Alluzience®: histidine) and an additional excipient (Innotox®: sodium phosphate as a buffer; Alluzience®: sucrose).

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

OBJECT OF THE INVENTION

[008] It is therefore an object of the present invention to provide a stable liquid formulation of botulinum toxin that is suitable for use in cosmetic and therapeutic applications.

SUMMARY

[009] The present disclosure is based on the surprising finding that human serum albumin (HSA) that has been pre-treated by incubating with a chelating agent, such as EDTA, followed by removing the chelating agent enables the preparation of a liquid formulation of botulinum toxin that not only exhibits an excellent storage stability but also a superior and improved light stability. Furthermore, the present disclosure is based on the surprising finding that iron ions, in particular Fe^{3+} ions, have a deleterious effect on the light stability of liquid botulinum toxin formulations containing HSA.

[0010] 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).

[0011] Thus, in a first example, the present disclosure provides a liquid formulation, comprising

(i) botulinum toxin and
(ii) human serum albumin (HSA),

wherein the liquid formulation contains Fe^{3+} ions in a concentration of less than 1 µM, preferably less than 500 nM, more preferably less than 250 nM, and most preferably less than 100 nM.

[0011a] In a first aspect, the present disclosure provides a liquid formulation, comprising

(i) botulinum toxin and
(ii) human serum albumin,

wherein the liquid formulation contains Fe^{3+} ions in a concentration of less than 1 µM, preferably less than 500 nM, more preferably less than 250 nM.

[0012] The liquid botulinum toxin formulation with low Fe^{3+} concentrations of, e.g., less than 1 µM, can be prepared by a method comprising step (a) of contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent, and step (b) of removing the chelating agent from the mixture.

[0013] In a second example, the present disclosure provides a liquid formulation, comprising

(i) botulinum toxin and
(ii) human serum albumin (HSA),

wherein the liquid formulation contains no tryptophan and N-acetyl-tryptophan or 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-acetyl-tryptophan (total concentration of both Trp and N-AcTrp).

[0013a] In a second aspect, the present disclosure provides a liquid formulation, comprising

(i) botulinum toxin and
(ii) human serum albumin,

wherein the liquid formulation contains no or no more than 50 µM tryptophan and N-acetyl-tryptophan.

[0014] The liquid formulation according to the first and second aspect and example of the present disclosure preferably further comprises (iii) a tonicity agent, in particular sodium chloride, and (iv) a buffering agent, in particular a histidine buffer,

phosphate buffer or a mixture thereof. The liquid botulinum toxin formulation free of or containing only very low concentrations of tryptophan and/or N-acetyl-tryptophan can be prepared by purifying the HSA starting material by a method such as dialysis and using the purified HSA material in the formulation of the liquid formulation of the present disclosure.

[0015] In a third example, the present disclosure provides a liquid formulation comprising:

(i) botulinum toxin and
(ii) human serum albumin (HSA),

wherein the human serum albumin is prepared by a method comprising the following steps:

(a) contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent, and
(b) removing the chelating agent from the mixture.

[0015a] In a third aspect, the present disclosure provides a liquid formulation, comprising:

(i) botulinum toxin and
(ii) human serum albumin,

wherein the liquid formulation is prepared by a method comprising the following steps:

(a) contacting human serum albumin with a chelating agent to obtain a mixture of human serum albumin and the chelating agent, and
(b) removing the chelating agent from the mixture.

[0016] The contacting step (a) is preferably carried out by adding the chelating agent

(e.g., EDTA) to a composition comprising HSA (e.g., a HSA solution) and incubating the resulting mixture for a predetermined time, optionally followed by dialysis against a buffer containing the chelating agent (e.g., EDTA). In step (b), the chelating agent is removed by any suitable method, for example by dialysis.

[0017] Furthermore, the liquid formulation according to the third aspect and example of the present disclosure preferably further comprises (iii) a tonicity agent, in particular sodium chloride, and/or (vi) a buffering agent, in particular a histidine buffer, phosphate buffer or a mixture thereof.

[0018] In a fourth example, the present disclosure relates to a method for preparing a liquid formulation according to the second aspect and example of the present disclosure, wherein the method comprises the following steps:

- purifying a human serum albumin starting material to obtain a purified human serum albumin material, e.g., by subjecting the human serum albumin starting material to dialysis, diafiltration, ultrafiltration, ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography or field-flow fractionation, preferably dialysis,
- mixing the obtained purified human serum albumin with botulinum toxin and optionally further components to obtain the liquid formulation.

[0019] An alternative method according to the fourth example of the disclosure comprises the following steps:

- purifying a liquid composition comprising botulinum toxin and human serum albumin to obtain a purified liquid composition, e.g., by subjecting the liquid composition comprising botulinum toxin and human serum albumin to dialysis, diafiltration, ultrafiltration, ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography or field-flow fractionation, preferably dialysis,
- mixing the obtained purified liquid composition with further components to obtain the liquid formulation.

[0019a] In a fourth aspect, the present disclosure relates to a method for preparing a liquid formulation according to the second aspect and example of the present disclosure, wherein the method comprises the following steps:

- purifying a human serum albumin starting material to obtain a purified human serum albumin material,
- mixing the obtained purified human serum albumin with botulinum toxin and optionally further components to obtain the liquid formulation,

or

- purifying a liquid composition comprising botulinum toxin and human serum albumin to obtain a purified liquid composition,
- mixing the obtained purified liquid composition with further components to obtain the liquid formulation

wherein the purifying step is preferably carried out by subjecting the human serum albumin starting material or the liquid composition comprising botulinum toxin and human serum albumin to dialysis, diafiltration, ultrafiltration, ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, field flow fractionation or precipitation (e.g., salt precipitation, ethanol precipitation).

[0020] In a fifth example, the present disclosure relates to a liquid formulation according to the present disclosure for use in therapy, especially for use in the treatment of

neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders.

[0020a] In a fifth aspect, the present disclosure relates to a liquid formulation as defined herein for use in therapy, particularly for use in the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders.

[0021] In a sixth example, the present disclosure relates to the cosmetic (aesthetic) use of the liquid formulation of the present disclosure for the treatment of a cosmetic condition, preferably a skin condition, in particular for the treatment of skin lines or folds or wrinkles.

[0021a] In a sixth aspect, the present disclosure relates to a cosmetic use of the liquid formulation as defined herein for the treatment of a cosmetic condition.

[0021b] In a seventh aspect, the present disclosure relates to use of the liquid formulation as defined herein in the manufacture of a medicament for the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, or neurological disorders.

[0022] In a seventh example, the present disclosure provides a method of treating a disease or condition, comprising administering an effective amount of the liquid formulation of the present disclosure to a person in need thereof.

[0023] Preferred examples of the liquid formulation, its use and methods of use according to the present disclosure are set forth in the appended claims.

[0024] The present disclosure may be understood more readily by reference to the following detailed description and the example included therein.

DETAILED DESCRIPTION OF THE INVENTION

[0025] The present invention is based on the unexpected finding that the use of a pre-treated 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), achieved by chelating agent treatment followed by dialysis, 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.

[0026] Furthermore, the present invention is based on the unexpected finding that tryptophan and/or N-acetyl-tryptophan impair the light stability (photostability) of human serum albumin (HSA)-containing liquid botulinum toxin formulations. In particular, it was found that the removal of N-acetyl-tryptophan from conventional HSA products by chelating agent treatment and subsequent dialysis resulted in liquid HSA-containing formulations of botulinum toxin having an improved light stability.

[0027] The liquid formulation of the present invention advantageously provides an

CLAIMS

1. A liquid formulation, comprising

   (i) botulinum toxin and

   (ii) human serum albumin,

   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.

2. A liquid formulation, comprising

   (i) botulinum toxin and

   (ii) human serum albumin,

   wherein the liquid formulation contains no or no more than 50 µM tryptophan and N-acetyl-tryptophan.

3. A liquid formulation, comprising

   (i) botulinum toxin and

   (ii) human serum albumin,

   wherein the liquid formulation is prepared by a method comprising the following steps:

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

   (b) removing the chelating agent from the mixture.

4. The liquid formulation of claim 1, wherein the liquid formulation is prepared by a method comprising the following steps:

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

   (b) removing the chelating agent from the mixture.

5. The liquid formulation of claim 3 or 4, wherein, in step (a), a human serum albumin starting material is contacted with a chelating agent to obtain a mixture of the human

serum albumin starting material and the chelating agent and, in step (b), the chelating agent is removed from the mixture to obtain a pre-treated human serum albumin material and, in a further step (c), botulinum toxin is mixed with the pre-treated human serum albumin material, wherein the chelating agent is preferably removed by dialysis, filtration, crossflow filtration or ultrafiltration,

or

wherein, in step (a), a liquid pre-formulation comprising botulinum toxin and human serum albumin is contacted with the chelating agent to obtain a mixture of the liquid pre-formulation and the chelating agent and, in step (b), the chelating agent is removed from the mixture to obtain the liquid formulation, wherein the chelating agent is preferably removed by dialysis, filtration, crossflow filtration or ultrafiltration.

6. The liquid formulation of any one of claims 1 to 5, wherein the human serum albumin is present in the liquid formulation in an amount of 0.001-1.00% w/v.

7. The liquid formulation of claim 2, wherein the liquid formulation contains Fe^{3+} ions in a concentration of less than 1000 nM, preferably less than 500 nM, and more preferably less than 250 nM.

8. The liquid formulation of any one of claims 1 to 7, wherein the liquid formulation further comprises:

(iii) a tonicity agent,

or wherein the liquid formulation further comprises:

(iii) a tonicity agent, wherein 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.

9. The liquid formulation of any one of claims 1 to 8, wherein the liquid formulation further comprises:

(iv) a buffering agent,

or wherein the liquid formulation further comprises:

(iv) a buffering agent, wherein the buffering agent is present in the liquid formulation in a concentration of 1-100 mM, or wherein the buffering agent is an amino acid, phosphate, or a mixture thereof, or wherein the buffering agent is histidine, or wherein the buffering 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.

10. A method for preparing a liquid formulation according to claim 2 or 7, or one of claims 8 and 9, as far as dependent on claim 2, wherein the method comprises the following steps:

- purifying a human serum albumin starting material to obtain a purified human serum albumin material,
- mixing the obtained purified human serum albumin with botulinum toxin and optionally further components to obtain the liquid formulation,

or

- purifying a liquid composition comprising botulinum toxin and human serum albumin to obtain a purified liquid composition,
- mixing the obtained purified liquid composition with further components to obtain the liquid formulation,

wherein the purifying step is preferably carried out by subjecting the human serum albumin starting material or the liquid composition comprising botulinum toxin and human serum albumin to dialysis, diafiltration, ultrafiltration, ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, field flow fractionation or precipitation (e.g., salt precipitation, ethanol precipitation).

11. The method of claim 10, wherein the purifying step is conducted as follows or wherein the purifying step additionally contains the following steps:

- contacting a human serum albumin starting material with a chelating agent to obtain a mixture of the human serum albumin starting material and the chelating agent, and
- removing the chelating agent from the mixture to obtain a chelating agent-treated purified human serum albumin material,

or

- contacting a liquid composition comprising botulinum toxin and human serum albumin with the chelating agent to obtain a mixture of the liquid composition and the chelating agent, and
- removing the chelating agent from the mixture to obtain a chelating agent-treated purified liquid formulation,

wherein the step of contacting in methods (i) and (ii) is preferably carried out such that the concentration of the chelating agent in mixture is 1 mM to 500 mM, and/or the removal of the chelating agent is carried out by dialysis, crossflow filtration or ultrafiltration.

12. The liquid formulation of any one of claims 3 to 6, 8 and 9 or the method according to claim 10 or 11, 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(b-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.

13. The liquid formulation of any one of claims 1 to 9 and 12 or the method according to claim 10 or 11, 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, or wherein the pH of the liquid formulation is in the range of 5.0-8.0.

14. A liquid formulation according to any one of claims 1 to 9, 12 and 13 for use in therapy, particularly for use in the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, and neurological disorders.

15. Cosmetic use of the liquid formulation of any one of claims 1 to 9, 12 and 13 for the treatment of a cosmetic condition.

16. Use of the liquid formulation according to any one of claims 1 to 9, 12 and 13 in the manufacture of a medicament for the treatment of neuromuscular diseases, pain, sialorrhea, hyperhidrosis, urological disorders, or neurological disorders.