

WHAT IS CLAIMED IS:

1. A method for purifying a botulinum toxin, comprising purifying the toxin from a solution comprising the toxin, wherein the process does not comprise precipitation, centrifugation or lyophilization.
2. The method of claim 1, wherein the botulinum toxin is serotype A.
3. The method of claim 1 or 2, wherein the purified botulinum toxin obtained is free, essentially free, or substantially free of botulinum toxin complexes.
4. The method of any one of claims 1-3, wherein the purified botulinum toxin obtained is free, essentially free, or substantially free of animal products.
5. The method of any one of claims 1-4, wherein the purified botulinum toxin obtained is free, essentially free, or substantially free of human albumin.
6. The method of any one of claims 1-5, wherein the purifying comprises a filtering step, preferably a tangential flow filtration step.
7. The method of claim 6, wherein the filtering step uses a hollow fiber filter.
8. The method of any one of claims 1-7, wherein the purifying comprises contacting a first chromatography column with a solution comprising the toxin to produce a toxin-containing fraction.
9. The method of claim 8, wherein the first chromatography column comprises an anion exchange chromatography column.
10. The method of claim 9, wherein the anion exchange chromatography column comprises Q Sepharose.
11. The method of any one of claims 1-10, wherein the purifying further comprises collecting the toxin-containing fraction, wherein the toxin-containing fraction does not adsorb to the first stationary phase.
12. The method of claim any one of claims 8-11, wherein the purifying further comprises contacting a second chromatography column with the toxin-containing fraction.

13. The method of claim 12, wherein the second chromatography column comprises a cation exchange chromatography column.
14. The method of claim 13, wherein the cation exchange chromatography column comprises SP Sepharose.
15. The method of any one of claims 12-14, further comprising eluting the botulinum toxin from the second chromatography column to produce a first toxin-containing eluant.
16. The method of any one of claims 12-15, further comprising filtering the first toxin-containing eluant to produce a toxin-containing retentate.
17. The method of claim 16, wherein filtering the toxin-containing eluant comprises a buffer exchange.
18. The method of claim 16 or 17, wherein filtering the first toxin-containing eluant separates botulinum toxin molecules from non-toxin proteins to produce free toxin molecules in the toxin-containing retentate.
19. The method of any one of claims 16-18, further comprising contacting a third chromatography column with the toxin-containing retentate.
20. The method of claim 19, wherein the third chromatography column comprises a second anion exchange chromatography column.
21. The method of claim 20, wherein the second anion exchange chromatography column comprises Q Sepharose.
22. The method of any one of claims 19-21, further comprising eluting the botulinum toxin from the third chromatography column to produce a second toxin-containing eluant.
23. The method of any one of claims 19-22, further comprising contacting a fourth chromatography column with the second toxin-containing eluant.
24. The method of claim 23, wherein the fourth chromatography column comprises a size exclusion chromatography column.
25. The method of claim 24, wherein the size exclusion chromatography column comprises a gel filtration chromatography column.

26. The method of claim 25, wherein the gel filtration chromatography column comprises Superdex 200.

27. The method of any one of claims 23-26, further comprising eluting the botulinum toxin from the fourth chromatography column to produce a purified botulinum toxin.

28. A method for purifying a botulinum toxin from a solution comprising the toxin, comprising:

- (a) filtering the solution comprising the toxin;
 - (b) contacting a first chromatography column with the filtered solution comprising the toxin from (a), wherein the first chromatography column is an ion exchange chromatography column;
 - (c) collecting a toxin-containing fraction, wherein the toxin-containing fraction flows through the first chromatography column without adsorbing to the stationary phase;
 - (d) contacting a second chromatography column with the toxin-containing fraction, wherein the second chromatography column is an ion exchange chromatography column;
 - (e) eluting the botulinum toxin from the second chromatography column to produce a first toxin-containing eluant;
 - (f) filtering the first toxin-containing eluant to produce a toxin-containing retentate;
 - (g) contacting a third chromatography column with the toxin-containing retentate from the filtering (f), wherein the third chromatography column is an ion exchange column;
 - (h) eluting the botulinum toxin from the third chromatography column to produce a second toxin-containing eluant;
 - (i) contacting a fourth chromatography column with the second toxin-containing eluant, wherein said fourth chromatography column is a size exclusion chromatography column;
 - (j) eluting the botulinum toxin from the fourth chromatography column, thereby producing a purified botulinum toxin,
- wherein the process does not comprise precipitating, centrifugation or lyophilizing the botulinum toxin.

29. The method of claim 28, wherein the botulinum toxin comprises botulinum neurotoxin serotype A.
30. The method of claim 28 or 29, wherein the purified botulinum toxin obtained is free, essentially free, or substantially free of botulinum toxin complexes.
31. The method of any one of claims 28-30, wherein the purified botulinum toxin obtained is free, essentially free, or substantially free of animal products.
32. The method of any one of claims 28-31, wherein the purified botulinum toxin is free, essentially free, or substantially free of human albumin.
33. The method of any one of claims 28-32, wherein the first chromatography column comprises an anion exchange chromatography column.
34. The method of any one of claims 28-33, wherein the first chromatography column comprises Q Sepharose.
35. The method of any one of claims 28-34, wherein the second chromatography column comprises a cation exchange chromatography column.
36. The method of any one of claims 28-35, wherein the second chromatography column comprises SP Sepharose.
37. The method of claim any one of claims 28-36, wherein the first chromatography column comprises an anion exchange chromatography column and the second chromatography column comprises a cation exchange chromatography column.
38. The method of any one of claims 28-37, wherein the first chromatography column comprises Q Sepharose and the second chromatography column comprises SP Sepharose.
39. The method of any one of claims 28-38, wherein the third chromatography column comprises an anion exchange chromatography column.
40. The method of any one of claims 28-39, wherein the third chromatography column comprises Q Sepharose.
41. The method of any one of claims 28-40, wherein the fourth chromatography column comprises a gel filtration column.

42. The method of any one of claims 28-41, wherein the fourth chromatography column comprises Superdex 200.
43. The method of any one of claims 28-42, wherein the third chromatography column comprises an anion exchange chromatography column and the fourth chromatography column comprises a gel filtration chromatography column.
44. The method of any one of claims 28-43, wherein the third chromatography column comprises Q Sepharose and the fourth chromatography column comprises Superdex 200.
45. The method of any one of claims 28-44, wherein the second toxin-containing eluant is directly injected onto the fourth chromatography column.
46. The method of any one of claims 28-45, wherein the third chromatography column and the fourth chromatography column are interconnected.
47. The method of any one of claims 28-46, wherein the first chromatography column is an anion exchange chromatography column, the second chromatography column is a cation exchange chromatography column, the third chromatography column is a second anion exchange chromatography column, and the fourth chromatography column is a gel filtration chromatography column.
48. The method of any one of claims 28-47, wherein the first chromatography column comprises Q Sepharose, the second chromatography column comprises SP Sepharose, the third chromatography column comprises Q Sepharose, and the fourth chromatography column comprises Superdex 200.
49. The method of any one of claims 28-48, wherein the first, second, third, and fourth chromatography columns are single-use chromatography systems.
50. The method of any one of claims 28-49, wherein the filtering (f) dissociates botulinum toxin protein molecules from non-toxin proteins to produce free toxin molecules.
51. The method of any one of claims 28-50, wherein the filtering (f) comprises a buffer exchange.

52. The method of any one of claims 1-27, wherein the solution comprising botulinum toxin is an animal product free, essentially animal product free, or substantially animal product free fermentation medium.
53. The method of any one of claims 28-51, wherein the solution comprising botulinum toxin is an animal product free, essentially animal product free, or substantially animal product free fermentation medium.
54. The method of any one of claims 1-27, further comprising contacting the botulinum toxin with a buffer solution, wherein the buffer solution has been filtered to reduce bioburden.
55. The method of any one of claims 28-51, further comprising contacting the botulinum toxin with a buffer solution, wherein said buffer solution has been filtered to reduce bioburden.
56. A purified botulinum toxin produced according to the method of any one of claims 1 to 27.
57. The purified botulinum toxin of claim 56, wherein the botulinum toxin is serotype A.
58. The purified botulinum toxin of claim 56 or 57, wherein the purified botulinum toxin is free, essentially free, or substantially free of toxin complexes.
59. The purified botulinum toxin of any one of claims 56-58, wherein the purified botulinum toxin is free, essentially free, or substantially free of animal products.
60. The purified botulinum toxin of any one of claims 56-59, wherein the purified botulinum toxin is free, essentially free, or substantially free of human albumin.
61. A purified botulinum toxin produced according to the method of any one of claims 28-51.
62. The purified botulinum toxin of claim 61, wherein the toxin is serotype A.
63. The purified botulinum toxin of claim 61 or 62, wherein the purified botulinum toxin is free, essentially free, or substantially free of toxin complexes.

64. The purified botulinum toxin of any one of claims 61 to 63, wherein the purified botulinum toxin is free, essentially free, or substantially free of animal products.
65. The purified botulinum toxin of any one of claims 61-64, wherein the purified botulinum toxin is free, essentially free, or substantially free of human albumin.
66. A composition comprising purified botulinum toxin in a buffer, the buffer comprising phosphate.
67. The composition of claim 66, wherein the buffer further comprises acetate.
68. The composition of claim 66 or 67, wherein the buffer further comprises at least one source of chloride ions.
69. The composition of claim 68, wherein the at least one source of chloride ions is sodium chloride.
70. The composition of any one of claims 66-69, wherein the buffer further comprises at least one surfactant.
71. The composition of claim 70, wherein the at least one surfactant comprises polysorbate 20.
72. The composition of any one of claims 66-71, wherein the botulinum toxin is botulinum neurotoxin serotype A.
73. The composition any one of claims 66-72, wherein the composition is free, essentially free, or substantially free of botulinum toxin complexes.
74. The composition of any one of claims 66-73, wherein the composition is free, essentially free, or substantially free of animal products.
75. The composition of any one of claims 66-74, wherein the composition is free, essentially free, or substantially free of human albumin.
76. The composition of any one of claims 66-75, wherein the composition has a pH of between about 6.6 and 6.9.