PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AKIN 100 PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/US2017/048174	International filing date *(day/month/year)* 23.08.2017	Priority date *(day/month/year)* 26.08.2016

International Patent Classification (IPC) or national classification and IPC
INV. A61K9/14

Applicant
AKINA, INC.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in the form of an Annex C/ST.25 text file, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*ter* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.06.2018	09.11.2018

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2017/048174

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-65 as originally filed

   Claims, Numbers

   1-51 filed with the demand for preliminary international examination

   Drawings, Sheets

   1/3-3/3 as originally filed

   ☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-*bis*)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).

6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
   - ☒ A top-up search was carried out by this Authority on 17.07.2018 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
     - ☐ Additional relevant documents have been discovered during the top-up search.
   - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.

7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* *If item 4 applies, some or all of those sheets may be marked "superseded".*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-51
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-51
Industrial applicability (IA)	Yes:	Claims	1-51
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Amendments (Art. 34(2)(b) PCT)

1.1 The amendments filed with the demand do not comply with the requirements of Article 34(2)(b) PCT. The reasons are the following:

Claims 1 and 19 now define "botulinum" as one of the proteins encapsulated in the microparticles. However, a basis can only be found for "botulinum toxin" (e.g. on page 5, lines 24-25 and throughout the whole description). Amending said claim 1 (multiple times) and claim 19 to specify botulinum toxin would overcome this objection.

2 Cited Documents

2.1 Reference is made to the following documents:

D3 US 6 312 708 B1 (DONOVAN STEPHEN [US]) 6 November 2001 (2001-11-06)cited in the application

D4 LU YING ET AL: "Microparticles produced by the hydrogel template method for sustained drug delivery",
INTERNATIONAL JOURNAL OF PHARMACEUTICS, ELSEVIER, AMSTERDAM, NL,
vol. 461, no. 1, 11 December 2013 (2013-12-11), pages 258-269, XP028810400,
ISSN: 0378-5173, DOI: 10.1016/J.IJPHARM.2013.11.058
cited in the application

D5 US 2016/175410 A1 (HUNT TERRENCE J [US]) 23 June 2016 (2016-06-23)

3 Novelty (Art. 33(2) PCT)

3.1 The subject-matter of claims 1-51 is new in the sense of Article 33(2) PCT.

3.2 Document D3 discloses (col. 8, lines 22-44; col. 22, lines 9-65; example 1): a method of formulating polymer microparticles for the controlled release of botulinum toxin. D3 does not disclose the precipitating agents included in claim 1. This will lead to a product which is also different from the ones described in present claims 36 and 47.

4 Inventive Step (Art. 33(3) PCT)

4.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-51 is not considered to involve an inventive step in the sense of Article 33(3) PCT.

4.2 D3, which is seen as the most relevant state of the art in relation to claim 1, discloses a method of formulating polymer microparticles for the controlled release of botulinum toxin (example 1). It comprises the steps of:

a) dissolving botulinum toxin and albumin in an aqueous solution (reconstituted Botox ®, see col. 28, lines 60-63; and col. 8, lines 22-44);

b) mixing or dispersing said solution in a solution of a polymer to form a polymer-botulinum toxin dispersion; and

c) preparing polymer microparticles encapsulating botulinum toxin

The subject-matter of claim 1 differs from D3 in that it additionally comprises the steps of: precipitating the botulinum toxin-protein from the solution with selected precipitating agents, and washing the obtained precipitant with a wash solvent.

The alleged effect of this difference is to overcome loss of activity of protein active agents associated with denaturation of water-dissolved proteins that occur in the interface between water and organic solvent (cf. description page 20, lines 8-11). However, the effect is not shown over the whole scope of the claim: the only precipitant used in the examples of the application is zinc chloride, whereas many other precipitating agents are claimed for which an effect is not shown. Therefore, the objective technical problem has to be formulated as the provision of an alternative method to formulate botulinum toxin polymer microparticles.

D3 already suggests the use of multivalent metal cations, preferably Zn++, as a stabilizer for botulinum toxin, avoiding a potential loss of activity (col. 22, lines 9-65; col. 23, lines 57-65). D3 does not specifically disclose zinc chloride, but that does not appear relevant as it is the zinc which will have the

precipitating effect. Moreover, washing of the precipitant is a common process step when precipitation is involved. Thus, taking D3 alone, the skilled person would be prompted to modify the method disclosed therein in order to solve the problem posed, arriving to the subject-matter of claim 1 without the exercise of inventive step (Art. 33(3) PCT).

4.3 The applicant argued that the claimed subject-matter is inventive, as none of the prior art leads one of the skill in the art to select from the myriad of available methods, reagents and formulations the specific ones that are claimed, much less with a reasonable expectation of success. However, this does not seem to be the case when starting from document D3 and applying the problem-solution approach to assess inventive step (see PCT Guidelines, 13.09 and Appendix to Chapter 13).

4.4 Moreover, the applicant argued that the claimed methods and resulting formulations required undue experimentation to determine the methods and reagents, to produce a safe and efficacious botulinum formulation. This Authority cannot see how this relates to inventive step, rather than to sufficiency of disclosure (Art. 5 PCT). It is also noted that the examples of the present application were limited to particular combinations of components, namely: botulinum toxin and albumin as proteins; zinc chloride as precipitant; and PLGA as polymer.

4.5 The same reasoning as for claim 1 applies *mutatis mutandis* to the subject-matter of independent claims 36 and 47, which are also not inventive (Art. 33(3) PCT).

4.6 Claim 50 refers to a method of treatment comprising administering the composition of claim 36. Methods of treatment are equally disclosed in D3; thus, the same reasoning as above applies to claim 50, which is also not inventive in the sense of Article 33(3) PCT.

4.7 Dependent claims 2-35, 37-46, 48, 49 and 51 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.

The reasons therefor are that the additional features of the said claims are a combination of features obvious to the man skilled in the art in consideration of the disclosure of the prior art named in the present proceedings, or they concern only minor modifications which lie within the normal practice of the man skilled in the art.

E.g., microparticles obtained via the micropatterned hydrogel template method, wherein the micropatterned template comprises poly(vinyl alcohol), are known from D4.

Compositions comprising thermosensitive polymers for sustained release of botulinum toxin are known from D5.

In the absence of a surprising effect, dependent claims claims 2-35, 37-46, 48, 49 and 51 lack an inventive step (Art. 33(3) PCT).

5 Industrial Applicability (Art. 33(4) PCT)

5.1 The subject-matter of claims 1-51 is industrially applicable in the sense of Article 33(4) PCT.

Re Item VIII

Certain observations on the international application

6 Clarity (Art. 6 PCT)

6.1 The application does not meet the requirements of Article 6 PCT, because claims 2, 4, 5 and 51 are not clear.

6.2 Claim 2 refers to the method of claim 1 step (b) wherein the bulking agent is (...). Step (b) of claim 1 does not relate to a bulking agent; step (a) does. Moreover, in relation to this, the readability of the claim would be improved if claim 2 would be redrafted such as: "The method of claim 1 wherein the bulking agent in step (a) is (...).

6.3 The same applies mutatis mutandis to claims 4 and 5. Their readability would be improved by redrafting said claims respectively to e.g.: "The method of claim 1 wherein the wash solvent in step (c) is (...)" and "The method of claim 1 wherein the polymer solvent in step (d) is (...)".

6.4 Claim 51 specifies some diseases and disorders twice, namely hemorrhagic cystitis and interstitial cystitis/ painful bladder syndrome (IC/PBS). The second instance these diseases appear should be deleted (Art. 6 PCT).

IN THE INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY/EP

Applicants: AKINA, INC.

International Application No.: *~~PCT/US2018/039318~~ PCT/US2017/048174 * IPEA/EP

International Filing Date: 23 August 2017

For: BIODEGRADABLE POLYMER FORMULATIONS FOR EXTENDED EFFICACY OF BOTULINUM TOXIN

European Patent Office
D-80298 Munich
GERMANY

RESPONSE TO WRITTEN OPINION AND AMENDMENT UNDER ARTICLE 34

Sir:

The following comments are in response to the Written Opinion mailed 11 December 2017. Please substitute pages 66-70 for pages 66-70 as originally filed.

Independent claim 1 was amended to define the first protein as botulinum and the second protein as a bulking agent protein and to incorporate the precipitation agents of claim 2. Claim 2 is amended to define the bulking agent protein as albumin. Support is found at the bottom of page 5 and original claim 19. Claim 3 is narrowed to zinc chloride as the precipitation agent (support is shown on page 23 and in the examples showing precipitation of the botulinum-albumin protein).

Dependent claims were amended for antecedent basis.

1. Method of Treatment

Claims 50 and 51 are directed to a method of treatment and diagnostic method practiced on the human body, so these claims have been searched on the alleged effects of the claimed composition. These claims will be amended as necessary prior to entry into the national and regional phase of the PCT to comply with foreign patent office regulations.

2. Novelty and Inventive Step

The following publications were cited by the examiner:

D1: US 2010/310669 by Paillard, et al.

D2: Wang, et al., Journal of Colloid And Interface Science, 271:1, 92-101 (March 1, 2004)

D3: US 6,312,708 by Donovan

D4: Ying, et al. International Journal of Pharmaceutics 461(1):258-269 (2013)

D5 US 2016/175410 by Hunt

The Claimed Invention

The claimed product is distinguished by a high degree of encapsulation, retention of activity and controlled release. This is achieved through the use of the primary method defined by claim 1, and further enhanced through the combination with the method of claims 7-19 (using a micropatterned template to form the microparticles), specific polymers, polymer ratios, and washing solvents (claims 26-28), and the conditions to reduce the particle size (claims 31-35). The prior art does not disclose any of these methods, especially for a protein as labile and difficult to incorporate in the very small amounts that are safe, as botulinum.

The release properties of the claimed formulation, and the safety and efficacy of the formulation, are demonstrated by the examples. Example 1, pages 45-49 demonstrates that botulinum is very labile, so that the method of encapsulation is critical for retention of activity, and that release properties require controlling precipitation conditions, solvent and polymer selection, and loading. See Figure 1. See also discussion at pages 48-49. Example 2, pages 49-51, and Figure 2, shows that safety was enhanced using the claimed particle formulations. Example 3, pages 51-52, and Figure 3, shows that efficacy was increased with the particles made using the claimed methods. Examples 4, pages 51-53, Figure 4, and 5, pages 53-55, and Figure 5 and Table 4 shows the increased efficacy of the botulinum particles made by the method of claim 1, further enhanced by formulation in the thermogels that form a gel at body temperature (Table 3). Example 10, pages 64-65, shows the increase in efficacy using crosslinked hyaluronic acid gels. Example 6, pages 56-58, and Tables

5 and 6, shows that the size of the botulinum precipitates (which results from selection of precipitating agents as well as conditions) influences efficacy. Example 7, pages 58-61, and Table 7, show the importance of solvent selection for washing of the precipitates. Example 8, pages 61-62, and Table 8, and Example 9, pages 62-64, Table 9, shows the importance of the emulsion methods used to make the botulinum loaded particles.

Absent these extensive studies, one skilled in the art could not have arrived at the claimed methods and resulting formulations.

Novelty

Claims 1, 5, 9, 25-28, 32, 36, and 50 are alleged to lack novelty over D1.

This rejection is mooted by the incorporation into claim 1 of the subject matter of claims 2 and 19, both of which were novel over D1.

Claims 1, 5, 9, 25-27, 31, 36, and 60 are alleged to lack novelty over D2

This rejection is mooted by the incorporation into claim 1 of the subject matter of claims 2 and 19, both of which were novel over D1.

Note also that D2 discloses precipitation with zinc acetate; the preferred compound in this case for botulinum and albumen is zinc chloride. These are distinct chemically and by charge, leading one skilled in the art not to extrapolate from

Claims 36, 45, 50 and 51 are alleged to lack novelty over D3

Claim 36 defines the composition of claims 1-35. D3 does not disclose the formulation of claim 1, as amended, and as recognized by the examiner in not rejecting claim 47 over D3.

There is no disclosure in D3 of a complex of botulinum, bulking agent protein and precipitating agent, which would be present in the final product.

Inventive Step

Claims 2-4, 6-8, 10-24, 29, 30, 33-35, 37-44, 46, 48 and 49 are alleged to lack inventive step over at least D3.

Claim 47 was objected to as lacking an inventive step over US 6,312,708 (D3). The Examiner alleges that the skilled person would be led to modify the microparticles disclosed in D3, and would arrive at the subject matter of claim 47.

The Examiner objected to dependent claims 2-4, 6-8, 10-24, 29, 30, 33-35, 37-44, 46, 48 and 49 as lacking an inventive step, asserting that the claims do not contain

any features which, in combination with the features of any claim to which they refer, meet the requirements of inventive step.

As discussed above, the claimed subject matter is novel over the prior art. The claimed subject matter is also inventive, none of the prior art leading one of skill in the art to select from the myriad of available methods, reagents, and formulations the specific ones that are claimed, much less with a reasonable expectation of success. The claimed methods, and resulting formulations required undue experimentation to determine the methods and reagents, to produce a safe and efficacious botulinum formulation.

Clarity and Lack of Sufficiency

Although it is not believed that breadth of claims is proof of a lack of clarity or sufficiency, the claims have been narrowed to microparticles containing botulinum in combination with a bulking agent proteins, precipitated using specific reagents.

Claim 10 was amended to correct antecedent basis.

The spelling of blends in claim 26 has been corrected.

Potential amendments to claim 51 and use of the term "about" for clarity will be addressed in the national and regional phases of the application.

The claims as amended, are clear, have sufficient basis, and are novel and contain an inventive step.

Respectfully submitted,

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We claim:

1. A method of formulating polymer microparticles for the controlled release of botulinum, comprising the steps of:

(a) dissolving the botulinum and a bulking agent protein in an aqueous solution to form a botulinum-protein solution;

(b) precipitating the botulinum-protein from the solution with a precipitating agent selected from the group consisting of L-histidine methyl ester, L-cysteine ethyl ester, Nα-(tert-butoxycarbonyl)-L-asparagine, L-proline benzyl ester, N-acetyl-L-tryptophan, gentisic acid, pentetic acid, octanoic acid, zinc chloride, and combinations thereof, to form a precipitant;

(c) washing the precipitant one or more times with a wash solvent to form a solvent-washed precipitant;

(d) mixing or dispersing the precipitant in a solution of a polymer to form a polymer-botulinum-protein dispersion; and

(e) preparing polymer microparticles encapsulating the botulinum-protein from the polymer-botulinum-protein dispersion.

2. The method of claim 1 step (b) wherein the bulking agent protein is albumin.

3. The method of claim 1 wherein the precipitating agent is zinc chloride.

4. The method of claim 1 step (c) wherein the wash solvent is selected from the group consisting of acetone, acetonitrile, dioxane, ethanol, 2-methoxy ethyl acetate, methoxy ethanol, ethoxy ethanol, butoxy ethanol, 2-propanol propylene glycol methyl ether, ethanediol, 1,2-propanediol, tert-butyl alcohol, diethylene glycol, and combinations thereof.

5. The method of claim 1 step (d) wherein the polymer solvent is selected from the group consisting of benzyl alcohol, n-butyl acetate, chlorobenzene, chloroform, dioxane, dichloromethane, ethyl acetate, ethyl benzoate, ethyl formate, methyl formate, methyl n-propyl ketone, phenethylamine, triacetin, trichloroethylene, and combinations thereof.

6. The method of claim 5 wherein the polymer solvent is a combination of dioxane and dichloromethane or ethyl acetate and dichloromethane.

7. The method of claim 1 wherein the polymer solvent dispersion is formed into microparticles using a micropatterned template.

AMENDED SHEET

8. The method of claim 7 wherein the micropatterned template comprises poly(vinyl alcohol).

9. The method of claim 1 wherein the microparticles are formed by adding the polymer-protein dispersion to a non-polymer solvent to form an emulsion.

10. The method of claim 9 wherein the non-polymer solvent is an aqueous solvent pre-conditioned with an organic solvent or solvents that dissolve the polymer.

11. The method of claim 10 wherein the organic solvent used for preconditioning is dichloromethane.

12. The method of claim 11 wherein the emulsion comprises dichloromethane in a concentration between 0.1% and 1.3% in an aqueous solution.

13. The method of claim 9 wherein the organic solvent used for preconditioning is ethyl acetate.

14. The method of claim 9 wherein the emulsion comprises ethyl acetate in a concentration between 0.1% and 8.7% in aqueous solution.

15. The method of claim 9 wherein the drug-polymer emulsion is mixed with an overhead stirrer or a high-speed homogenizer.

16. The method of claim 9 wherein the emulsion is an aqueous emulsion containing a dissolution prevention agent selected from the group consisting of L-histidine methyl ester, L-cysteine ethyl ester, Nα-(tert-butoxycarbonyl)-L-asparagine, L-proline benzyl ester, N-acetyl-L-tryptophan, gentisic acid, pentetic acid, octanoic acid, and zinc chloride.

17. The method of claim 16 wherein the dissolution prevention agent is zinc chloride.

18. The method of claim 17 wherein the concentration of zinc chloride is between 0.1% to 10% w/v.

19. The method of claim 1 wherein the botulinum comprises a botulinum toxin selected from the group consisting of botulinum toxin types A, B, C, D, E, F, G, and mixtures thereof.

20. The method of claim 19, wherein the botulinum toxin is a botulinum toxin type A.

21. The method of claim 20, wherein between about 1 unit and about 50,000 units of botulinum toxin A are associated with the microparticles.

22. The method of claim 21, wherein the quantity of the botulinum toxin associated with the microparticles is between about 100 units and about 30,000 units of botulinum toxin A.

23. The method of claim 19, wherein between about 100 units and about 30,000 units of botulinum toxin B are associated with the microparticles.

24. The method of claim 1, wherein the protein comprises a serum albumin protein.

25. The method of claim 1, wherein the polymer is a biodegradable polymer.

26. The method of claim 25, wherein the biodegradable polymer is selected from the group consisting of polylactide, poly(lactide-co-glycolide), poly(lactide-co-glycolide)-poly(ethylene glycol) block copolymers, blends and copolymers thereof.

27. The method of claim 26, wherein the polymer is a poly(lactide-co-glycolide) having the lactide:glycolide (L:G) ratio of between about 50:50 and 100:1, inclusive.

28. The method of claim 27 wherein the polymer is a poly(lactide-co-glycolide) having the L:G ratio of between about 75:25 and 85:15, inclusive.

29. The method of claim 1, where solvent washing is performed using a solvent selected from the group consisting of acetone, acetonitrile, dioxane, ethanol, 2-methoxy ethyl acetate, methoxy ethanol, ethoxy ethanol, butoxy ethanol, 2-propanol, propylene glycol methyl ether, ethanediol, 1,2-propanediol, tert-butyl alcohol, diethylene glycol, methanol, N-methylpyrrolidone, dimethylacetamide, dimethylformamide, dimethylsulfoxide, pyridine, tetrahydrofurane, or combinations thereof.

30. The method of claim 1, where solvent washing maintains protein activity and the solvent is selected from the group consisting of acetone, acetonitrile, dioxane, ethanol, 2-methoxy ethyl acetate, methoxy ethanol, ethoxy ethanol, butoxy ethanol, 2-propanol, propylene glycol methyl ether, ethanediol, 1,2-propanediol, tert-butyl alcohol, diethylene glycol, and combinations thereof.

31. The method of claim 1, where the precipitant is dried by freeze-drying in the absence of solvent washing or after solvent washing.

32. The method of claim 1, wherein solvent-washed precipitate is directly mixed with polymer solution.

33. The method of claim 1, further comprising reducing the size of precipitants by dry or wet milling.

34. The method of claim 33, wherein the milling temperature is controlled at the temperature of solidified carbon dioxide or liquid nitrogen.

35. The method of claim 33 comprising wet milling of precipitate mixed with n-butyl acetate, dioxane, dichlomethane, ethyl acetate, or combinations thereof.

36. A pharmaceutical composition, comprising a plurality of polymer microparticles encapsulating botulinum toxin, formulated according to the method of any one of claims 1-35.

37. The pharmaceutical composition of claim 36 dispersed in a diluent consisting of an aqueous thermosensitive polymer solution.

38. The pharmaceutical composition of claim 37 wherein the aqueous thermosensitive polymer solution transitions between liquid and gel within the range of 4 °C to 40 °C.

39. The pharmaceutical composition of claim 38 wherein the aqueous thermosensitive polymer solution transitions between liquid and gel within the range of 20 °C to 38 °C.

40. The pharmaceutical composition of claim 37 wherein the aqueous thermosensitive polymer is selected from poly(lactide-co-glycolide)-b-poly(ethylene glycol)-b-poly(lactide-co-glycolide), poly(lactide)-b-poly(ethylene glycol)-b-poly(lactide), poly(lactide-co-caprolactone)-b-poly(ethylene glycol)-b-poly(lactide-co-caprolactone), poly(caprolactone)-b-poly(ethylene glycol)-b-poly(caprolactone), methoxy poly(ethylene glycol)-b-poly(caprolactone), or combinations thereof

41. The pharmaceutical composition of claim 37 wherein the aqueous thermosensitive polymer solution is included at a concentration between 5% and 40% (w/v) in the aqueous solution.

42. The pharmaceutical composition of claim 41 wherein the aqueous thermosensitive polymer solution is added into the aqueous solution to a concentration between 10% and 30% (w/v).

43. The pharmaceutical composition of claim 36 dispersed in a diluent consisting of a gel-forming solution or a gel.

44. The pharmaceutical composition of claim 43 wherein the gel-forming solution is hyaluronic acid and the gel is crosslinked hyaluronic acid.

45. The pharmaceutical composition of claim 36, wherein the protein comprises a botulinum toxin selected from the group consisting of botulinum toxin types A, B, C, D, E, F, G, and mixtures thereof.

46. The pharmaceutical composition of claim 36 further comprising one or more therapeutic, prophylactic, or diagnostic agents.

47. A pharmaceutical composition, comprising a plurality of Zn-precipitated botulinum toxin/albumin, formulated according to the method of claim 1.

48. The pharmaceutical composition of claim 47 dispersed in a diluent consisting of an aqueous thermosensitive polymer solution.

49. The pharmaceutical composition of claim 47 dispersed in a diluent consisting of an aqueous gel.

50. A method of treating one or more diseases, disorders of cosmetic defects comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 36 to reduce or prevent one or more symptoms of a diseases, disorder or cosmetic defects in the subject.

51. The method of claim 50, wherein one or more diseases, disorders or cosmetic defects to be treated is selected from the group consisting of hemorrhagic cystitis, interstitial cystitis/painful bladder syndrome, hemorrhagic cystitis, IC/PBS, hematuria, urinary urgency, supra pubic pain, inflammation, and urinary retention, crossed eyes (strabismus), uncontrolled blinking (blepharospasm), muscle stiffness/spasms, cervical dystonia, torticollis, uncontrollable sweating, undesirable wrinkles, headaches and migraines.