and wherein the surfactant excipient is a poloxamer, a polysorbate, a polyoxyethylene glycol dodecyl ether, or a polyoxyethylene octyl phenyl ether. The composition is buffered to about pH 5.5 to about pH 6.5 using a citrate buffer, a phosphate buffer, a histidine buffer, or a histidine phosphate buffer. The composition may further comprise an effective amount of sodium chloride. The non-protein polymer excipient is a dextran, a polyethylene glycol, a polyethylene imine, a polyvinyl pyrrolidone, a polyvinyl acetate, an inulin, a starch, or a starch derivative. The composition further comprises an effective amount of a non-protein polymer excipient. The Clostridial toxin active ingredient is stable for at least one-year when stored at either ambient or below freezing temperatures.

[0013] WO2017/148915 finally discloses a botulinum toxin containing composition comprising non-crosslinked hyaluronic acid and human serum albumin.

[0014] Despite these advances in the preparation of liquid botulinum toxin formulations, there is still a need for new options to develop ready-to-use liquid formulations of botulinum toxin, which are stable during transport and storage.

OBJECT OF THE INVENTION

[0015] It is an object of the present invention to provide a stable liquid formulation of botulinum toxin that is ready-to-use for therapeutic and cosmetic treatments.

SUMMARY

[0016] The present disclosure is based on the unexpected finding that the use of a pre-treated protein stabilizer based on human serum albumin (HSA) in the formulation of a liquid botulinum toxin formulation results in a reduced light sensitivity and, thus, in an improved light stability. Furthermore, it was unexpectedly found that very low amounts of iron ions (Fe^{3+} ions) drastically increase the light sensitivity of liquid botulinum toxin formulations containing human serum albumin (HSA). This is surprising since iron is relatively common and ubiquitous, and moreover, it was found that other metal ions such as copper, cobalt or nickel do not show this destabilizing effect.

[0017] Furthermore, the present disclosure is based on the surprising finding that tryptophan and N-acetyl-tryptophan decrease the light stability of liquid botulinum toxin

formulations containing human serum albumin (HSA). This finding is highly surprising since commercial HSA products, in addition to water and sodium chloride, usually contain sodium caprylate and N-acetyl-tryptophan to stabilize HSA at high temperatures, which in this way get into the botulinum toxin formulation. It is also surprising because tryptophan is described as stabilizing additive for botulinum toxin (see, e.g., EP 3 679 946).

[0018] It has been further found that the presence of a non-proteinaceous stabilizer comprising a hyaluronic acid and the presence of an alditol contribute to the improved light stability of the liquid formulation.

[0019] Accordingly, in an aspect, the present disclosure provides for a liquid formulation comprising water and the following components:

(i) botulinum toxin,
(ii) human serum albumin,
(iii) hyaluronic acid,
(iv) alditol;

wherein:

(I) the liquid formulation comprises a chelating agent; or
(II) the liquid formulation comprises a chelating agent and a metal salt selected from a salt of calcium, magnesium, zinc, and mixtures thereof; or
(III) said human serum albumin has been subjected to the following process comprising steps (a) and (c) or steps (b) and (c):
    (a) contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent;
    (b) contacting human serum albumin with a chelating agent and a metal salt selected from calcium, magnesium or zinc, and mixtures thereof to obtain a mixture of human serum albumin, the chelating agent, and the metal salt;
    (c) removing the chelating agent from the mixture obtained in step (a) or step (b) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis; or

(IV) the liquid formulation has been subjected to the following process comprising steps (d) and (f) or steps (e) and (f):

(d) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent to obtain a mixture of said liquid formulation and the chelating agent;

(e) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent and a metal salt selected from calcium, magnesium or zinc, or a mixture thereof to obtain a mixture of said liquid formulation, the chelating agent, and the metal salt;

(f) removing the chelating agent from the mixture obtained in step (d) or step (e) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis.

[0020] In another aspect, the disclosure provides for a liquid formulation comprising water and the following components:

(i) botulinum toxin,
(ii) human serum albumin,
(iii) hyaluronic acid,
(iv) alditol, wherein the alditol is sorbitol.

[0021] In one example, the liquid formulation further comprises one or both of components (v) and (vi):

(v) tonicity agent,
(vi) buffer agent.

[0022] In one example, the botulinum toxin is a botulinum neurotoxin complex; or

the botulinum toxin is botulinum neurotoxin free of complexing proteins; or

wherein the botulinum toxin is of serotype A; or

the botulinum toxin is botulinum neurotoxin of serotype A free of complexing proteins; or

the botulinum toxin is of serotype A and is present at a concentration of 1-1000 U/ml; or

the botulinum toxin is botulinum neurotoxin of serotype A free of complexing proteins and is present at a concentration of 1-1000 U/ml.

[0023] In one example, the human serum albumin is present in the liquid formulation at a concentration of 0.001-1.00% w/v.

[0024] In one example and the examples referring thereto, the chelating agent is selected from the group consisting of aminopolycarboxylic acids having three to six carboxylic acid functional groups, citrate, porphyrins, N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN), triethylenetetramine (TETA), and mixtures thereof,

preferably wherein the chelating agent is an aminopolycarboxylic acids of general formula (I):

$$(HO_2CCH_2)_2N\text{-}R\text{-}N(CH_2CO_2H)_2 \qquad (I),$$

wherein R comprises no carboxylic acid group or one or two carboxylic acid groups, the aminopolycarboxylic acid preferably being selected from the group consisting of ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(α-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), diethylenetriaminepentaacetic acid (DTPA), triethylenetetramine-hexaacetate (TTHA), and mixture thereof; or

wherein the chelating agent is 2,2',2''-nitrilotriacetic acid.

[0025] In one example and the examples referring thereto, the metal salt is present in the liquid formulation in an amount of 0.01-100 mM; or wherein the metal salt is calcium chloride and is present in the liquid formulation in an amount of 0.01-100 mM.

[0026] In one example or the examples referring thereto, the liquid formulation contains Fe^{3+} ions in a concentration of less than 1 μM, preferably less than 500 nM, and more preferably less than 250 nM.

[0027] In one example or the examples referring thereto, the concentration of tryptophan and N-acetyl-tryptophan in the liquid formulation is no more than 50 µM, preferably no more than 20 µM, more preferably no more than 10 µM, still more preferably no more than 1 µM, and most preferably 0 µM, of tryptophan and N-acetyltryptophan (total concentration of both Trp and N-AcTrp).

[0028] In one example or the examples referring thereto, the hyaluronic acid is not crosslinked; or

hyaluronic acid is present at a concentration of 2 to 10 mg/ml and wherein the hyaluronic acid is not crosslinked.

[0029] In a further example, hyaluronic acid is present at a concentration of 2 to 10 mg/ml and wherein the hyaluronic acid is crosslinked.

[0030] In one example and the examples referring thereto, the alditol is selected from glycerol, mannitol, isomalt, lactitol, sorbitol, xylitol, threitol, erythritol and arabitol, preferably from mannitol and sorbitol.

[0031] In one example or the examples referring thereto, the alditol is present in a concentration of from 20 to 100 mg/ml.

[0032] In one example and the examples referring thereto,

(v) the tonicity agent is present in the liquid formulation in an amount of 0.01-2.0 % w/v, or the tonicity agent is sodium chloride, or the tonicity agent is sodium chloride and is present in the liquid formulation in an amount of 0.01-2.0 % w/v;

(vi) the buffer agent is present in the liquid formulation in a concentration of 1-100 mM, or the buffer agent is citric acid, an amino acid, phosphate, or a mixture thereof, or the buffer agent is histidine, or the buffer agent is histidine, phosphate, or a mixture thereof, and the histidine and phosphate are present in the liquid formulation in a concentration of 1-100 mM.

[0033] In one example or the examples referring thereto, the pH of the liquid formulation is in the range of from of 5.0 to 8, preferably from 5.5 to 6.5.

[0034] In another aspect, the disclosure relates to a method of making the liquid formulation as disclosed herein, comprising:

mixing components (i) to (iv) and optionally (v) and/or (vi) in a liquid comprising water; and

(I) a chelating agent; or

(II) a chelating agent and a metal salt selected from a salt of calcium, magnesium, zinc, and mixtures thereof; or

(III) wherein the method comprises steps (a) and (c) or steps (b) and (c):

(a) contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent;

(b) contacting said human serum albumin with a chelating agent and a metal salt selected from calcium, magnesium or zinc, or a mixture thereof, to obtain a mixture of human serum albumin, the chelating agent, and the metal salt; and

(c) removing the chelating agent from the mixture obtained in step (a) or step (b) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis; or

(IV) wherein the method comprises steps (d) and (f) or steps (e) and (f):

(d) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent to obtain a mixture of said liquid formulation and the chelating agent;

(e) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent and a metal salt selected from calcium, magnesium or zinc,

or mixtures thereof to obtain a mixture of said liquid formulation, the chelating agent, and the metal salt;

(f) removing the chelating agent from the mixture obtained in step (d) or step (e) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis;

preferably wherein the contacting in step (a) or step (b) is carried out by mixing the composition comprising human serum albumin with the chelating agent or the chelating agent and the metal salt or wherein the contacting in step (d) and (e) is carried out by mixing a liquid formulation comprising components (i) to (iv) with the chelating agent or the chelating agent and the metal salt such that the concentration of the chelating agent in the liquid formulation is 1 mM – 500 mM;

or the disclosure relates to a method of making the liquid formulation as disclosed herein, comprising:

mixing components (i) to (iv) and optionally (v) and/or (vi) in a liquid comprising water.

[0035] In an example, the disclosure relates to the liquid formulation as disclosed herein for use in therapy, particularly for use in the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders,

[0036] In an example, the disclosure relates to the use of the liquid formulation as disclosed herein in a cosmetic / non-therapeutic application.

[0037] In an example, the disclosure relates to a method of treating a disease or condition, comprising administering an effective amount of the liquid formulation as disclosed herein to a person in need thereof.

[0037a] In another aspect, the disclosure relates to the liquid formulation as disclosed herein for use in therapy, particularly for use in the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders; or

the use of the liquid formulation as disclosed herein in a cosmetic application; or

use of the liquid formulation as disclosed herein in the manufacture of a medicament for the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders in a person in need thereof.

DETAILED DESCRIPTION OF THE INVENTION

[0038] Definitions

CLAIMS

1. A liquid formulation comprising water and the following components:

   (i) botulinum toxin,
   (ii) human serum albumin,
   (iii) hyaluronic acid,
   (iv) alditol;

   wherein:

   (I) the liquid formulation comprises a chelating agent; or
   (II) the liquid formulation comprises a chelating agent and a metal salt selected from a salt of calcium, magnesium, zinc, and mixtures thereof; or
   (III) said human serum albumin has been subjected to the following process comprising steps (a) and (c) or steps (b) and (c):
      (a) contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent;
      (b) contacting human serum albumin with a chelating agent and a metal salt selected from calcium, magnesium or zinc, and mixtures thereof to obtain a mixture of human serum albumin, the chelating agent, and the metal salt;
      (c) removing the chelating agent from the mixture obtained in step (a) or step (b) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis; or
   (IV) the liquid formulation has been subjected to the following process comprising steps (d) and (f) or steps (e) and (f):
      (d) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent to obtain a mixture of said liquid formulation and the chelating agent;
      (e) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent and a metal salt selected from calcium, magnesium or zinc, or a mixture thereof to obtain a mixture of said liquid formulation, the chelating agent, and the metal salt;

(f) removing the chelating agent from the mixture obtained in step (d) or step (e) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis.

2. A liquid formulation comprising water and the following components:

(i) botulinum toxin,
(ii) human serum albumin,
(iii) hyaluronic acid,
(iv) alditol, wherein the alditol is sorbitol.

3. The liquid formulation of claim 1 or claim 2, further comprising one or both of components (v) and (vi):

(v) tonicity agent,
(vi) buffer agent.

4. The liquid formulation of any one of claims 1 to 3, wherein the botulinum toxin is a botulinum neurotoxin complex; or
wherein the botulinum toxin is botulinum neurotoxin free of complexing proteins; or wherein the botulinum toxin is of serotype A; or
wherein the botulinum toxin is botulinum neurotoxin of serotype A free of complexing proteins; or
wherein the botulinum toxin is of serotype A and is present at a concentration of 1-1000 U/ml; or
wherein the botulinum toxin is botulinum neurotoxin of serotype A free of complexing proteins and is present at a concentration of 1-1000 U/ml.

5. The liquid formulation of any one of claims 1 to 4, wherein the human serum albumin is present in the liquid formulation at a concentration of 0.001-1.00% w/v.

6. The liquid formulation of claim 1 or any one of claims 3 to 5, as far as depending on claim 1, wherein the chelating agent is selected from the group consisting of aminopolycarboxylic acids having three to six carboxylic acid functional groups,

citrate, porphyrins, N,N,N',N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN), triethylenetetramine (TETA), and mixtures thereof,

preferably wherein the chelating agent is an aminopolycarboxylic acids of general formula (I):

$$(HO_2CCH_2)_2N\text{-}R\text{-}N(CH_2CO_2H)_2 \qquad (I),$$

wherein R comprises no carboxylic acid group or one or two carboxylic acid groups, the aminopolycarboxylic acid preferably being selected from the group consisting of ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetic acid (BAPTA), diethylenetriaminepentaacetic acid (DTPA), triethylenetetraminehexaacetate (TTHA), and mixture thereof; or

wherein the chelating agent is 2,2',2''-nitrilotriacetic acid.

7. The liquid formulation of claim 1 or any one of claims 3 to 6, as far as depending on claim 1, wherein the metal salt is present in the liquid formulation in an amount of 0.01-100 mM; or wherein the metal salt is calcium chloride and is present in the liquid formulation in an amount of 0.01-100 mM.

8. The liquid formulation of any one of claims 1 to 7, wherein the liquid formulation contains Fe^{3+} ions in a concentration of less than 1 µM, preferably less than 500 nM, and more preferably less than 250 nM and/or the concentration of tryptophan and N-acetyl-tryptophan in the liquid formulation is no more than 50 µM, preferably no more than 20 µM, more preferably no more than 10 µM, still more preferably no more than 1 µM, and most preferably 0 µM, of tryptophan and N-acetyltryptophan (total concentration of both Trp and N-AcTrp).

9. The liquid formulation of any one of claims 1 to 8, wherein
hyaluronic acid is not crosslinked; or
hyaluronic acid is present at a concentration of 2 to 10 mg/ml and wherein the hyaluronic acid is not crosslinked; or

hyaluronic acid is present at a concentration of 2 to 10 mg/ml and wherein hyaluronic acid is crosslinked.

10. The liquid formulation of claim 1 or any one of claims 3 to 9, as far as depending on claim 1, wherein the alditol is selected from glycerol, mannitol, isomalt, lactitol, sorbitol, xylitol, threitol, erythritol and arabitol, preferably from mannitol and sorbitol.

11. The liquid formulation of any one of claims 1 to 10, wherein the alditol is present in a concentration of from 20 to 100 mg/ml.

12. The liquid formulation of any one of claims 3 to 11, wherein

    (v) the tonicity agent is present in the liquid formulation in an amount of 0.01-2.0% w/v, or wherein the tonicity agent is sodium chloride, or wherein the tonicity agent is sodium chloride and is present in the liquid formulation in an amount of 0.01-2.0% w/v;

    (vi) the buffer agent is present in the liquid formulation in a concentration of 1-100 mM, or wherein the buffer agent is citric acid, an amino acid, phosphate, or a mixture thereof, or wherein the buffer agent is histidine, or wherein the buffer agent is histidine, phosphate, or a mixture thereof, and the histidine and phosphate are present in the liquid formulation in a concentration of 1-100 mM.

13. The liquid formulation of any one of claims 1 to 12, wherein the pH of the liquid formulation is in the range of from of 5.0 to 8, preferably from 5.5 to 6.5.

14. A method of making the liquid formulation as defined in claim 1 or any one of claims 3 to 13, as far as depending on claim 1, comprising:

    mixing components (i) to (iv) and optionally (v) and/or (vi) in a liquid comprising water; and

    (l) a chelating agent; or

(II) a chelating agent and a metal salt selected from a salt of calcium, magnesium, zinc, and mixtures thereof; or

(III) wherein the method comprises steps (a) and (c) or steps (b) and (c):

(a) contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent;

(b) contacting said human serum albumin with a chelating agent and a metal salt selected from calcium, magnesium or zinc, or a mixture thereof, to obtain a mixture of human serum albumin, the chelating agent, and the metal salt; and

(c) removing the chelating agent from the mixture obtained in step (a) or step (b) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis; or

(IV) wherein the method comprises steps (d) and (f) or steps (e) and (f):

(d) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent to obtain a mixture of said liquid formulation and the chelating agent;

(e) contacting a liquid formulation comprising components (i) to (iv) with a chelating agent and a metal salt selected from calcium, magnesium or zinc, or mixtures thereof to obtain a mixture of said liquid formulation, the chelating agent, and the metal salt;

(f) removing the chelating agent from the mixture obtained in step (d) or step (e) by dialysis, filtration, crossflow filtration or ultrafiltration, preferably by dialysis;

preferably wherein the contacting in step (a) or step (b) is carried out by mixing the composition comprising human serum albumin with the chelating agent or the chelating agent and the metal salt or wherein the contacting in step (d) and (e) is carried out by mixing a liquid formulation comprising components (i) to (iv) with the chelating agent or the chelating agent and the metal salt such that the concentration of the chelating agent in the liquid formulation is 1 mM – 500 mM;

or

a method of making the liquid formulation as defined in claim 2 or any one of claims 3 to 5 and 8 to 13, as far as depending on claim 2, comprising:

mixing components (i) to (iv) and optionally (v) and/or (vi) in a liquid comprising water.

15. The liquid formulation according to any one of claims 1 to 13 for use in therapy, particularly for use in the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders; or

the use of the liquid formulation of any one of claims 1 to 13 in a cosmetic application; or

use of the liquid formulation of any one of claims 1 to 13 in the manufacture of a medicament for the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders in a person in need thereof.