about 2.1U, about 2.2U, about 2.3U, about 2.4U, about 2.5U, or more, per injection site to achieve a therapeutic response. In embodiments, lower doses are used for treatment of small deviations. In embodiments, vertical muscles and horizontal strabismus of less than 20 prism diameters can be treated with 1.25 to 2.5U per injection site. A treatment session can comprise multiple treatments.

[0084] In an embodiment, the neuromuscular disease is muscle spasticity. A subject suffering from muscle spasticity, for example, receives between about 20 to 200U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 20 to 30U, 20 to 40U, 20 to 60U, 20 to 80U, 20 to 100U, 20 to 125U, 20 to 150U, or 20 to 175U per treatment are administered to a patient with muscle spasticity. In a still further example, the subject receives about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, about 150U, about 155U, about 160U, about 165U, about 170U, about 175U, about 180U, about 185U, about 190U, about 195U, or about 200U per treatment are administered to a patient with muscle spasticity. In an embodiment, the biceps brachii can be injected with between 100U and 200U divided into 4 injection sites. In an embodiment, the flexor carpi radialis can be injected with between 12.5U and 50U in 1 injection site. In an embodiment, the flexor carpi ulnaris can be injected with between 12.5U and 50U in 1 injection site. In an embodiment, the flexor digitorum profundus can be injected with between 30U and 50U in one injection site. In an embodiment, the flexor digitorum sublimis can be injected with between 30U and 50 in a single injection site. Dosages greater than 200U per treatment may also be administered to patients with muscle spasticity to achieve a therapeutic response. A treatment session can comprise multiple treatments.

Treatment of pain

[0085] In another embodiment, the present invention provides methods for treating pain comprising the step of administering a pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to reduce pain. In another embodiment, the patient suffers from myofascial pain, migraine headache pain, tension headache pain, neuropathic pain, facial pain, lower-back pain, sinus-headache pain, pain associated with temporomandibular joint disease, pain associated with spasticity or cervical dystonia, post-surgical wound pain, or neuralgia. A treatment session can comprise multiple treatments.

[0086] In an embodiment, the patient suffers from facial pain. A subject suffering from facial pain, for example, receives between about 4 to 40U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 4 to 10U, 4 to 15U, 4 to 20U, 4 to 25U, 4 to 30U, 4 to 35U, 7 to 15U, 7 to 20U, 7 to 25U, 7 to 30U, 7 to 35U, or 7 to 40U per treatment are administered to a patient suffering from facial pain. In a still further example, the subject receives about 4U, about 5U, about 7.5U, about 10U, about 12.5U, about 15U, about 17.5U, about 20.0U, about 22.5U, about 25.0U, about 27.5U, about 30.0U, about 32.5U, about 35U, about 37.5U, or about 40U per treatment are administered to a patient with facial pain. Dosages greater than 40U per treatment may also be administered to patients with facial pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0087] In an embodiment, the patient suffers from myofascial pain. A subject suffering from myofascial pain, for example, receives between about 5 to 100U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject recieves between about 5 to 10U, 5 to 20U, 5 to 30U, 5 to 40 Units, 5 to 50 Units, 5 to 60 Units, 5 to 70 Units, 5 to 80 Units, 5 to 90U, 10 to 20U, 10 to 30U, 10 to 50U, or 10 to 60U, or 10 to 70U, or 10 to 80U, 10 to 90U, or 10 to 100U per treatment are administered to a patient suffering from myofascial pain. In a further example, the subject receives about 5U, about 10U, about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, or about 100U per treatment are administered to a patient with myofascial pain. Dosages greater than 100U per treatment may also be administered to patients with myofascial pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0088] In an embodiment, the subject suffers from lower-back pain. A subject suffering from lower-back pain, for example, receives between about 15 to 150U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 15 to

30U, 15 to 50U, 15 to 75U, 15 to 100U, 15 to 125U, 15 to 150U, 20 to 100U, 20 to 150U, or 100 to 150U per treatment are administered to a patient with lower-back pain. In a still further example, the subject receives about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, or about 150U per treatment are administered to a patient with lower-back pain. Dosages greater than 150U per treatment may also be administered to patients with lower-back pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0089] In an embodiment, the patient suffers from migraine headache pain, including wherein the patient suffers from migraine headaches of 4 hours or more 15 or more days per month. A subject suffering from migraine-headache pain, for example, receives between about 0.5 to 200U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 5 to 190U, 15 to 180U, 25 to 170U, 35 to 160U, 45 to 150U, 55 to 140U, 65 to 130U, 75 to 120U, 85 to 110U, or 95 to 105U per treatment are administered to a patient suffering from migraineheadache pain. A treatment session can comprise multiple treatments.

[0090] For example, about 0.5U, about 1.0U, about 1.5U, about 2.0U, about 2.5U, about 3.0U, about 3.5U, about 4.0U, about 4.5U, about 5.0U, about 5.5U, about 6.0U, about 6.5U, about 7.0U, about 7.5U, about 8.0U, about 8.5U, about 9.0U, about 9.5U, about 10.0U, about 12U, about 15U, about 17U, about 20U, about 22U, about 25U, about 27U, about 30U, about 32U, about 35U, about 37U, about 40U, about 42U, about 45U, about 47U, or about 50U per treatment site are administered to a patient with migraine-headache pain. A patient can be treated at multiple sites, such as, for example, 2 sites, 3 sites, 4 sites, 5 sites, 6 sites, 7 sites, 8 sites, 9 sites, 10 sites, 11 sites, 12 sites, 13 sites, 14 sites, 15 sites, 16 sites, 17 sites, 18 sites, 19 sites, 20 sites, 21 sites, 22 sites, 23 sites, 24 sites, 25 sites, 26 sites, 27 sites, 28 sites, 29 sites, 30 sites, 31 sites, 32 sites, or more, or the like. In an embodiment, a patient suffering from migraine is injected 31 times with 5U per 0.1mL injection, across the corrugator (2 injections of 5U each), procerus (1 injection of 5U), frontalis (4 injections of 5U each), temporalis (8 injections of 5U each), occipitalis (6 injections of 5U each), cervical paraspinal (4 injections of 5U each), and trapezius (6 injections of 5U each) muscles. With the exception of the procerus muscle which can be injected at the midline, all muscles can, in certain embodiments, be injected bilaterally with half of the injection sites to the left and half to the right side of the head and neck. Dosages greater than 200U per treatment may also be administered to patients with migraine-headache pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0091] In an embodiment, the patient suffers from sinus-headache pain. A subject suffering from sinus-headache pain, for example, receives between about 4 to 40U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 4 to 10U, 4 to 15U, 4 to 20U, 4 to 25U, 4 to 30U, 4 to 35U, 7 to 15U, 7 to 20U, 7 to 25U, 7 to 30U, 7 to 35U, or 7 to 40U per treatment are administered to a patient suffering from sinus-headache pain. In a still further example, the subject receives about 4U, about 5U, about 7.5U, about 10U, about 12.5U, about 15U, about 17.5U, about 20.0U, about 22.5U, about 25.0U, about 27.5U, about 30.0U, about 32.5U, about 35U, about 37.5U, or about 40U per treatment are administered to a patient with sinus-headache pain. Dosages greater than 40U per treatment may also be administered to patients with sinus headache-pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0092] In an embodiment, the patient suffers from tension-headache pain. A subject suffering from tension-headache pain, for example, receives between about 5 to 50U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 5 to 10U, 5 to 15U, 5 to 20U, 5 to 25U, 5 to 30U, 5 to 35U, 5 to 40U, 5 to 45U, 10 to 20U, 10 to 25U, 10 to 30U, 10 to 35U, 10 to 40U, or 10 to 45U per treatment are administered to a patient with tension-headache pain. In a still further example, the subject receives about 5U, about 10U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, or about 50U per treatment are administered to a patient with tension-headache pain. In an embodiment, a patient suffering from tension headache is injected 31 times with 5U per 0.1mL injection, across the corrugator (2 injections of 5U each), procerus (1 injection of 5U), frontalis (4 injections of 5U each), temporalis (8 injections of 5U each), occipitalis (6 injections of 5U each), cervical paraspinal (4 injections of 5U each), and trapezius (6 injections of 5U each) muscles. With the exception of the procerus muscle which can be injected at the midline, all muscles can, in certain embodiments, be injected bilaterally with half of the injection sites to the left and half to the right side of the head and neck. Dosages greater than 200U per treatment may also be administered to patients with tension headache pain to achieve a therapeutic response. A treatment session can comprise multiple treatments.

[0093] In an embodiment, the patient suffers from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis. For example a pharmaceutical formulation of the present invention can be administered to the nasal mucosa or to the subcutaneous structures overlying the sinuses, wherein the administration of the formulation reduces the headache and/or facial pain associated with acute recurrent or chronic sinusitis. In further embodiments, any of the pharmaceutical formulations of the present invention can be administered to the nasal mucosa or to

the subcutaneous structures overlying the sinuses, such as over one or more of the sinuses selected from the group consisting of: ethmoid; maxillary; mastoid; frontal; and sphenoid. In another embodiment, subcutaneous structures overlying the sinuses lie within one or more of the areas selected from the group consisting of: forehead; malar; temporal; post auricular; and lip. In embodiments, multiple injections of 5U each are administered to treat the sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis.

[0094] In another embodiment, a patient suffering from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis is treated by administering any of the pharmaceutical formulations of the present invention to an afflicted area of the patient. In a further embodiment, the pharmaceutical formulations disclosed herein are administered to the projections of a trigeminal nerve innervating a sinus.

[0095] Patients suffering from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis often exhibit symptoms including rhinitis, sinus hypersecretion and/or purulent nasal discharge. In one embodiment, patients treated with the pharmaceutical formulations of the present invention exhibit symptoms of sinus hypersecretion and purulent nasal discharge.

[0096] Embodiments of the present invention also provide methods for treating a patient suffering from sinus headache pain or facial pain associated with acute or recurrent chronic sinusitis, wherein the subject suffers from neuralgia. In certain embodiments the neuralgia is trigeminal neuralgia. In another embodiment, the neuralgia is: associated with compressive forces on a sensory nerve; associated with intrinsic nerve damage, demyelinating disease, or a genetic disorder; associated with a metabolic disorder; associated with central neurologic vascular disease; or associated with trauma. In another embodiment of the present invention, the pain is associated with dental extraction or reconstruction.

Treatment of urological disorders

[0097] In an embodiment, the invention also provide methods for treating a patient suffering from overactive bladder (OAB), such as, for example, that due to a neurologic condition (NOAB), or idiopathic OAB (IOAB). For example, pharmaceutical formulations of the present invention can be administered to the bladder or its vicinity, *e.g.* the detrusor, wherein the administration of the formulation reduces the urge incontinence associated with overactive bladder. In certain embodiments, the dosage can be, for example, 200U, or more, or less, or the like. For example, the dosage can be about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about

130U, about 135U, about 140U, about 145U, about 150U, about 160U, about 170U, about 180U, about 190U, about 200U, about 210U, about 220, about 230U, about 240U, or more, or the like, per treatment. A patient can be injected at multiple sites, such as, for example, 2 sites, 3 sites, 4 sites, 5 sites, 6 sites, 7 sites, 8 sites, 9 sites, 10 sites, 11 sites, 12 sites, 13 sites, 14 sites, 15 sites, 16 sites, 17 sites, 18 sites, 19 sites, 20 sites, 21 sites, 22 sites, 23 sites, 24 sites, 25 sites, 26 sites, 27 sites, 28 sites, 29 sites, 30 sites, 31 sites, 32 sites, 33 sites, 34 sites, 35 sites, 36 sites, 37 sites, 38 sites, or more, or the like. In an embodiment, patients suffering from OAB are treated with 30 1mL injections of approximately 6.7U per injection into the detrusor muscle.

[0098] In an embodiment, the invention also provides methods for treating a patient suffering from neurogenic detrusor overactivity (NDO), such as that due to a neurologic condition. For example, pharmaceutical formulations of the present invention can be administered to the bladder or its vicinity, *e.g.* the detrusor, wherein the administration of the formulation reduces the urge incontinence associated with overactive bladder. In certain embodiments, the dosage can be, for example, 200U, or more, or less, or the like. For example, the dosage can be about 15U, about 20U, about 25U, about 30U, about 35U, about 40U, about 45U, about 50U, about 55U, about 60U, about 65U, about 70U, about 75U, about 80U, about 85U, about 90U, about 95U, about 100U, about 105U, about 110U, about 115U, about 120U, about 125U, about 130U, about 135U, about 140U, about 145U, about 150U, about 160U, about 170U, about 180U, about 190U, about 200U, about 210U, about 220, about 230U, about 240U, or more, or the like, per treatment. A patient can be injected at multiple sites, such as, for example, 2 sites, 3 sites, 4 sites, 5 sites, 6 sites, 7 sites, 8 sites, 9 sites, 10 sites, 11 sites, 12 sites, 13 sites, 14 sites, 15 sites, 16 sites, 17 sites, 18 sites, 19 sites, 20 sites, 21 sites, 22 sites, 23 sites, 24 sites, 25 sites, 26 sites, 27 sites, 28 sites, 29 sites, 30 sites, 31 sites, 32 sites, or more, or the like. In an embodiment, patients suffering from NDO are treated with 30 1mL injections of approximately 6.7U per injection into the detrusor muscle.

Treatment of cosmetic features

[0099] In another embodiment, the present invention provides methods for cosmetically modifying soft-tissue features comprising the step of administering at least one pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to modify said features. In a further embodiment, the pharmaceutical formulation is administered via transcutaneous or transmucosal injection either at a single focus or multiple foci.

[0100] In embodiments, pharmaceutical formulations of the present invention are administered to the face or neck of the subject. In a further embodiment, the pharmaceutical formulations of the present invention are administered to the subject in an amount sufficient to reduce rhytides. For

example, the formulation can be administered between eyebrows of the subject in an amount sufficient to reduce vertical lines between the eyebrows and on a bridge of a nose. The pharmaceutical formulations can also be administered near either one or both eyes of the subject in an amount sufficient to reduce lines at corners of the eyes. In an embodiment, compositions of the invention can be injected locally to smooth skin. In another embodiment, the pharmaceutical formulations of the present invention can also be administered to a forehead of the subject in an amount sufficient to reduce horizontal lines on said forehead. In yet another embodiment of the present invention the pharmaceutical formulation is administered to the neck of the subject in an amount sufficient to reduce muscle bands in the neck. In an embodiment, a pharmaceutical composition is applied to the masseter muscle to relax the muscle and / or decerase masseter mass.

[0101] In a further embodiment, the patient suffers from facial wrinkles. A subject suffering from facial wrinkles, for example, can receive between about 1 to 100U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receives between about 1 to 10U, 1 to 20U, 1 to 30U, 1 to 40U, 1 to 50U, 1 to 60U, 1 to 70U, 1 to 80U, 1 to 90U, 5 to 20U, 5 to 30U, 5 to 40U, 5 to 50U, 5 to 60U, 5 to 70U, 5 to 80U, 5 to 90U, or 5 to 100U per treatment are administered to a patient with an inflammatory disorder. In a still further example, the subject receives about 1U, about 10U, about 20U, about 30U, about 40U, about 50U, about 60U, about 70U, about 80U, about 90U, or about 100U per treatment are administered to a patient. Dosages greater than 100U per treatment may also be administered to patients suffering from inflammation or an inflammatory disorder to achieve a therapeutic response.

Treatment of inflammation

[0102] In another embodiment, the present invention provides methods for treating inflammation comprising the step of administering a pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to reduce inflammation. In certain embodiments, pharmaceutical formulations of the present invention are administered to a patient without producing muscle weakness. In an embodiment, the pharmaceutical formulations of the present invention are administered to patients with an inflammatory condition. In certain embodiments the inflammatory condition is neurogenic inflammation. In another embodiment, the subject suffers from rheumatoid arthritis or a gastro-intestinal inflammatory disease.

[0103] In a further embodiment, the patient suffers from an inflammatory disorder. A subject suffering from an inflammatory disorder, for example, receives between about 1 to 100U per treatment of a pharmaceutical formulation of the present invention. In a further example, the subject receivesbetween about 1 to 10U, 1 to 20U, 1 to 30U, 1 to 40U, 1 to 50U, 1 to 60U, 1 to 70U, 1 to 80U, 1 to 90U, 5 to 20U, 5 to 30U, 5 to 40U, 5 to 50U, 5 to 60U, 5 to 70U, 5 to 80U, 5 to 90U, or 5

to 100U per treatment are administered to a patient with an inflammatory disorder. In a still further example, the subject receives about 1U, about 10U, about 20U, about 30U, about 40U, about 50U, about 60U, about 70U, about 80U, about 90U, or about 100U per treatment are administered to a patient. Dosages greater than 100U per treatment may also be administered to patients suffering from inflammation or an inflammatory disorder to achieve a therapeutic response.

Treatment of skin conditions

[0104] A method within the scope of the present invention for treating a skin disorder can have the step of local administration of a botulinum neurotoxin to a location of a skin disorder of a patient, such as to a face, hand or foot of a patient. The neurotoxin can be locally administered in an amount of between about 10^{-3} units/kg of patient weight and about 35 units/kg of patient weight. For example, the neurotoxin is locally administered in an amount of between about 10^{-2} U/kg and about 25 U/kg of patient weight. In a further example, the neurotoxin is administered in an amount of between about 10^{-1} U/kg and about 15 U/kg. In one method within the scope of the present invention, the neurotoxin is locally administered in an amount of between about 1 U/kg and about 10 U/kg. In a clinical setting it can be advantageous to administer from 1U to 3000U of a neurotoxin, such as botulinum toxin type A or B, to a skin disorder location by topical application or by subdermal administration, to effectively treat the skin disorder.

[0105] Administration of botulinum toxin can be carried out at multiple sites in the skin, wherein the sites of adjacent injections are separated by about 0.1 to 10cm, or about 0.5 to about 5cm, for example, by about 1.5 to about 3cm. The toxins may be any of the botulinum toxins A, B, C, D, E, F or G. The amounts administered may vary between 0.1 and 1000U, or about 1 to about 40, or from about 5 to about 10U, depending on the manufactures specifications, the class of the toxin and the mode of administration. The repeat time range for these administrations for maintenance of the desired change varies substantially according to the location of the injection, the condition to be adjusted and the condition of the patient. Thus the repeat time may vary from about 1 week to about 50 weeks, however a common range is about 4 to about 25 weeks, or even about 12 weeks to about 16 weeks.

[0106] The distances between administrations, for example, injections, can vary from about 1 mm to about 10cm, suitably from about 5mm to about 5cm, and more usually from about 1cm to about 3cm. Thus for example botulinum A may be suitably administered by intradermal injection between about 0.1 to about 10U at a separation of from about 0.5 to about 10cm.

[0107] In another embodiment, the present invention provides methods for treating cutaneous disorders comprising the step of administering a pharmaceutical formulation of the present invention to a subject in need thereof in an amount sufficient to reduce a sebaceous or mucous secretion. In

further embodiments, the pharmaceutical formulations of the present invention are administered to a patient without producing muscle weakness. In certain embodiments the pharmaceutical formulations of the present invention are injected into one or more sites of an eyelid or conjunctiva. In another embodiment, the formulations of the present invention are administered to a body surface.

[0108] In another embodiment, the pharmaceutical formulations are administered in an amount sufficient to reduce cutaneous bacterial or fungal growth, including but not limited to Staphylococcus; Streptococcus and Moraxella. For example, the pharmaceutical formulations of the present invention are administered to an area selected from the group consisting of: eyelid; scalp; feet; groin; and armpit to reduce cutaneous infection.

EXAMPLES

[0109] The following examples illustrate embodiments and aspects of the present invention and are not intended to limit the scope of the present invention.

[0110] Example 1: Activities and stabilities of exemplary solid clostridial pharmaceutical compositions relative to prior art formulations.

[0111] Bulk solutions of botulinum toxin were prepared by mixing an appropriate aliquot of a botulinum toxin type A with several vehicle solutions as described in the following Tables 1-3. The solutions were filled into glass vials and lyophilized using conventional freeze-drying conditions. Potency of the lyophilized formulations was tested by cell based potency assay (CBPA) after reconstitution of the lyophiles with saline. Potency recovery results after freeze-drying and after storage at indicated temperatures are provided in the tables and normalized to target potency (known amount of toxins that were added to the formulations). The potencies of the solid compositions prepared according to aspects of the present invention were compared to prior art formulations as shown in Tables 1-3.

[0112] Table 1.1: Lyophilized formulations

	Excipient, % w/w							**Normalized potency, storage at 25 °C**		
Formulation	**Treh**	**TWEEN® 20**	**P 188**	**NaCl**	**Met**	**NAC**	**Buffer**	**T0**	**3 mo**	**6 mo**
Comparator 1	3	0.04		0.9	0.2	-	Water	80.6%	86.4%	80.8%
Comparator 2	2	-	4	-	-	-	20mM Histidine pH 5.5	81.5%	68.6%	68.0%
Formulation 1	2	-	4	-	-	0.03	20mM Histidine pH 5.5	98.6%	91.9%	86.9%

Treh = trehalose; P 188 = poloxamer P 188; Met = L-methionine; NAC = N-acetyl-L-cysteine.

[0113] Table 1.2: Lyophilized formulations

	Excipient, % w/w							Normalized potency, storage at 40 °C		
Formulation	**Treh**	**TWEEN® 20**	**P 188**	**NaCl**	**Met**	**NAC**	**Buffer**	**1 mo**	**3 mo**	**6 mo**
Comparator 1	3	0.04		0.9	0.2		Water	74.8%	70.3%	24.0%
Comparator 2	2		4				20mM Histidine pH 5.5	64.8%	57.8%	46.8%
Formulation 1	2		4			0.03	20mM Histidine pH 5.5	86.5%	77.9%	62.3%

Treh = trehalose; P 188 = poloxamer P 188; Met = L-methionine; NAC = N-acetyl-L-cysteine.

[0114] Table 2: Lyophilized formulations

	Excipient, % w/w								Normalized potency (% of target)		
Formulation	Treh	Sucr	TWEEN® 20	P188	NaCl	Met	NAC	**Buffer**	**T0**	**1 mo -20 °C**	**1 mo 40 °C**
Comparator 2	2			4				20mM Histidine pH 5.5	87.15%	88.95%	76.32%
Comparator 3		3	0.04		0.9	0.2		Water	84.06%	85.18%	72.86%
Formulation 2	2			4		0.2		20mM Histidine pH 6.0	98.60%	120.85 %	91.17%
Formulation 3	8		0.04				0.03	20mM Histidine pH 6.0	105.2%	110.05 %	96.89%

Treh = trehalose; Sucr = sucrose; P188 = poloxamer P 188; Met = L-methionine; NAC = N-acetyl-L-cysteine.

[0115] Table 3: Lyophilized formulations

	Excipient, % w/w								Normalized potency, storage at 25 °C		
Formulation	Treh	Sucr	TWEEN® 20	P188	NaCl	Met	NAC	**Buffer**	**T0**	**3 mo**	**7.5 mo**
Comparator 2	2			4				20mM Histidine, pH 5.5	87.1%	78.0%	78.0%
Formulation 2	2			4		0.2		20mM Histidine, pH 6.0	98.6%	97.0%	98.0%
Formulation 4	8			0.6		0.2		20mM Histidine, pH 6.0	86.5%	83.0%	84.0%

P-188 = Poloxamer P-188; Met = L-methionine.

[0116] Example 2: Activities of liquid clostridial pharmaceutical compositions in the presence or absence of antioxidants

[0117] Bulk drug product solutions were prepared by mixing an appropriate aliquot of the BoNT/A drug substance (DS) with three different vehicle solutions as shown in Table 4. All three formulations contained 8% w/w trehalose, 4% w/w P188 and 20 mM Histine buffer at pH 6.0. Formulation 10 contained no antioxidant. Formulations 11 and 12 contained NAC and methionine, respectively. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by cell based potency assay (CBPA) after filling (time zero, t0) and storage for one month at four temperatures (-70, 5, 25, and 40°C). Potency test results are given in Table 4. Briefly, methionine-containing formulation (#12) retained its potency after one month storage at all four temperatures, including 40°C, whereas the formulation w/o an antioxidant lost approx. 15 % potency at 25°C and lost essentially all activity (i.e., complete inactivation) at 40°C. A second antioxidant tested, N-acetyl-L-cysteine, accelerated loss of potency as compared with the antioxidant-free formulation, demonstrating that N-acetyl-L-cysteine acted as pro-oxidant in this formulation.

[0118] Table 4: Liquid formulations

		Potency, U/mL				
Formulation No.*	**Antioxidant**	**T0**	**1 mo. -70°C**	**1 mo. 5°C**	**1 mo. 25°C**	**1 mo. 40°C**
Formulation 10	none	128	135	135	106	0.225
Formulation 11	N-acetyl-L-cysteine, 0.2 % w/w	128	133	129	61	0.2
Formulation 12	L-methionine, 0.2% w/w	133	146	146	145	138

*Each formulation contained the same amount of botulinum toxin, 8 w/w% trehalose, and 4 w/w% poloxamer P188 in histidine buffer.

[0119] Example 3: Impact of exemplary antioxidants on the stability of exemplary liquid formulations.

[0120] Bulk drug product solutions were prepared by mixing an appropriate aliquot of the BoNT/A drug substance (DS) with different antioxidants as shown in Table 5. All formulations contained 8% w/w trehalose, 4% w/w P188, 20 mM Histine buffer at pH 6.0 and one or more antioxidants as listed. Target potency was 100 U/mL. The bulk solutions were filled into 2 mL glass vials (1.25 mL fill), and sealed with rubber stopper and aluminum shell. Potency of the formulations was tested by cell based potency assay (CBPA) after filling (time zero, t0) and storage for two weeks and 1 month at 40°C. Potency test results are given in Table 5.

[0121] Table 5: Liquid formulations

	Antioxidant[2]							**Potency U/mL**		
Formulation No.[1]	NAC %	Met %	TRP %	GSH %	NaSul %	PrpGal %	EDTA %	T0	2 wks 40 °C	1 mo. 40 °C
Formulation 20		0.2						126	129	130
Formulation 21			0.2					127	13.71	NT[3]
Formulation 22				0.2				123	3.76	NT
Formulation 23					0.2			23.7	0.161	NT
Formulation 24						0.2		0.164	0.150	NT
Formulation 25	0.2		0.2					133	0.253	NT
Formulation 26	0.2						0.03	129	127	127
Formulation 27	0.2		0.2				0.03	129	125	122
Formulation 28			0.2	0.2				126	2.45	NT

[1] Each formulation contained 100 U/mL botulinum toxin, 8 w/w% trehalose, and 4 w/w% poloxamer P188 in 20 mM histidine buffer, pH 6.0 and the specified antioxidant.

[2] NAC = N-acetyl-L-cysteine; Met = L-methionine; TRP = L-tryptophan; GSH = L-glutathione; NaSul = sodium sulfite; PrpGal = propyl gallate; EDTA = ethylene diamine tetraacetic acid, sodium salt.

[3] NT=not tested

[0122] Many alterations and modifications may be made by those having ordinary skill in the art, without departing from the spirit and scope of the disclosure. Therefore, it must be understood that the described embodiments have been set forth only for the purposes of examples, and that the embodiments should not be taken as limiting the scope of the following claims. The following claims are, therefore, to be read to include not only the combination of elements which are literally set forth, but all equivalent elements for performing substantially the same function in substantially the same way to obtain substantially the same result. The claims are thus to be understood to include those that have been described above, those that are conceptually equivalent, and those that incorporate the ideas of the disclosure.

What is claimed is:

1. A pharmaceutical composition comprising:
(i) a Clostridial toxin active ingredient;
(ii) a tonicity agent;
(iii) a poloxamer and/or a polysorbate; and
(iv) an antioxidant.

2. The composition according to claim 1, comprising botulinum toxin.

3. The composition according to claim 1 or claim 2, comprising trehalose.

4. The composition according to any one of claims 1 to 3, comprising poloxamer 188 and/or polysorbate 20.

5. The composition according to any one of claims 1 to 4, comprising one or more of methionine and N-Acetyl-cysteine.

6. The composition according to any one of claims 1 to 5, comprising botulinum toxin, trehalose, one of poloxamer 188 or polysorbate 20, and one of methionine or N-Acetyl-cysteine.

7. The composition according to any one of claims 1 to 6, comprising botulinum toxin, trehalose, poloxamer 188, and methionine.

8. The composition according to claim 7, wherein the relative weight amounts (%, w/w) of trehalose, poloxamer 188, and methionine are within the following ranges:

trehalose	1 to 10
poloxamer 188	0.5 to 5
methionine	0.1 to 0.3

9. The composition according to any one of claims 1 to 6, comprising botulinum toxin, trehalose, polysorbate 20, and methionine.

10. The composition according to claim 9, wherein the relative weight amounts (%, w/w) of trehalose, polysorbate 20, and methionine are within the following ranges:

trehalose	1 to 10
polysorbate 20	0.02 to 0.06
methionine	0.1 to 0.3

11. The composition according to any one of claims 1 to 6, comprising botulinum toxin, trehalose, poloxamer 188, and N-Acetyl-cysteine.

12. The composition according to claim 11, wherein the relative weight amounts (%, w/w) of trehalose, poloxamer 188, and N-Acetyl-cysteine are within the following ranges:

trehalose	1 to 10
poloxamer 188	0.5 to 5
N-Acetyl-cysteine	0.01 to 0.5

13. The composition according to any one of claims 1 to 6, comprising botulinum toxin, trehalose, polysorbate 20, and N-Acetyl-cysteine.

14. The composition according to 13, wherein the relative weight amounts (%, w/w) of trehalose, polysorbate 20, and N-Acetyl-cysteine are within the following ranges:

trehalose	1 to 10
polysorbate 20	0.02 to 0.06
N-Acetyl-cysteine	0.01 to 0.5

15. The composition according to any one of claims 1 to 14, comprising histidine.

16. The composition according to any one of claims 1 to 15, comprising no animal-derived protein.

17. The composition according to any one of claims 1 to 4; comprising ethylene diamine tetraacetic acid sodium salt (EDTA) or an EDTA analog.

18. The composition according to any one of claims 5 and 6, further comprising ethylene diamine tetraacetic acid sodium salt (EDTA) or an EDTA analog.

19. The composition according to any one of claims 11 to 14, further comprising ethylene diamine tetraacetic acid sodium salt (EDTA) or an EDTA analog.

20. The composition according to any one of claims 1-19, whereint the relative weight amount (%, w/w) of EDTA ranges from about 0.01 to 0.10.

21. The composition according to any one of claims 1 to 20, wherein the composition is a solid formulation.

22. The composition according to any one of claims 12, 14, 19 and 20, wherein the composition is a solid formulation and the relative weight amount (%, w/w) of N-Acetyl-cysteine is 0.01 to 0.05.

23. The composition according to any one of claims 1 to 20, wherein the composition is a liquid formulation and has a pH of from 5 to 7.

24. The composition according to any one of claims 11 to 14, 19 and 20, wherein the composition is a liquid formulation and has a pH of from 5 to 7, wherein the relative weight amount of N-Acetyl-cysteine is 0.1 to 0.5.