JUDGE JONES

08 CIV 7611

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

TEVA PHARMACEUTICALS USA, INC.,

TEVA PHARMACEUTICAL INDUSTRIES LTD.,

TEVA NEUROSCIENCE, INC.,

and

YEDA RESEARCH AND DEVELOPMENT CO. LTD.,

Plaintiffs,

v.

SANDOZ, INC.,

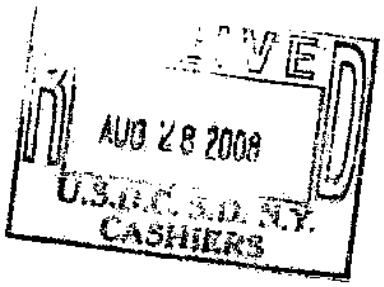
SANDOZ INTERNATIONAL GMBH,

NOVARTIS AG,

and

MOMENTA PHARMACEUTICALS, INC.

Defendants.



Civil Action No. _____

COMPLAINT

Plaintiffs Teva Pharmaceuticals USA, Inc., Teva Pharmaceutical Industries Ltd., Teva Neuroscience, Inc., and Yeda Research and Development Co. Ltd. ("Plaintiffs"), bring this action for patent infringement, trade secret misappropriation, unfair competition and declaratory judgment against Defendants Sandoz, Inc., Sandoz International GmbH, Novartis AG, and Momenta Pharmaceuticals, Inc. ("Defendants").

THE PARTIES

1. Teva Pharmaceuticals USA, Inc. ("Teva USA") is a Delaware corporation with its principal place of business at 1090 Horsham Road, North Wales, Pennsylvania 19454-1090.

2. Teva Pharmaceutical Industries Ltd. ("Teva Ltd.") is an Israeli company with its principal place of business at 5 Basel Street, P.O. Box 3190, Petah Tikva, 49131, Israel.

3. Teva Neuroscience, Inc. ("Teva Neuroscience"), is a Delaware corporation with its principal place of business at 901 E. 104th Street, Suite 900, Kansas City, MO 64131.

4. Yeda Research and Development Co. Ltd. ("Yeda") markets and commercializes new developments emerging from the laboratories of the Weizmann Institute of Science, and its principal place of business is at P.O. Box 95, Rehovot, 76100, Israel.

5. Upon information and belief, Sandoz, Inc., is a Colorado corporation with its principal place of business at 506 Carnegie Center, Suite 400, Princeton, NJ 08540, and is a wholly owned subsidiary of Novartis AG.

6. Upon information and belief, Sandoz, Inc., is doing business in the State of New York, including in this Judicial District. Sandoz, Inc., is registered to do business in New York, has designated Corporate Service Company at 80 State Street, Albany, New York, 12207-2543 for receipt of service, and maintains a place of business at 227-15 N. Conduit Avenue, Laurelton, New York 11413. Sandoz, Inc. has engaged in continuous and systematic contacts with the State of New York and purposefully availed itself of this forum by, among other things, making, shipping, using, offering to sell or selling, or causing others to use, offer to sell, or sell, pharmaceutical products in the State of New York including in this Judicial District and deriving revenue from such activities, and by filing claims and counterclaims in this Judicial District.

7. Upon information and belief, Sandoz International GmbH ("Sandoz International") is a German company with its principal place of business at Industriestrasse 25, 83607 Holzkirchen, Germany, and is a wholly owned subsidiary of Novartis AG. Upon information and belief, Sandoz International has engaged in continuous and systematic contacts with the State of New York and purposefully availed itself to this forum by doing business, directly or through its subsidiaries (including Sandoz, Inc.), including, among other things, by making, shipping, using, offering to sell or selling, or causing others to use, offer to sell, or sell, pharmaceutical products in the State of New York including in this Judicial District, and deriving revenue from such activities.

8. Upon information and belief, Novartis AG is a Swiss company with its principal place of business at Lichtestrasse 35, CH-4056 Basel, Switzerland. Upon information and belief, Novartis AG has engaged in continuous and systematic contacts with the State of New York and purposefully availed itself of this forum by doing business, directly or through its subsidiaries, including, among other things, by making, shipping, using, offering to sell or selling, or causing others to use, offer to sell, or sell, pharmaceutical products in the State of New York including in this Judicial District and deriving revenue from such activities, and by filing claims in this Judicial District.

9. Upon information and belief, Momenta Pharmaceuticals, Inc., ("Momenta") is a Delaware corporation with its principal place of business at 675 West Kendall Street, Cambridge, MA 02142. Upon information and belief, Momenta has engaged in continuous and systematic contacts with the State of New York and purposefully availed itself of this forum by doing business, including, among other things, soliciting business in the State of New York, including in this Judicial District.

JURISDICTION

10. This action for patent infringement arises under 35 U.S.C. § 271(e).

11. This Court has jurisdiction over Counts I-VIII of this action pursuant to 28 U.S.C. §§ 1331 and 1338(a), and the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202.

12. This Court has jurisdiction over Counts IX and X of this action pursuant to 28 U.S.C. §§ 1332, 1338(b), and 1367.

13. Venue is proper in this Judicial District under 28 U.S.C. § 1400(b) and § 1391.

14. This Court has personal jurisdiction over Sandoz, Inc., Sandoz International, Novartis AG, and Momenta under the New York long-arm statute, N.Y.C.P.L.R. § 301, et seq.

BACKGROUND

15. United States Patent No. 7,199,098 ("the '098 patent"), entitled "Copolymer-1 improvements in compositions of copolymers," was duly and legally issued to Yeda by the United States Patent and Trademark Office on April 3, 2007, and expires on May 24, 2014. A true and correct copy of the '098 patent is attached as Exhibit A. Since its date of issue, Yeda has been and still is the owner of that patent.

16. Teva Ltd. is the exclusive licensee of the '098 patent.

17. United States Patent No. 6,939,539 ("the '539 patent"), entitled "Copolymer-1 improvements in compositions of copolymers," was duly and legally issued to Yeda by the United States Patent and Trademark Office on September 6, 2005, and expires on May 24, 2014. A true and correct copy of the '539 patent is attached as Exhibit B. Since its date of issue, Yeda has been and still is the owner of that patent.

18. Teva Ltd. is the exclusive licensee of the '539 patent.

19. United States Patent No. 6,054,430 ("the '430 patent"), entitled "Copolymer-1 improvements in compositions of copolymers," was duly and legally issued to Yeda by the United States Patent and Trademark Office on April 25, 2000, and expires on May 24, 2014. A true and correct copy of the '430 patent is attached as Exhibit C. Since its date of issue, Yeda has been and still is the owner of that patent.

20. Teva Ltd. is the exclusive licensee of the '430 patent.

21. United States Patent No. 6,620,847 ("the '847 patent"), entitled "Copolymer-1 improvements in compositions of copolymers," was duly and legally issued to Yeda by the United States Patent and Trademark Office on September 16, 2003, and expires on May 24, 2014. A true and correct copy of the '847 patent is attached as Exhibit D. Since its date of issue, Yeda has been and still is the owner of that patent.

22. Teva Ltd. is the exclusive licensee of the '847 patent.

23. Plaintiffs researched, developed, applied for, obtained approval of, and market the glatiramer acetate product known around the world as Copaxone®.

24. Teva USA is the holder of a New Drug Application ("NDA") number 02-0622 approved by the United States Food and Drug Administration ("FDA") for the use of glatiramer acetate, marketed as Copaxone®, for reducing the frequency of relapses in patients with relapsing-remitting multiple sclerosis.

25. Upon information and belief, Sandoz, Inc., filed with the FDA, in Rockville, Maryland, an Abbreviated New Drug Application ("ANDA") under 21 U.S.C. § 355(j), to obtain approval for glatiramer acetate, for injection, 20 mg/mL, 1 mL pre-filled syringes, purported to be generic to Teva USA's Copaxone® ("Sandoz's generic glatiramer acetate product"). Upon information and belief, Sandoz, Inc., filed the ANDA, assigned ANDA No. 90-218 ("the Sandoz,

Inc. ANDA), to obtain approval to market Sandoz's generic glatiramer acetate product before the expiration of the '098, '539, '430 and '847 patents ("the patents in suit").

26. Upon information and belief, Sandoz, Inc. also filed with the FDA, pursuant to 21 U.S.C. § 355(j)(2)(A)(vii)(IV), a certification alleging that the claims of the patents in suit are invalid, unenforceable, and/or would not be infringed by the manufacture, use, importation, sale or offer for sale of Sandoz's generic glatiramer acetate product.

27. Upon information and belief, Momenta worked in active concert and participation with Sandoz, Inc. to manufacture Sandoz's generic glatiramer acetate product and to prepare the Sandoz, Inc. ANDA.

28. Sandoz, Inc., caused to be sent to Teva USA, Teva Ltd., Teva Neuroscience (collectively, "Teva"), and Yeda a letter ("the Notice Letter"), dated July 10, 2008, notifying them that Sandoz, Inc., had filed an ANDA for glatiramer acetate and was providing information to Teva pursuant to 21 U.S.C. § 355(j)(2)(B)(ii). Teva USA received the Notice Letter on or about July 14, 2008; Teva Ltd. received the Notice Letter on or about July 22, 2008; Teva Neuroscience received the Notice Letter on or about July 14, 2008; and Yeda received the Notice Letter on or about July 21, 2008.

SANDOZ, INC.'S FAILURE TO COMPLY WITH THE OFFER OF CONFIDENTIAL ACCESS PROVISIONS OF 21 U.S.C § 355 (j)

29. In its Notice Letter, Sandoz, Inc., purported to offer Teva and Yeda confidential access to portions of the Sandoz, Inc. ANDA for the purpose of determining whether to bring an action with respect to the patents in suit, among other patents listed in the Orange Book ("Sandoz's Offer of Confidential Access").

30. Sandoz's Offer of Confidential Access, however, contained far stricter restrictions as to persons entitled to access than would apply had a protective order been entered for the

purpose of protecting trade secrets and other confidential business information. With respect to outside counsel, Sandoz, Inc. limited access to only two designated attorneys at a single outside law firm not directly or indirectly involved in the prosecution of any patent applications relating to glatiramer acetate and/or the regulatory approval of glatiramer acetate. In addition, Sandoz, Inc. required as a condition for access that the two designated outside attorneys be specifically identified and that they enter into an agreement that contains confidentiality and non-use obligations.

31. Sandoz, Inc. refused to remove these unduly restrictive provisions on outside counsel despite Teva's requests.

32. Sandoz, Inc. refused to provide access to relevant portions of the Sandoz, Inc. ANDA, despite requests from Teva to do so and Teva's willingness to agree to reasonable restrictions on access to such information.

33. Sandoz, Inc. also refused to provide access to the Drug Master File ("DMF"), if any, relating to the active pharmaceutical ingredient ("API") in Sandoz's generic glatiramer acetate product. Sandoz, Inc. further refused to provide samples of the API and Sandoz's generic glatiramer acetate product.

34. Sandoz's Offer of Confidential Access failed to satisfy the requirements of 21 U.S.C. §355(j)(5)(C)(i)(I)(cc) and 21 U.S.C. §355(j)(5)(C)(i)(III).

COUNT I FOR INFRINGEMENT OF UNITED STATES PATENT NO. 7,199,098

35. The allegations of proceeding paragraphs 1-34 are realleged and incorporated herein by reference.

36. Under 35 U.S.C. § 271(e)(2)(A), Sandoz, Inc.'s, submission to the FDA of its ANDA No. 90-218 to obtain approval for Sandoz's generic glatiramer acetate product before the

expiration of the '098 patent constitutes an act of infringement of the '098 patent, and if approved, Sandoz, Inc.'s commercial manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product would infringe one or more claims of the '098 patent under at least sections (a)-(c) of 35 U.S.C. § 271.

37. Upon information and belief, Sandoz International, Novartis AG, and Momenta have, under 35 U.S.C. § 271(b), acted in concert, actively supporting, participating in, encouraging, and inducing Sandoz, Inc.'s, filing of ANDA No. 90-218 for glatiramer acetate, and in the preparation to sell, in the United States, pharmaceutical products containing glatiramer acetate.

COUNT II FOR INFRINGEMENT OF UNITED STATES PATENT NO. 6,939,539

38. The allegations of paragraphs 1-37 are realleged and incorporated herein by reference.

39. Under 35 U.S.C. § 271(e)(2)(A), Sandoz, Inc.'s, submission to the FDA of its ANDA No. 90-218 to obtain approval for Sandoz's generic glatiramer acetate product before the expiration of the '539 patent constitutes an act of infringement of the '539 patent, and if approved, Sandoz, Inc.'s commercial manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product would infringe one or more claims of the '539 patent under at least sections (a)-(c) of 35 U.S.C. § 271.

40. Upon information and belief, Sandoz International, Novartis AG, and Momenta have, under 35 U.S.C. § 271(b), acted in concert, actively supporting, participating in, encouraging, and inducing Sandoz, Inc.'s, filing of ANDA No. 90-218 for glatiramer acetate, and in the preparation to sell, in the United States, pharmaceutical products containing glatiramer acetate.

COUNT III FOR INFRINGEMENT OF UNITED STATES PATENT NO. 6,054,430

41. The allegations of paragraphs 1-40 are realleged and incorporated herein by reference.

42. Under 35 U.S.C. § 271(e)(2)(A), Sandoz, Inc.'s, submission to the FDA of its ANDA No. 90-218 to obtain approval for Sandoz's generic glatiramer acetate product before the expiration of the '430 patent constitutes an act of infringement of the '430 patent, and if approved, Sandoz, Inc.'s commercial manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product would infringe one or more claims of the '430 patent under at least sections (a)-(c) of 35 U.S.C. § 271.

43. Upon information and belief, Sandoz International, Novartis AG, and Momenta have, under 35 U.S.C. § 271(b), acted in concert, actively supporting, participating in, encouraging, and inducing Sandoz, Inc.'s, filing of ANDA No. 90-218 for glatiramer acetate, and in the preparation to sell, in the United States, pharmaceutical products containing glatiramer acetate.

COUNT IV FOR INFRINGEMENT OF UNITED STATES PATENT NO. 6,620,847

44. The allegations of paragraphs 1-43 are realleged and incorporated herein by reference.

45. Under 35 U.S.C. § 271(e)(2)(A), Sandoz, Inc.'s, submission to the FDA of its ANDA No. 90-218 to obtain approval for Sandoz's generic glatiramer acetate product before the expiration of the '847 patent constitutes an act of infringement of the '847 patent, and if approved, Sandoz, Inc.'s commercial manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product would infringe one or more claims of the '847 patent under at least sections (a)-(c) of 35 U.S.C. § 271.

46. Upon information and belief, Sandoz International, Novartis AG, and Momenta have, under 35 U.S.C. § 271(b), acted in concert, actively supporting, participating in, encouraging, and inducing Sandoz, Inc.'s, filing of ANDA No. 90-218 for glatiramer acetate, and in the preparation to sell, in the United States, pharmaceutical products containing glatiramer acetate.

COUNT V FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF UNITED STATES PATENT NO. 7,199,098

47. The allegations of paragraphs 1-46 are realleged and incorporated herein by reference.

48. Upon information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Sandoz's generic glatiramer acetate product soon after FDA approval.

49. Such conduct will constitute direct infringement of one or more claims of the '098 patent under 35 U.S.C. § 271(a), inducement of infringement of the '098 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

50. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the ANDA.

51. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Plaintiffs and Defendants as to liability for the infringement of the '098 patent. Defendants' actions have created in Plaintiffs a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

COUNT VI FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF UNITED STATES PATENT NO. 6,939,539

52. The allegations of paragraphs 1-51 are realleged and incorporated herein by reference.

53. Upon information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Sandoz's generic glatiramer acetate product soon after FDA approval.

54. Such conduct will constitute direct infringement of one or more claims of the '539 patent under 35 U.S.C. § 271(a), inducement of infringement of the '539 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

55. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the ANDA.

56. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Plaintiffs and Defendants as to liability for the infringement of the '539 patent. Defendants' actions have created in Plaintiffs a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

COUNT VII FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF UNITED STATES PATENT NO. 6,054,430

57. The allegations of paragraphs 1-56 are realleged and incorporated herein by reference.

58. Upon information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Sandoz's generic glatiramer acetate product soon after FDA approval.

59. Such conduct will constitute direct infringement of one or more claims of the '430 patent under 35 U.S.C. § 271(a), inducement of infringement of the '430 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

60. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the ANDA.

61. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Plaintiffs and Defendants as to liability for the infringement of the '430 patent. Defendants' actions have created in Plaintiffs a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

COUNT VIII FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF UNITED STATES PATENT NO. 6,620,847

62. The allegations of paragraphs 1-61 are realleged and incorporated herein by reference.

63. Upon information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Sandoz's generic glatiramer acetate product soon after FDA approval.

64. Such conduct will constitute direct infringement of one or more claims of the '847 patent under 35 U.S.C. § 271(a), inducement of infringement of the '847 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

65. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the ANDA.

66. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Plaintiffs and Defendants as to liability for the infringement of

the '847 patent. Defendants' actions have created in Plaintiffs a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

COUNT IX FOR MISAPPROPRIATION OF TRADE SECRETS
(Asserted by Teva Ltd. Against Sandoz, Inc., Sandoz International, and Novartis AG)

67. The allegations of paragraphs 1-66 are realleged and incorporated herein by reference.

68. On or about May 7, 1997, Teva Ltd. entered into a Supply and Distribution Agreement with Lek Pharmaceutical and Chemical Company d.d. ("Lek"), pursuant to which Teva Ltd. appointed Lek as its exclusive distributor in certain specified countries for its glatiramer acetate product (the "Teva-Lek Agreement").

69. Upon information and belief, Lek is, and has been, in the business of developing and manufacturing pharmaceutical products, and operates as a supply center for the markets of Central and Eastern Europe, South Eastern Europe, and the Commonwealth of Independent States.

70. Pursuant to the terms of the Teva-Lek Agreement, Lek agreed on behalf of itself and its affiliates, including entities controlling or under common control with Lek, to keep secret, *inter alia*, all knowledge, data and information received from Teva Ltd. relating to the Teva-Lek Agreement, and all information concerning Teva Ltd.'s glatiramer acetate product, for a period of ten years following termination of the Teva-Lek Agreement. The Teva-Lek Agreement was terminated on January 22, 2005.

71. Teva Ltd.'s trade secrets pertaining to its glatiramer acetate product were, and continue to be, of significant economic value to Teva Ltd., and divulgence of its trade secrets to competitors would subject Teva Ltd. to severe economic detriment. Thus, Teva Ltd. has taken, and continues to take, every precaution to prevent the disclosure of trade secrets pertaining to its

glatiramer acetate product. This information is not in the public domain and cannot be readily discovered.

72. In connection with the Teva-Lek Agreement, Teva Ltd. disclosed to Lek over a period of several years extensive and highly confidential information concerning its glatiramer acetate product, including, but not limited to, detailed information concerning its manufacturing process and analytical testing methodology. Such confidential information concerning Teva Ltd.'s glatiramer acetate product constitutes trade secrets owned by Teva Ltd.

73. By April of 2002, Teva Ltd. had sent to Lek substantial parts of its European Marketing Authorization Application containing thousands of pages of highly confidential information concerning the manufacture and characterization of Copaxone®, Teva Ltd.'s brand of glatiramir acetate, which gave a complete blueprint of how to characterize Copaxone®, and, thus, a generic version of Copaxone®. These portions of the application contained numerous and valuable trade secrets of Teva Ltd.

74. Copaxone® is a very complex mixture of polypeptides that has not been completely characterized. Copaxone® requires a consistent and well-controlled manufacturing process and scientifically convincing and validated characterization tests to establish its chemical, pharmacological, immunological, and biological activities. It has unique mechanisms of action and unique pharmacologic characteristics which prevent the use of pharmacokinetic or pharmacodynamic studies alone to establish comparable bioequivalence or bioavailability. Thus Copaxone® is extremely difficult to duplicate, and the trade secrets provided by Teva Ltd. to Lek would greatly facilitate the development of a generic version of Copaxone®.

75. Upon information and belief, at the time Teva Ltd. provided its trade secrets to Lek, Lek was a relatively small generic drug company operating principally in Eastern Europe,

but with limited operations in the major markets in which Teva Ltd., Teva USA and Teva Neuroscience operated, including the United States.

76. Upon information and belief, Novartis AG acquired Lek in November of 2002. Novartis AG was at that time, and is today, one of the largest pharmaceutical companies in the world, and was, and is, one of Teva Ltd.'s chief competitors both inside and outside of the United States.

77. Upon information and belief, since the acquisition of Lek, pharmaceutical development and manufacturing strategy is freely shared and communicated by and between Lek and Sandoz, Inc., Sandoz International and Novartis AG.

78. Upon information and belief, Lek disclosed the trade secrets of Teva Ltd. referred to in paragraphs 72 and 73 above to Sandoz, Inc., Sandoz International and Novartis AG without the express or implied consent of Teva Ltd.

79. Upon information and belief, Sandoz, Inc., Sandoz International and Novartis AG unlawfully caused Lek to disclose Teva Ltd.'s trade secrets to them or to someone acting for them or on their behalf, or to a third party which later disclosed the information to one of them.

80. Upon information and belief, Sandoz, Inc., Sandoz International and Novartis AG used Teva Ltd.'s trade secrets to develop Sandoz's generic glatiramer acetate product and to prepare and file the Sandoz, Inc. ANDA, without the express or implied consent of Teva Ltd.

81. Teva USA's NDA for Copaxone® was first approved on December 20, 1996, and an ANDA could have been filed as early as December 20, 2000. However, on information and belief, no ANDA was accepted for filing by the FDA until Sandoz filed its ANDA in December 2007.

82. Upon information and belief, Sandoz, Inc. was the first, and thus far the only, company to file an ANDA for a generic glatiramir acetate product.

83. Upon information and belief, Sandoz, Inc. was able to file its ANDA before any of its generic pharmaceutical competitors because of the misappropriation of Teva Ltd.'s trade secrets by Sandoz, Inc., Sandoz International and Novartis AG.

84. Upon information and belief, at the time they received Teva Ltd.'s trade secrets, and/or at the time use was made of these trade secrets, Sandoz, Inc., Sandoz International and Novartis AG knew or had reason to know that Lek and its affiliates owed a duty to Teva Ltd. to maintain the secrecy of the confidential information and prevent any use or disclosure.

85. Upon information and belief, Sandoz, Inc., Sandoz International and Novartis AG were aware of the Teva-Lek Agreement prior to and concurrently with the development of Sandoz's generic glatiramer acetate product and the preparation and filing of Sandoz, Inc.'s ANDA.

86. Upon information and belief, Sandoz, Inc., Sandoz International and Novartis AG misappropriated such trade secrets of Teva Ltd. in breach of a confidential relationship.

87. Upon information and belief, Sandoz, Inc., Sandoz International and Novartis AG were unjustly enriched by their misappropriation of Teva Ltd.'s trade secrets.

88. Teva Ltd. has suffered, and will continue to suffer, damages as a result of Sandoz, Inc.'s, Sandoz International's and Novartis AG's misappropriation of Teva Ltd.'s trade secrets.

89. Upon information and belief, Sandoz, Inc.'s, Sandoz International's and Novartis AG's misappropriation of Teva Ltd.'s trade secrets was willful.

COUNT X FOR UNFAIR COMPETITION
(Asserted by Teva Ltd. Against Sandoz, Inc., Sandoz International, and Novartis AG)

90. The allegations of paragraphs 1-89 are realleged and incorporated herein by reference.

91. The allegations of paragraphs 1-90 constitute, and state a cause of action for, unfair competition by Sandoz, Inc., Sandoz International and Novartis AG as contemplated by 28 U.S.C. § 1338(b).

WHEREFORE, Plaintiffs demand judgment against Defendants as follows:

(a) declaring that the '098 patent is valid and enforceable;

(b) declaring that Defendants have infringed one or more claims of the '098 patent by the filing of ANDA No. 90-218 and would infringe one or more of the claims of the '098 patent by the threatened acts of importation, manufacture, use, offering to sell and sale of Sandoz's generic glatiramer acetate product prior to the expiration of said patent;

(c) declaring that the '539 patent is valid and enforceable;

(d) declaring that Defendants have infringed one or more claims of the '539 patent by the filing of ANDA No. 90-218 and would infringe one or more of the claims of the '539 patent by the threatened acts of importation, manufacture, use, offering to sell and sale of Sandoz's generic glatiramer acetate product prior to the expiration of said patent;

(e) declaring that the '430 patent is valid and enforceable;

(f) declaring that Defendants have infringed one or more claims of the '430 patent by the filing of ANDA No. 90-218 and would infringe one or more of the claims of the '430 patent by the threatened acts of importation, manufacture, use, offering

to sell and sale of Sandoz's generic glatiramer acetate product prior to the expiration of said patent;

(g) declaring that the '847 patent is valid and enforceable;

(h) declaring that Defendants have infringed one or more claims of the '847 patent by the filing of ANDA No. 90-218 and would infringe one or more of the claims of the '847 patent by the threatened acts of importation, manufacture, use, offering to sell and sale of Sandoz's generic glatiramer acetate product prior to the expiration of said patent;

(i) ordering that the effective date of the approval of Sandoz's generic glatiramer acetate product shall not be before the date of expiration of the patents in suit, in accordance with 35 U.S.C. § 271(e)(4)(A);

(j) enjoining Defendants from the commercial manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product, in accordance with 35 U.S.C. § 271(e)(4)(B);

(k) awarding Plaintiffs damages or other monetary relief in accordance with 35 U.S.C. § 271(e)(4)(C) to compensate Plaintiffs for any and all commercial manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product prior to the expiration of the patents in suit;

(l) declaring this to be an exceptional case and awarding Plaintiffs attorney's fees under 35 U.S.C. §§ 285 and 271(e)(4);

(m) in the event that Sandoz, Inc. obtains final approval for Sandoz's generic glatiramer acetate product prior to judgment being entered in this action, enjoining, including preliminarily enjoining, Defendants from the commercial

manufacture, use, offer to sell, sale, or importation of Sandoz's generic glatiramer acetate product in the U.S. before the date of expiration of the patents in suit in accordance with 35 U.S.C. § 283;

(n) Entering judgment for Teva Ltd. on Counts IX and X of this Complaint;

(o) Ordering Sandoz, Inc. to withdraw its ANDA No. 90-218;

(p) Entering preliminary and permanent judgment enjoining Sandoz, Inc., Sandoz International, Novartis AG and Momenta from (a) using or disclosing Teva Ltd.'s confidential information or (b) using, for any purpose, any part of, or any information contained in, or developed for, ANDA No. 90-218, which was based in whole or in part on Teva Ltd's confidential information;

(q) Awarding Teva Ltd. monetary and exemplary damages, together with interest, to compensate it for Sandoz, Inc.'s, Sandoz International's and Novartis AG's misappropriation of Teva Ltd.'s trade secrets; and

(r) Awarding Plaintiffs any further and additional relief as this Court deems just and proper.

Dated: August 28, 2008

Respectfully submitted,

By: _____

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Exhibit A


US007199098B2

(12) **United States Patent**
Konfino et al.

(10) **Patent No.: US 7,199,098 B2**
(45) **Date of Patent: *Apr. 3, 2007**

(54) **COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS**

(75) Inventors: **Eliezer Konfino**, Ramat Gan (IL); **Michael Sela**, Rehovot (IL); **Dvora Teitelbaum**, Rehovot (IL); **Ruth Arnon**, Rehovot (IL)

(73) Assignee: **Yeda Research and Development Co., Ltd.**, Rehovot (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **11/098,432**

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to an improved composition of copolymer-1 comprising copolymer-1 substantially free of species having a molecular weight of over 40 kilodaltons.

20 Claims, 2 Drawing Sheets

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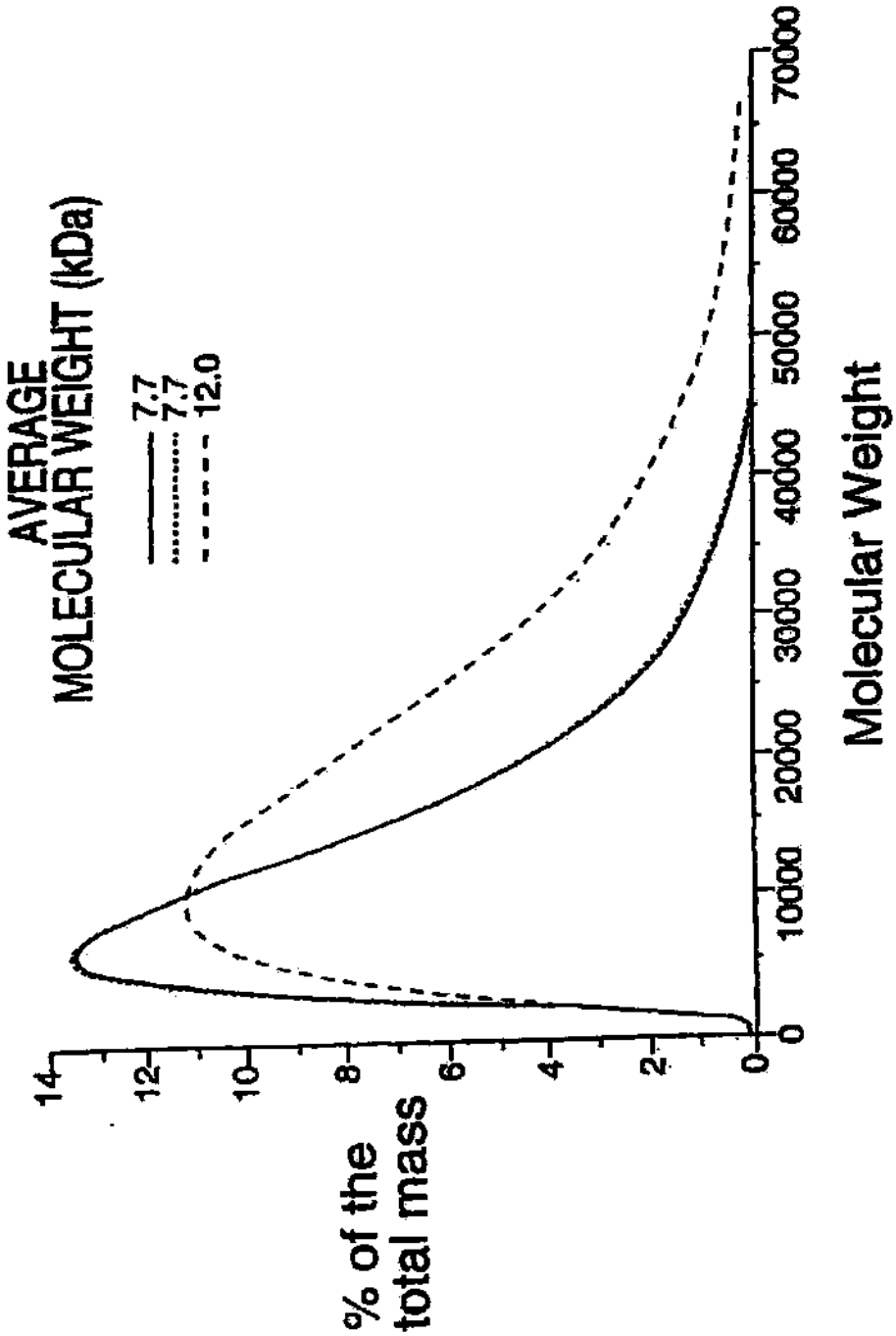
AVERAGE MOLECULAR WEIGHT (kDa)
7.7
7.7
12.0
% of the total mass
0
2
4
6
8
10
12
14
0
10000
20000
30000
40000
50000
60000
70000
Molecular Weight

FIG. 1

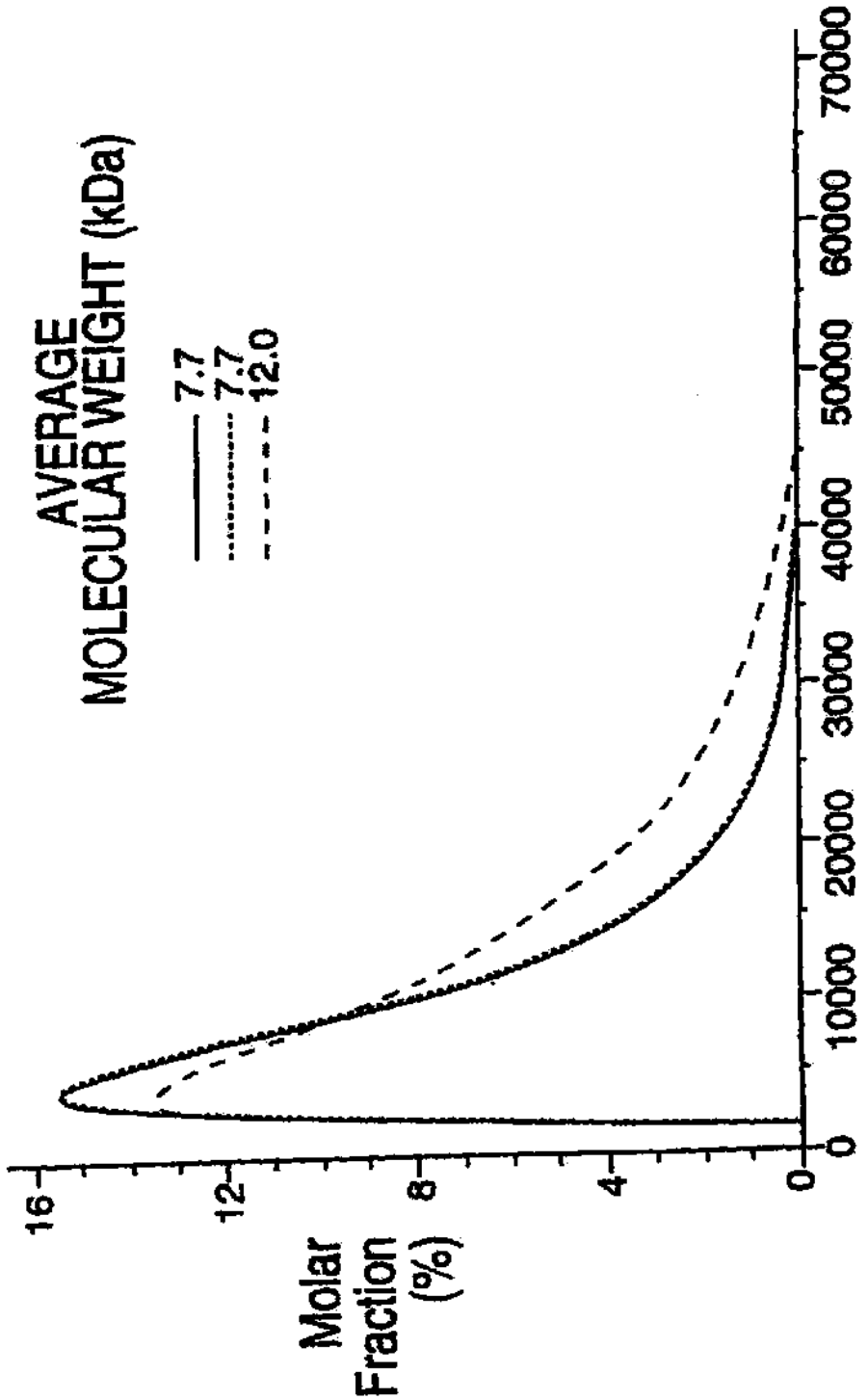
AVERAGE MOLECULAR WEIGHT (kDa)
7.7
7.7
12.0
Molar Fraction (%)
16
12
8
4
0
0
10000
20000
30000
40000
50000
60000
70000
Molecular Weight

FIG. 2

COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS

This application is a continuation application of U.S. Ser. No. 10/615,865, filed Jul. 10, 2003, now U.S. Pat. No. 6,939,539, which is a continuation of application U.S. Ser. No. 10/014,477, filed Dec. 14, 2001, now U.S. Pat. No. 6,620,847, which is a continuation of application Ser. No. 09/510,466, filed Feb. 22, 2000, now U.S. Pat. No. 6,362,161, which is a continuation of U.S. Ser. No. 09/032,334 filed Feb. 27, 1998, now U.S. Pat. No. 6,048,898, which is a continuation of U.S. Ser. No. 08/447,146, filed May 22, 1995, now U.S. Pat. No. 5,800,808, which is a continuation-in-part of U.S. Ser. No. 08/344,248, filed Nov. 23, 1994, now abandoned, which is a continuation of U.S. Ser. No. 08/248,037, filed May 24, 1994, now abandoned.

BACKGROUND OF THE INVENTION

Copolymer-1 is a synthetic polypeptide analog of myelin basic protein (MBP), which is a natural component of the myelin sheath. It has been suggested as a potential therapeutic agent for multiple sclerosis (Eur. J. Immunol. [1971] 1:242; and J. Neurol. Sci. [1977] 31:433). All references cited herein are hereby incorporated by reference in their entirety. Interest in copolymer-1 as an immunotherapy for multiple sclerosis stems from observations first made in the 1950's that myelin components such as MBP prevent or arrest experimental autoimmune encephalomyelitis (EAE). EAE is a disease resembling multiple sclerosis that can be induced in susceptible animals.

Copolymer-1 was developed by Drs. Sela, Arnon, and their co-workers at the Weizmann Institute (Rehovot, Israel). It was shown to suppress EAE (Eur. J. Immunol. [1971] 1:242; U.S. Pat. No. 3,849,550). More recently, copolymer-1 was shown to be beneficial for patients with the exacerbating-remitting form of multiple sclerosis (N. Engl. J. Med. [1987] 317:408). Patients treated with daily injections of copolymer-1 had fewer exacerbations and smaller increases in their disability status than the control patients.

Copolymer-1 is a mixture of polypeptides composed of alanine, glutamic acid, lysine, and tyrosine in a molar ratio of approximately 6:2:5:1, respectively. It is synthesized by chemically polymerizing the four amino acids forming products with average molecular weights of 23,000 daltons (U.S. Pat. No. 3,849,550).

It is an object of the present invention to provide an improved composition of copolymer-1.

SUMMARY OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa).

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa.

Moreover, the invention relates to a pharmaceutical composition and a method for the treatment of multiple sclerosis, using the above-discussed copolymer-1.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 displays the molecular weight distribution of three batches of copolymer-1, showing the proportion of species with molecular weight above 40 KDa. FIG. 2 shows similar data relating to the molar fraction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa). Preferably, the composition contains less than 5% of species of copolymer-1 having a molecular weight of 40 KDa or more. More preferably, the composition contains less than 2.5% of species of copolymer-1 having a molecular weight of 40 KDa, or more.

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa. In particular, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8 KDa and a copolymer-1 having an average molecular weight of about 6.25 to about 8.4 KDa.

Copolymer-1, according to the present invention, may be prepared by methods known in the art, for example, the process disclosed in U.S. Pat. 3,849,550, wherein the N-carboxyanhydrides of tyrosine, alanine, γ-benzyl glutamate and ε-N-trifluoro-acetyllysine are polymerised at ambient temperature in anhydrous dioxane with diethylamine as initiator. The deblocking of the γ-carboxyl group of the glutamic acid is effected by hydrogen bromide in glacial acetic acid and is followed by the removal of the trifluoroacetyl groups from the lysine residues by 1M piperidine. For the purposes of the application, the terms "ambient temperature" and "room temperature" should be understood to mean a temperature ranging from about 20 to about 26° C.

The copolymer-1 with the required molecular weight profile can be obtained either by methods known per se. Such methods include chromatography of copolymer-1 containing high molecular weight species and collecting the fractions without the undesired species or by partial acid or enzymatic hydrolysis to remove the high molecular weight species with subsequent purification by dialysis or ultrafiltration. A further method to obtain copolymer-1 with the desired molecular weight profile is by preparing the desired species while the amino acids are still protected and then obtain the correct species directly upon removing the protection. The compositions of the present invention may be formulated by conventional methods known in the art. Preferably, the composition is lyophilized and formed into an aqueous solution suitable for sub-cutaneous injection. Alternatively, copolymer-1 may be formulated in any of the forms known in the art for preparing oral, nasal, buccal, or rectal formulations of peptide drugs.

Typically, copolymer-1 is administered daily to patients suffering from multiple sclerosis at a dosage of 20 mg.

The invention will be exemplified but not necessarily limited by the following Examples.

EXAMPLE 1

Chromatographic method of preparation of low-toxicity copolymer-1 Two batches of copolymer-1 were prepared according to the methods known in the art, for example, U.S. Pat. No. 3,849,550.

One batch was then subjected to chromatographic separation, as described below.

A column for gel filtration, FRACTOGEL TSK HW55 (600×26 mm) was prepared in a Superformance 26 Merck cartridge according to the manufacturer's instructions. The column was equilibrated with water and acetone solution was injected for total volume determination. The column was equilibrated with 0.2M ammonium acetate buffer pH 5.0. 30 ml copolymer-1 samples (20 mg/ml, in 0.2M ammonium acetate pH 5.0) were loaded on the column and fractions were collected every 10 minutes. A fraction having an average molecular weight of 7–8 KDa was isolated between 120–130 minutes (Batch A).

Molecular Weight Analysis

UV absorbance at 275 nm was determined in a UVIKON 810 spectrophotometer. Samples were diluted to obtain a UV absorbance lower than 1 Absorption Unit. The molecular distribution of the 2 batches was determined on a calibrated gel filtration column (Superose 12).

Copolymer-1 batch A was found to have an average molecular weight of 7–8 KDa. 2.5% of this batch had a molecular weight above 32 KDa, but no copolymer-1 species present in this batch had a molecular weight of over 40 KDa.

The other batch of copolymer-1 which was not subjected to chromatography, had an average molecular weight of 12 KDa. 2.5% of the batch had a molecular weight above 42 KDa and 5% of the total copolymer-1 species in this batch had a molecular weight of over 40 KDa.

EXAMPLE 2

Toxicity Analysis

A: In Vivo

Three batches of copolymer-1 having an average molecular weight of 7.3 and 8.4 KDa (less than 2.5% copolymer-1 species over 40 KDa) and 22 KDa (more than 5% copolymer-1 species over 40 KDa) were subjected to the toxicity test described below. In each case 5 mice were used in each experimental group.

Method

Copolymer-1 was dissolved in distilled water to yield a solution of 2 mg/ml of the active ingredient. Each mouse was injected with 0.5 ml of the test solution into the lateral tail vein. Mice were observed for mortality and relevant clinical signs over a 48 hour period. Observations were recorded 10 minutes, 24 hours and 48 hours post-injection. If, at the end of 48 hours, all the animals were alive and no adverse signs had been observed, then the batch was designated "non-toxic". If, however, one or more of the mice had died or had shown adverse signs, then the batch was designated "toxic".

The batches with the average molecular weight of 7.3 and 8.4 KDa were both designated "non-toxic", whereas in the batch with the average molecular weight of 22 KDa, 3 out of 5 mice had died at the end of 48 hours, and it was consequently designated "toxic".

B: In Vitro

RBL—Degranulation Test

I. Introduction

Histamine (or serotonin) release from basophile is an in vitro model for immediate hypersensitivity. The Rat Basophilic Leukemia cell line (RBL-2H$_3$) was developed and characterized as a highly sensitive, uniform, easy to maintain in culture and reproducible system (E. L. Basumian, C. Isersky, M. G. Petrino and R. P. Siraganian. Eur. J. Immunol. 11, 317 (1981)). The physiological stimulus for histamine release involves binding of the antigen to membrane-bound IgE molecules, resulting in the latter's cross-linking and the consequent triggering of an intricate biochemical cascade. Beside these physiological, immunoglobulin-mediated triggers, degranulation can be induced by different non-IgE-mediated stimuli. Among these are various peptides and synthetic polymers, e.g. polylysine (R. P. Siraganian. Trends in Pharmacological Sciences, October 432 (1983)). The RBL degranulation testis, therefore, used in order to screen out those batches of copolymer-1 which evoke substantial degranulation and thus might elicit undesirable local and/or systemic side effects.

II. Principle of the Test Method

Rat Basophilic Leukemia cells (RBL-2H$_3$), are loaded with [^{3}H]-serotonin, followed by incubation with 100 μg of the copolymer-1 to be tested. Batches of copolymer-1 which induce non-specific degranulation, release [^{3}H]-serotonin into the medium. The radioactivity in the medium is counted by a scintillation counter and the total radiolabeled serotonin incorporated into the cells is determined in the pelleted cells. Percent degranulation is calculated as the percentage of serotonin released out of the total incorporated.

III. Results

Four batches of copolymer-1, with average molecular weight between 6,250–14,500 were analyzed for both % of the species with molecular weight over 40 KDa and for degranulation of RBL's. Results are summarized in the following table.

Average M.W. (Daltons)	% of species with M.W. over 40 KDa	% Serotonin Release
6,250	<2.5	12.4
7,300	<2.5	21.0
13,000	>5	66.9
14,500	>5	67.8

As can be seen, when the % of high molecular weight species is low (<2.5), the % release of serotonin, indicative of toxicity, is low, and vice versa.

EXAMPLE 3

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), γ-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried. The removal of the gamma-benzyl blocking groups from the glutamate residue is carried out by treating the protected copolymer-1 with 33% hydrobromic acid in glacial acetic acid at room temperature for 6–12 hours with stirring. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

EXAMPLE 4

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), γ-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried.

Protected copolymer-1 is treated with 33% HBr in acetic acid which removes the omega benzyl protecting group from the 5-carboxylate of the glutamate residue and cleaves the polymer to smaller polypeptides. The time needed for obtaining copolymer-1 of molecular weight 7,000±2,000 Da depends on the reaction temperature and the size of protected copolymer-1. At temperatures of between 20–28° C. a test reaction is performed on every batch at different time periods for example, from 10–50 hours.

The results concerning the molecular weights of these small scale reactions are calculated and a curve of molecular weight against time is drawn. The time needed for obtaining molecular-weight 7,000±2,000 Da is calculated from the curve and performed on larger scale reaction. On average, working at 26° C. the time period is 17 hours. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

Preparation of Low-toxicity Copolymer-1

20 g of trifluoroacetyl-copolymer-1 are dispersed in 1 liter of water to which 100 g piperidine are added. The mixture is stirred for 24 hours at room temperature and filtered. The solution of crude copolymer-1 is distributed into dialysis bags and dialyzed at 10°–20° C. against water until a pH=8 is attained. It is then dialyzed against about 0.3% acetic acid and again water until a pH=5.5–6.0 is obtained. This solution is then concentrated and lyophilized to dryness.

The invention claimed is:

1. A copolymer-1 composition comprising a mixture of copolymers of alanine, glutamic acid, lysine and tyrosine, the copolymer species in the mixture being non-uniform with respect to molecular weight and sequence, wherein over 75% of the copolymers in the mixture, on a molar fraction basis, have a molecular weight in the range of 2 kDa to 20 kDa and less than 5% of the copolymers have a molecular weight above 40 kDa, and wherein the composition is suitable for treating multiple sclerosis.

2. The composition of claim 1, wherein the mixture has an average molecular weight of 7000 Da±2000 Da.

3. The composition of claim 1, wherein the mixture has an average molecular weight of 4 to 8.6 kDa.

4. The composition of claim 1, wherein the mixture has an average molecular weight of 6.25 to 8.4 kDa.

5. The composition of claim 1, wherein the mixture has an average molecular weight of 4 to 8 kDa.

6. The composition of claim 1, wherein the mixture has an average molecular weight of 7 to 8 kDa.

7. The composition of claim 1, wherein the mixture has a molecular weight distribution as depicted in FIG. 1 for the mixture with an average molecular weight of 7.7 kDa.

8. The composition of claim 1, wherein less than 2.5% of the copolymers in the mixture have a molecular weight above 40 kDa.

9. The composition of claim 8, wherein the mixture has an average molecular weight of 7000 Da±2000 Da.

10. The composition of claim 8, wherein the mixture has an average molecular weight of 4 to 8.6 kDa.

11. The composition of claim 8, wherein the mixture has an average molecular weight of 6.2 5 to 8.4 kDa.

12. The composition of claim 8, wherein the mixture has an average molecular weight of 4 to 8 kDa.

13. The composition of claim 8, wherein the mixture has an average molecular weight of 7 to 8 kDa.

14. The composition of claim 8, wherein the mixture has a molecular weight distribution as depicted in FIG. 1 for the mixture with an average molecular weight of 7.7 kDa.

15. A process for preparing a copolymer-1 composition having an average molecular weight of 4 to about 8.6 kDa comprising:

polymerizing a mixture of an N-carboxyanhydride of tyrosine, an N-carboxyanhydride of alanine, γ-benzyl glutamate and trifluoroacetyllysine in a molar ratio of 1:5:2:4 to form a mixture of protected polypeptides;

deprotecting the protected polypeptides to form a mixture of unprotected polypeptides; and

isolating a copolymer-1 composition of unprotected polypeptides composed of glutamic acid, lysine, alanine and tyrosine, wherein the copolymer-1 composition has an average molecular weight of 4 to 9 kDa.

16. The process of claim 15, wherein the copolymer-1 composition has an average molecular weight of 6.25 to 8.4 kilodaltons.

17. A copolymer-1 composition having an average molecular weight of about 4 to about 8.6 kDa preparable by a process comprising:

polymerizing a mixture of an N-carboxyanhydride of tyrosine, an N-carboxyanhydride of alanine, γ-benzyl glutamate and trifluoroacetyllysine in a molar ratio of 1:5:2:4 to form a mixture of protected polypeptides;

deprotecting the protected polypeptides to form a mixture of unprotected polypeptides; and

isolating a mixture of unprotected polypeptides composed of glutamic acid, lysine, alanine and tyrosine, wherein the mixture has an average molecular weight of 4 to 9 kDa.

18. The composition of claim 17, wherein the mixture has an average molecular weight of 6.25 to 8.4 kilodaltons.

19. A copolymer-1 composition comprising a mixture of polypeptides composed of glutamic acid, lysine, alanine and tyrosine, wherein the mixture has an average molecular weight of 4 to about 8.6 kilodaltons, wherein the mixture of polypeptides is non-uniform with respect to molecular weight and sequence, and wherein the composition exhibits lower toxicity than a copolymer-1 composition having an average molecular weight greater than about 8.6 kilodaltons.

20. The composition of claim 19, wherein the mixture has an average molecular weight of 6.25 to 8.4 kilodaltons.

* * * * *

Exhibit B

US006939539B2

(12) **United States Patent**
Konfino et al.

(10) **Patent No.: US 6,939,539 B2**
(45) **Date of Patent: Sep. 6, 2005**

(54) **COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS**

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(21) Appl. No.: **10/615,865**

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Related U.S. Application Data

(63) Continuation of application No. 10/014,477, filed on Dec. 14, 2001, now Pat. No. 6,620,847, which is a continuation of application No. 09/510,466, filed on Feb. 22, 2000, now Pat. No. 6,362,161, which is a continuation of application No. 09/032,334, filed on Feb. 27, 1998, now Pat. No. 6,048,898, which is a continuation of application No. 08/447,146, filed on May 22, 1995, now Pat. No. 5,800,808, which is a continuation-in-part of application No. 08/344,248, filed on Nov. 23, 1994, now abandoned, which is a continuation of application No. 08/248,037, filed on May 24, 1994, now abandoned.

(51) Int. Cl.[7] **A61F 2/00**

(52) U.S. Cl. **424/78.08**; 424/78.37; 424/78.17; 424/78.26; 424/78.29; 514/2; 514/12; 514/561; 514/903; 525/420; 525/434; 525/435; 528/328

(58) **Field of Search** 424/78.08, 78.17, 424/78.26, 78.29, 78.37; 514/2, 12, 561, 903; 525/420, 434, 435; 528/328

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(57) **ABSTRACT**

The present invention relates to an improved composition of copolymer-1 comprising copolymer-1 substantially free of species having a molecular weight of over 40 kilodaltons.

33 Claims, 2 Drawing Sheets

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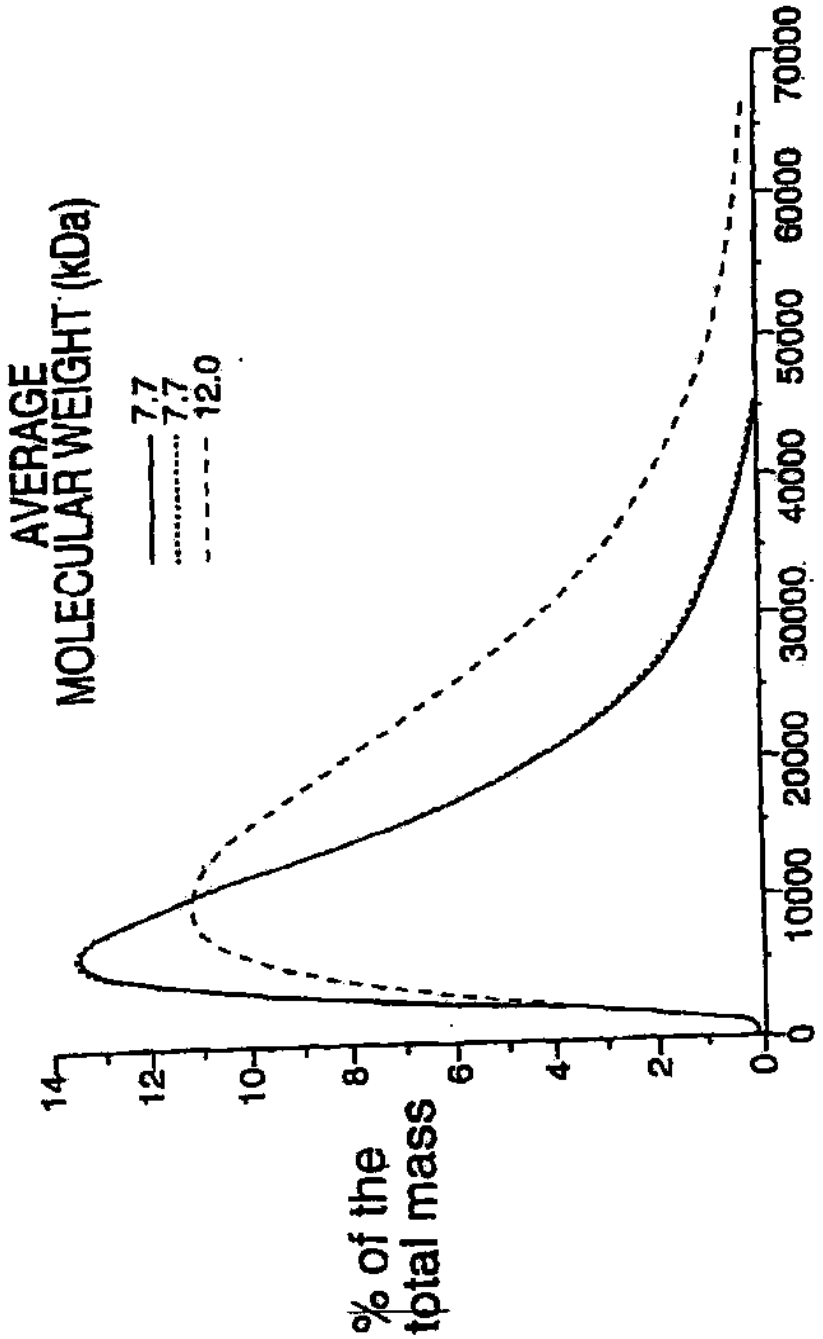
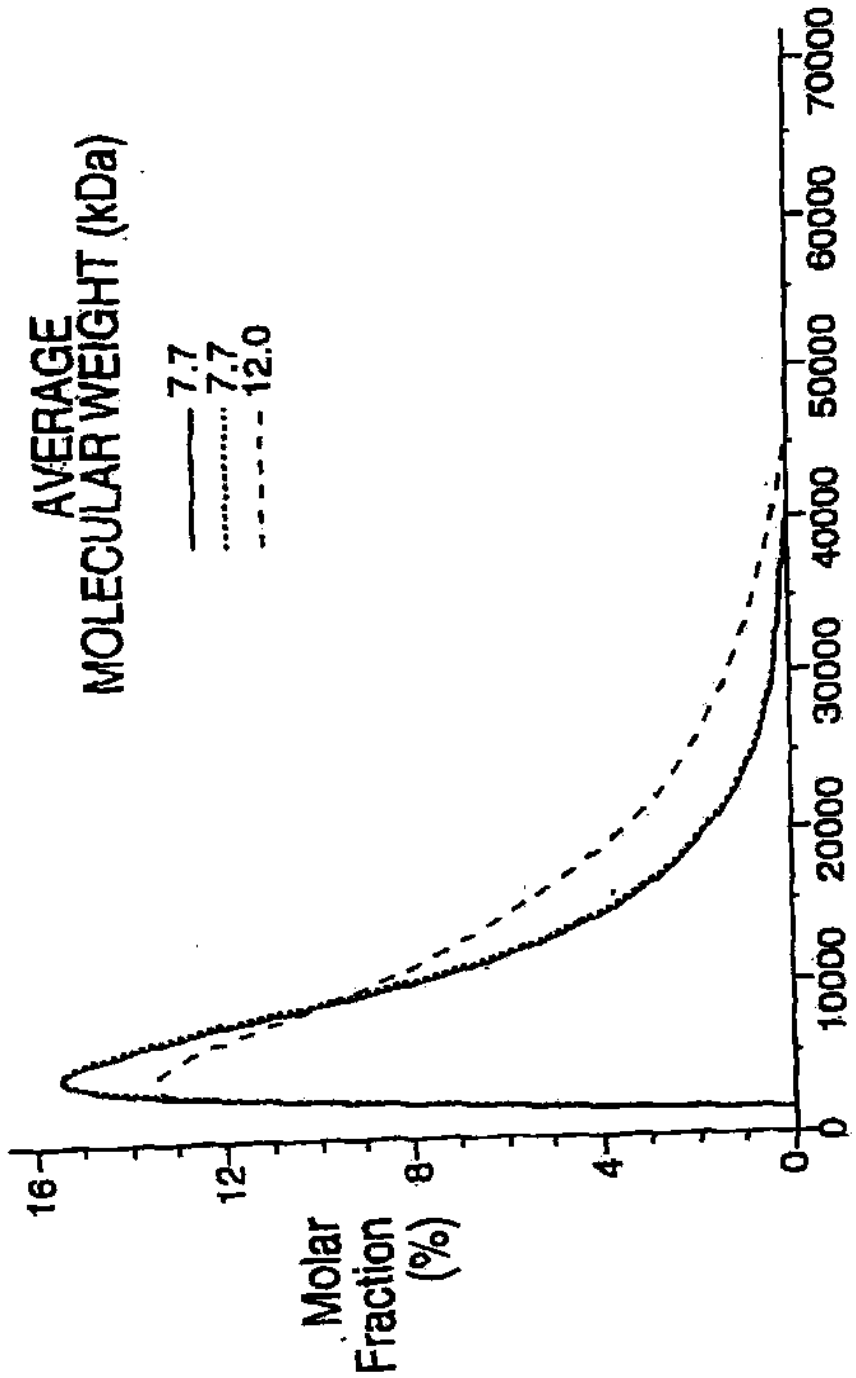

AVERAGE MOLECULAR WEIGHT (kDa)
7.7
7.7
12.0
% of the total mass
0
2
4
6
8
10
12
14
0
10000
20000
30000
40000
50000
60000
70000
Molecular Weight

FIG. 1


AVERAGE MOLECULAR WEIGHT (kDa)
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7.7
12.0
Molar Fraction (%)
16
12
8
4
0
0
10000
20000
30000
40000
50000
60000
70000
Molecular Weight

FIG. 2

COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS

This application is a continuation of application U.S. Ser. No. 10/014,477 filed Dec. 14, 2001 now U.S. Pat. No. 6,620,847, which is a continuation of application Ser. No. 09/510,466, filed Feb. 22, 2000, now U.S. Pat. No. 6,362,161, which is a continuation of U.S. Ser. No. 09/032,334 filed Feb. 27, 1998, now U.S. Pat. No. 6,048,898, which is a continuation of U.S. Ser. No. 08/447,146, filed May 22, 1995, now U.S. Pat. No. 5,800,808, which is a continuation-in-part of U.S. Ser. No. 08/344,248, filed Nov. 23, 1994, now abandoned, which is a continuation of U.S. Ser. No. 08/248,037, filed May 24, 1994, now abandoned.

BACKGROUND OF THE INVENTION

Copolymer-1 is a synthetic polypeptide analog of myelin basic protein (MBP), which is a natural component of the myelin sheath. It has been suggested as a potential therapeutic agent for multiple sclerosis (Eur. J. Immunol. [1971] 1:242; and J. Neurol. Sci. [1977] 31:433). All references cited herein are hereby incorporated by reference in their entirety. Interest in copolymer-1 as an immunotherapy for multiple sclerosis stems from observations first made in the 1950's that myelin components such as MBP prevent or arrest experimental autoimmune encephalomyelitis (EAE). EAE is a disease resembling multiple sclerosis that can be induced in susceptible animals.

Copolymer-1 was developed by Drs. Sela, Arnon, and their co-workers at the Weizmann Institute (Rehovot, Israel). It was shown to suppress EAE (Eur. J. Immunol. [1971] 1:242; U.S. Pat. No. 3,849,550). More recently, copolymer-1 was shown to be beneficial for patients with the exacerbating-remitting form of multiple sclerosis (N. Engl. J. Med. [1987] 317:408). Patients treated with daily injections of copolymer-1 had fewer exacerbations and smaller increases in their disability status than the control patients.

Copolymer-1 is a mixture of polypeptides composed of alanine, glutamic acid, lysine, and tyrosine in a molar ratio of approximately 6:2:5:1, respectively. It is synthesized by chemically polymerizing the four amino acids forming products with average molecular weights of 23,000 daltons (U.S. Pat. No. 3,849,550).

It is an object of the present invention to provide an improved composition of copolymer-1.

SUMMARY OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa).

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa.

Moreover, the invention relates to a pharmaceutical composition and a method for the treatment of multiple sclerosis, using the above-discussed copolymer-1.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 displays the molecular weight distribution of three batches of copolymer-1, showing the proportion of species with molecular weight above 40 KDa.

FIG. 2 shows similar data relating to the molar fraction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa). Preferably, the composition contains less than 5% of species of copolymer-1 having a molecular weight of 40 KDa or more. More preferably, the composition contains less than 2.5% of species of copolymer-1 having a molecular weight of 40 KDa, or more.

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa. In particular, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8 KDa and a copolymer-1 having an average molecular weight of about 6.25 to about 8.4 KDa.

Copolymer-1, according to the present invention, may be prepared by methods known in the art, for example, the process disclosed in U.S. Pat. No. 3,849,550, wherein the N-carboxyanhydrides of tyrosine, alanine, γ-benzyl glutamate and E-N-trifluoro-acetyllysine are polymerised at ambient temperature in anhydrous dioxane with diethylamine as initiator. The deblocking of the γ-carboxyl group of the glutamic acid is effected by hydrogen bromide in glacial acetic acid and is followed by the removal of the trifluoroacetyl groups from the lysine residues by 1M piperidine. For the purposes of the application, the terms "ambient temperature" and "room temperature" should be understood to mean a temperature ranging from about 20 to about 26° C.

The copolymer-1 with the required molecular weight profile can be obtained either by methods known per se. Such methods include chromatography of copolymer-1 containing high molecular weight species and collecting the fractions without the undesired species or by partial acid or enzymatic hydrolysis to remove the high molecular weight species with subsequent purification by dialysis or ultrafiltration. A further method to obtain copolymer-1 with the desired molecular weight profile is by preparing the desired species while the amino acids are still protected and then obtain the correct species directly upon removing the protection. The compositions of the present invention may be formulated by conventional methods known in the art. Preferably, the composition is lyophilized and formed into an aqueous solution suitable for sub-cutaneous injection. Alternatively, copolymer-1 may be formulated in any of the forms known in the art for preparing oral, nasal, buccal, or rectal formulations of peptide drugs.

Typically, copolymer-1 is administered daily to patients suffering from multiple sclerosis at a dosage of 20 mg.

The invention will be exemplified but not necessarily limited by the following Examples.

EXAMPLE 1

Chromatographic Method of Preparation of Low-Toxicity Copolymer-1

Two batches of copolymer-1 were prepared according to the methods known in the art, for example, U.S. Pat. No. 3,849,550.

One batch was then subjected to chromatographic separation, as described below.

A column for gel filtration, FRACTOGEL TSK HW55 (600×26 mm) was prepared in a Superformance 26 Merck cartridge according to the manufacturer's instructions. The column was equilibrated with water and acetone solution was injected for total volume determination. The column

was equilibrated with 0.2M ammonium acetate buffer pH 5.0. 30 ml copolymer-1 samples (20 mg/ml, in 0.2M ammonium acetate pH 5.0) were loaded on the column and fractions were collected every 10 minutes. A fraction having an average molecular weight of 7–8 KDa was isolated between 120–130 minutes (Batch A).

Molecular Weight Analysis

UV absorbance at 275 nm was determined in a UVIKON 810 spectrophotometer. Samples were diluted to obtain a UV absorbance lower than 1 Absorption Unit. The molecular distribution of the 2 batches was determined on a calibrated gel filtration column (Superose 12).

Copolymer-1 batch A was found to have an average molecular weight of 7–8 KDa. 2.5% of this batch had a molecular weight above 32 KDa, but no copolymer-1 species present in this batch had a molecular weight of over 40 KDa.

The other batch of copolymer-1 which was not subjected to chromatography, had an average molecular weight of 12 KDa. 2.5% of the batch had a molecular weight above 42 KDa and 5% of the total copolymer-1 species in this batch had a molecular weight of over 40 KDa.

EXAMPLE 2

Toxicity Analysis

A: In Vivo

Three batches of copolymer-1 having an average molecular weight of 7.3 and 8.4 KDa (less than 2.5% copolymer-1 species over 40 KDa) and 22 KDa (more than 5% copolymer-1 species over 40 KDa) were subjected to the toxicity test described below. In each case 5 mice were used in each experimental group.

Method

Copolymer-1 was dissolved in distilled water to yield a solution of 2 mg/ml of the active ingredient. Each mouse was injected with 0.5 ml of the test solution into the lateral tail vein. Mice were observed for mortality and relevant clinical signs over a 48 hour period. Observations were recorded 10 minutes, 24 hours and 48 hours post-injection. If, at the end of 48 hours, all the animals were alive and no adverse signs had been observed, then the batch was designated "non-toxic". If, however, one or more of the mice had died or had shown adverse signs, then the batch was designated "toxic".

The batches with the average molecular weight of 7.3 and 8.4 KDa were both designated "non-toxic", whereas in the batch with the average molecular weight of 22 KDa, 3 out of 5 mice had died at the end of 48 hours, and it was consequently designated "toxic".

B: In Vitro

RBL—Degranulation Test

I. Introduction

Histamine (or serotonin) release from basophile is an in vitro model for immediate hypersensitivity. The Rat Basophilic Leukemia cell line (RBL-2H$_3$) was developed and characterized as a highly sensitive, uniform, easy to maintain in culture and reproducible system (E. L. Basumian, C. Isersky, M. G. Petrino and R. P. Siraganian. Eur. J. Immunol. 11, 317 (1981)). The physiological stimulus for histamine release involves binding of the antigen to membrane-bound IgE molecules, resulting in the latter's cross-linking and the consequent triggering of an intricate biochemical cascade. Beside these physiological, immunoglobulin-mediated triggers, degranulation can be induced by different non-IgE-mediated stimuli. Among these are various peptides and synthetic polymers, e.g. polylysine (R. P. Siraganian. Trends in Pharmacological Sciences, October 432 (1983)). The RBL degranulation test is, therefore, used in order to screen out those batches of copolymer-1 which evoke substantial degranulation and thus might elicit undesirable local and/or systemic side effects.

II. Principle of the Test Method

Rat Basophilic Leukemia cells (RBL-2H$_3$), are loaded with [^{3}H]-serotonin, followed by incubation with 100 μg of the copolymer-1 to be tested. Batches of copolymer-1 which induce non-specific degranulation, release [^{3}H]-serotonin into the medium. The radioactivity in the medium is counted by a scintillation counter and the total radiolabeled serotonin incorporated into the cells is determined in the pelleted cells. Percent degranulation is calculated as the percentage of serotonin released out of the total incorporated.

III. Results

Four batches of copolymer-1, with average molecular weight between 6,250–14,500 were analyzed for both % of the species with molecular weight over 40 KDa and for degranulation of RBL's. Results are summarized in the following table.

Average M.W. (Daltons)	% of species with M.W. over 40 KDa	% Serotonin Release
6,250	<2.5	12.4
7,300	<2.5	21.0
13,000	>5	66.9
14,500	>5	67.8

As can be seen, when the % of high molecular weight species is low (<2.5), the % release of serotonin, indicative of toxicity, is low, and vice versa.

EXAMPLE 3

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), γ-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried. The removal of the gamma-benzyl blocking groups from the glutamate residue is carried out by treating the protected copolymer-1 with 33% hydrobromic acid in glacial acetic acid at room temperature for 6–12 hours with stirring. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

EXAMPLE 4

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), γ-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried.

Protected copolymer-1 is treated with 33% HBr in acetic acid which removes the omega benzyl protecting group from the 5-carboxylate of the glutamate residue and cleaves the polymer to smaller polypeptides. The time needed for obtaining copolymer-1 of molecular weight 7,000±2,000 Da depends on the reaction temperature and the size of protected copolymer-1. At temperatures of between 20–26° C. a test reaction is performed on every batch at different time periods for example, from 10–50 hours.

The results concerning the molecular weights of these small scale reactions are calculated and a curve of molecular weight against time is drawn. The time needed for obtaining molecular weight 7,000±2,000 Da is calculated from the curve and performed on larger scale reaction. On average, working at 26° C. the time period is 17 hours. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

Preparation of Low-toxicity Copolymer-1

20 g of trifluoroacetyl-copolymer-1 are dispersed in 1 liter of water to which 100 g piperidine are added. The mixture is stirred for 24 hours at room temperature and filtered. The solution of crude copolymer-1 is distributed into dialysis bags and dialyzed at 10°–20° C. against water until a pH=8 is attained. It is then dialyzed against about 0.3% acetic acid and again water until a pH=5.5–6.0 is obtained. This solution is then concentrated and lyophilized to dryness.

What is claimed is:

1. A copolymer-1 composition comprising a mixture of polypeptides composed of glutamic acid, lysine, alanine and tyrosine, wherein the mixture has an average molecular weight of about 4 to about 9 kilodaltons, wherein the mixture of polypeptides is non-uniform with respect to molecular weight and sequence, and wherein the composition is suitable for treating multiple sclerosis.

2. The composition of claim 1, wherein over 75% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight in a range of about 2 kilodaltons to about 20 kilodaltons.

3. The composition of claim 1, wherein less than 5% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight of over 40 kilodaltons.

4. The composition of claim 3, wherein over 75% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight in a range of about 2 kilodaltons to about 20 kilodaltons.

5. The composition of claim 4, wherein the mixture has an average molecular weight of 6.25 to 8.4 kilodaltons.

6. The composition of claim 1, wherein the mixture has an average molecular weight of about 4 to about 8.6 kilodaltons.

7. The composition of claim 1, wherein the mixture has an average molecular weight of about 5 to about 9 kilodaltons.

8. The composition of claim 1, wherein less than 2.5% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight of over 40 kilodaltons.

9. The composition of claim 8, wherein over 75% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight in a range of about 2 kilodaltons to about 20 kilodaltons.

10. The composition of claim 9, wherein the mixture has an average molecular weight of 6.25 to 8.4 kilodaltons.

11. The composition of claim 1, wherein the mixture has a molecular weight distribution substantially as depicted in the curves of FIG. 1 or FIG. 2 in which the average molecular weight is about 7.7 kDa.

12. A pharmaceutical composition comprising:

a dose therapeutically effective to treat multiple sclerosis of a copolymer-1 composition, wherein the copolymer-1 composition comprises a mixture of polypeptides composed of glutamic acid, lysine, alanine and tyrosine, wherein the mixture has an average molecular weight of about 4 to about 9 kilodaltons, wherein the mixture of polypeptides is non-uniform with respect to molecular weight and sequence; and

a pharmaceutically acceptable excipient.

13. The pharmaceutical composition of claim 12, wherein over 75% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight in a range of about 2 kilodaltons to about 20 kilodaltons.

14. The pharmaceutical composition of claim 12, wherein less than 5% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight of over 40 kilodaltons.

15. The pharmaceutical composition of claim 14, wherein over 75% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight in a range of about 2 kilodaltons to about 20 kilodaltons.

16. The pharmaceutical composition of claim 15, wherein the mixture has an average molecular weight of 6.25 to 8.4 kilodaltons.

17. The pharmaceutical composition of claim 12, wherein the mixture has an average molecular weight of about 4 to about 8.6 kilodaltons.

18. The pharmaceutical composition of claim 12, wherein the mixture has an average molecular weight of about 5 to about 9 kilodaltons.

19. The pharmaceutical composition of claim 12, wherein less than 2.5% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight of over 40 kilodaltons.

20. The pharmaceutical composition of claim 19, wherein over 75% of the polypeptides of the mixture, on a molar fraction basis, have a molecular weight in a range of about 2 kilodaltons to about 20 kilodaltons.

21. The pharmaceutical composition of claim 20, wherein the mixture has an average molecular weight of 6.25 to 8 4 kilodaltons.

22. The pharmaceutical composition of claim 12, wherein the mixture has a molecular weight distribution substantially as depicted in the curves of FIG. 1 or FIG. 2 in which the average molecular weight is about 7.7 kDa.

23. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 12.

24. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 13.

25. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 14.

26. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 15.

27. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 16.

28. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 17.

29. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 18.

30. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 19.

31. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 20.

32. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 21.

33. A method for treating a patient suffering from multiple sclerosis comprising administering to a patient in need thereof the pharmaceutical composition of claim 22.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,939,539 B2
DATED : September 6, 2005
INVENTOR(S) : Konfino et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

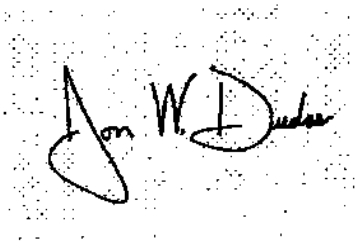
Title page,
Item [56], **References Cited**, OTHER PUBLICATIONS, "D. Titelbaum et al." reference, change "Copolymre 1" to -- Copolymer 1 --.

Column 4,
Line 34, change "trifluoroacetyllysine" to -- trifluoroacetyllysine --.

Column 6,
Line 28, change "84 kilodaltons" to -- 8.4 kilodaltons --.

Signed and Sealed this

First Day of November, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

Exhibit C

US006054430A

United States Patent [19]

Konfino et al.

[11] Patent Number: 6,054,430

[45] Date of Patent: Apr. 25, 2000

[54] **COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS**

[75] Inventors: **Eliezer Konfino**, Ramat Gan; **Michael Sela**, Rehovot; **Dvora Teitelbaum**, Rehovot; **Ruth Arnon**, Rehovot, all of Israel

[73] Assignee: **Yeda Research and Development Co., Ltd.**, Rehovot, Israel

[21] Appl. No.: 09/032,647

[22] Filed: Feb. 27, 1998

Related U.S. Application Data

[60] Division of application No. 08/447,146, May 22, 1995, Pat. No. 5,800,808, which is a continuation-in-part of application No. 08/344,248, Nov. 23, 1994, abandoned, which is a continuation of application No. 08/248,037, May 24, 1994, abandoned.

[51] Int. Cl.[7] A61K 31/785; A61K 38/16

[52] U.S. Cl. 514/12; 424/78.17; 514/903; 525/420

[58] Field of Search 514/903, 12; 424/78.26

[56] **References Cited**

FOREIGN PATENT DOCUMENTS

9531990 11/1995 WIPO .

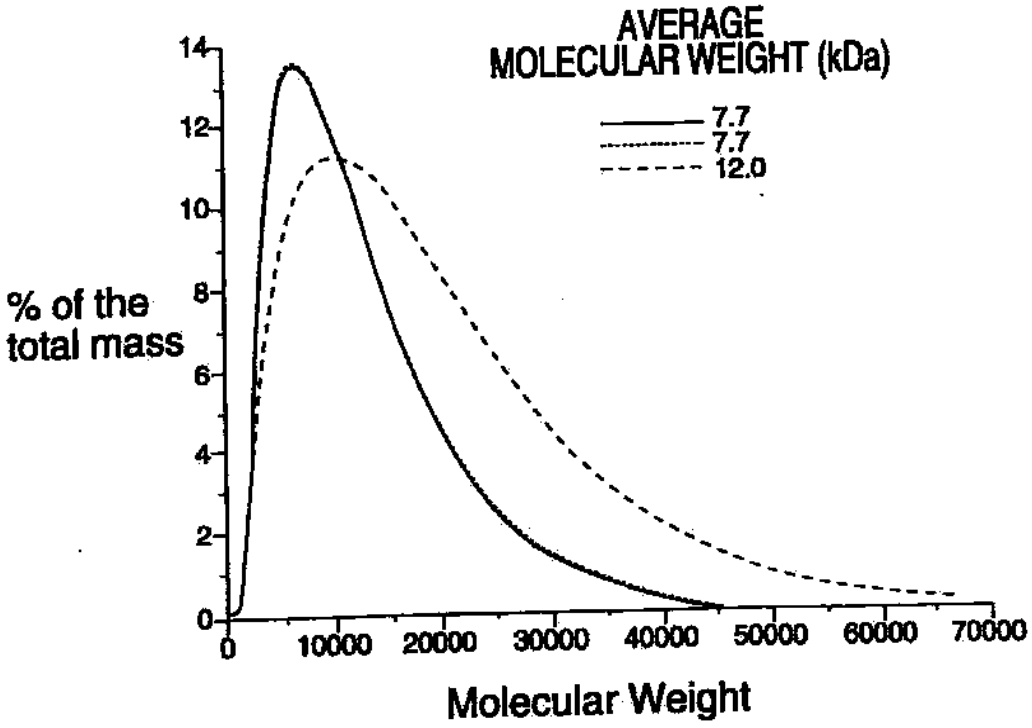
Primary Examiner—Peter F. Kulkosky

Attorney, Agent, or Firm—Kenyon & Kenyon

[57] **ABSTRACT**

The present invention relates to an improved composition of copolymer-1 comprising copolymer-1 substantially free of species having a molecular weight of over 40 kilodaltons.

6 Claims, 2 Drawing Sheets



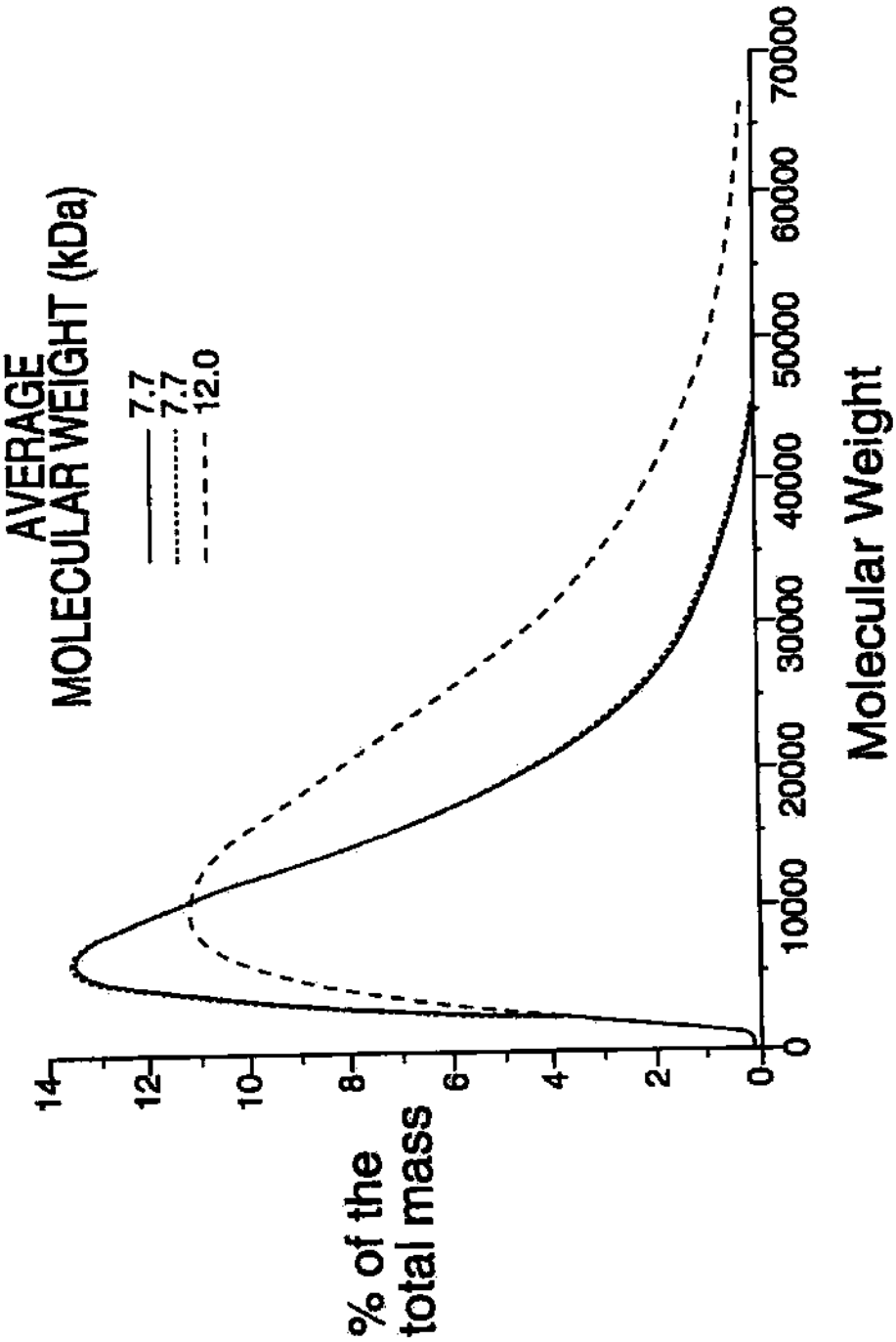

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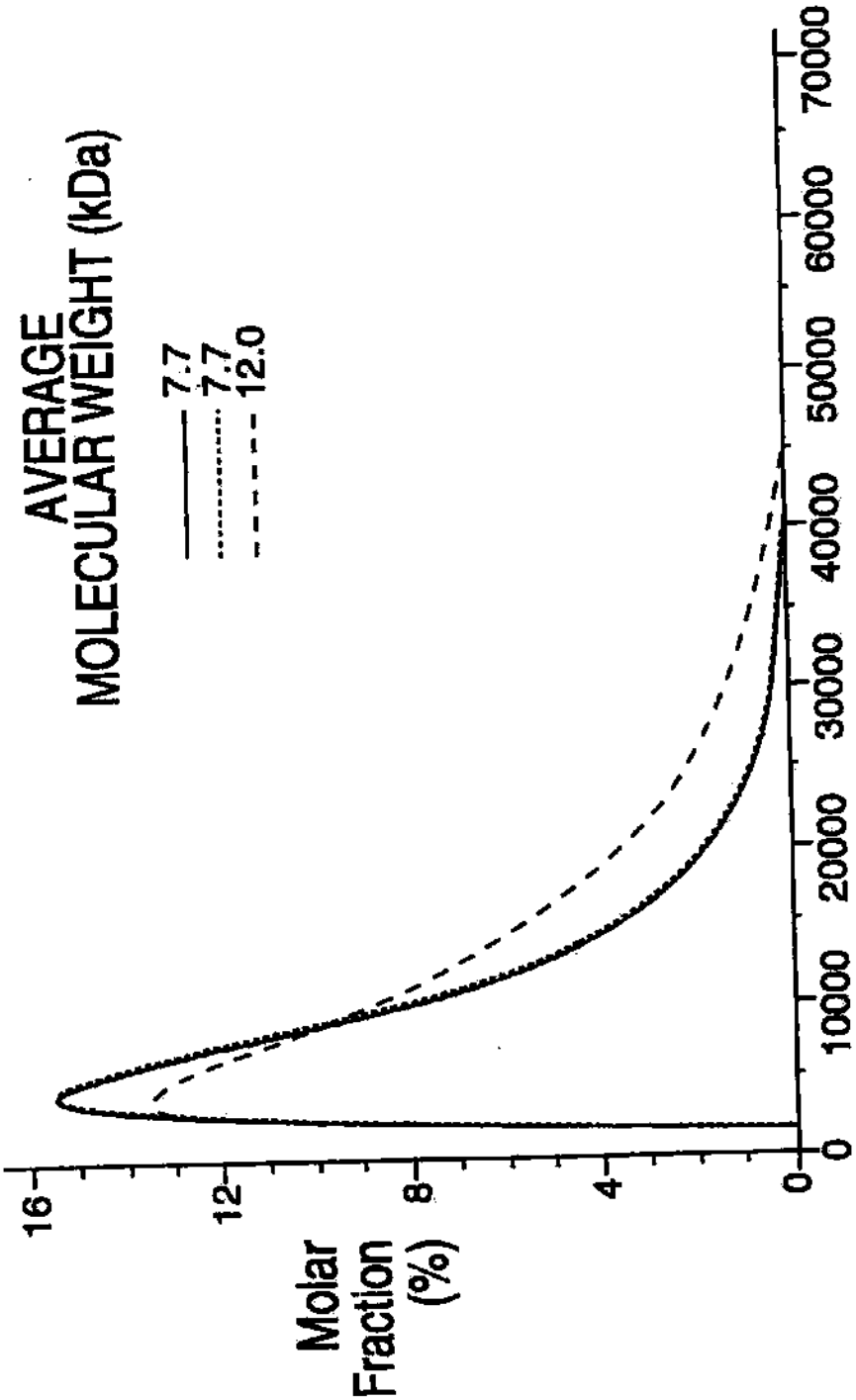
FIG. 1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Copolymer-1 is a mixture of polypeptides composed of alanine, glutamic acid, lysine, and tyrosine in a molar ratio of approximately 6:2:5:1, respectively. It is synthesized by chemically polymerizing the four amino acids forming products with average molecular weights of 23,000 daltons (U.S. Pat. No. 3,849,550).

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The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

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Moreover, the invention relates to a pharmaceutical composition and a method for the treatment of multiple sclerosis, using the above-discussed copolymer-1.

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The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa. In particular, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8 KDa and a copolymer-1 having an average molecular weight of about 6.25 to about 8.4 KDa.

Copolymer-1, according to the present invention, may be prepared by methods known in the art, for example, the process disclosed in U.S. Pat. No. 3,849,550, wherein the N-carboxyanhydrides of tyrosine, alanine, γ-benzyl glutamate and ε-N-trifluoro-acetyllysine are polymerised at ambient temperature in anhydrous dioxane with diethylamine as initiator. The deblocking of the γ-carboxyl group of the glutamic acid is effected by hydrogen bromide in glacial acetic acid and is followed by the removal of the trifluoroacetyl groups from the lysine residues by 1M piperidine. For the purposes of the application, the terms "ambient temperature" and "room temperature" should be understood to mean a temperature ranging from about 20 to about 26° C.

The copolymer-1 with the required molecular weight profile can be obtained either by methods known per se. Such methods include chromatography of copolymer-1 containing high molecular weight species and collecting the fractions without the undesired species or by partial acid or enzymatic hydrolysis to remove the high molecular weight species with subsequent purification by dialysis or ultrafiltration. A further method to obtain copolymer-1 with the desired molecular weight profile is by preparing the desired species while the amino acids are still protected and then obtain the correct species directly upon removing the protection. The compositions of the present invention may be formulated by conventional methods known in the art. Preferably, the composition is lyophilized and formed into an aqueous solution suitable for sub-cutaneous injection. Alternatively, copolymer-1 may be formulated in any of the forms known in the art for preparing oral, nasal, buccal, or rectal formulations of peptide drugs.

Typically, copolymer-1 is administered daily to patients suffering from multiple sclerosis at a dosage of 20 mg.

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Two batches of copolymer-1 were prepared according to the methods known in the art, for example, U.S. Pat. No. 3,849,550.

One batch was then subjected to chromatographic separation, as described below.

A column for gel filtration, FRACTOGEL TSK HW55 (600×26 mm) was prepared in a Superformance 26 Merck cartridge according to the manufacturer's instructions. The column was equilibrated with water and acetone solution was injected for total volume determination. The column was equilibrated with 0.2M ammonium acetate buffer pH 5.0. 30 ml copolymer-1 samples (20 mg/ml, in 0.2M ammo-

nium acetate pH 5.0) were loaded on the column and fractions were collected every 10 minutes. A fraction having an average molecular weight of 7–8 KDa was isolated between 120–130 minutes (Batch A).

Molecular Weight Analysis

UV absorbance at 275 nm was determined in a UVIKON 810 spectrophotometer. Samples were diluted to obtain a UV absorbance lower than 1 Absorption Unit. The molecular distribution of the 2 batches was determined on a calibrated gel filtration column (Superose 12).

Copolymer-1 batch A was found to have an average molecular weight of 7–8 KDa. 2.5% of this batch had a molecular weight above 32 KDa, but no copolymer-1 species present in this batch had a molecular weight of over 40 KDa.

The other batch of copolymer-1 which was not subjected to chromatography, had an average molecular weight of 12 KDa. 2.5% of the batch had a molecular weight above 42 KDa and 5% of the total copolymer-1 species in this batch had a molecular weight of over 40 KDa.

EXAMPLE 2

Toxicity Analysis

A: In Vivo

Three batches of copolymer-1 having an average molecular weight of 7.3 and 8.4 KDa (less than 2.5% copolymer-1 species over 40 KDa) and 22 KDa (more than 5% copolymer-1 species over 40 KDa) were subjected to the toxicity test described below. In each case 5 mice were used in each experimental group.

Method

Copolymer-1 was dissolved in distilled water to yield a solution of 2 mg/ml of the active ingredient. Each mouse was injected with 0.5 ml of the test solution into the lateral tail vein. Mice were observed for mortality and relevant clinical signs over a 48 hour period. Observations were recorded 10 minutes, 24 hours and 48 hours post-injection. If, at the end of 48 hours, all the animals were alive and no adverse signs had been observed, then the batch was designated "non-toxic". If, however, one or more of the mice had died or had shown adverse signs, then the batch was designated "toxic".

The batches with the average molecular weight of 7.3 and 8.4 KDa were both designated "non-toxic", whereas in the batch with the average molecular weight of 22 KDa, 3 out of 5 mice had died at the end of 48 hours, and it was consequently designated "toxic".

B: In Vitro

RBL—Degranulation Test

I. Introduction

Histamine (or serotonin) release from basophile is an in vitro model for immediate hypersensitivity. The Rat Basophilic Leukemia cell line (RBL-2H$_3$) was developed and characterized as a highly sensitive, uniform, easy to maintain in culture and reproducible system (E. L. Basumian, C. Isersky, M. G. Petrino and R. P. Siraganian. Eur. J. Immunol. 11, 317 (1981)). The physiological stimulus for histamine release involves binding of the antigen to membrane-bound IgE molecules, resulting in the latter's cross-linking and the consequent triggering of an intricate biochemical cascade. Beside these physiological, immunoglobulin-mediated triggers, degranulation can be induced by different non-IgE-mediated stimuli. Among these are various peptides and synthetic polymers, e.g. polylysine (R. P. Siraganian. Trends in Pharmacological Sciences, October 432 (1983)). The RBL degranulation test is, therefore, used in order to screen out those batches of copolymer-1 which evoke substantial degranulation and thus might elicit undesirable local and/or systemic side effects.

II. Principle of the Test Method

Rat Basophilic Leukemia cells (RBL-2H$_3$), are loaded with [^{3}H]-serotonin, followed by incubation with 100 μg of the copolymer-1 to be tested. Batches of copolymer-1 which induce non-specific degranulation, release [^{3}H]-serotonin into the medium. The radioactivity in the medium is counted by a scintillation counter and the total radiolabeled serotonin incorporated into the cells is determined in the pelleted cells. Percent degranulation is calculated as the percentage of serotonin released out of the total incorporated.

III. Results

Four batches of copolymer-1, with average molecular weight between 6,250–14,500 were analyzed for both % of the species with molecular weight over 40 KDa and for degranulation of RBL's. Results are summarized in the following table.

Average M.W. (Daltons)	% of species with M.W. over 40 KDa	% Serotonin Release
6,250	<2.5	12.4
7,300	<2.5	21.0
13,000	>5	66.9
14,500	>5	67.8

As can be seen, when the % of high molecular weight species is low (<2.5), the % release of serotonin, indicative of toxicity, is low, and vice versa.

EXAMPLE 3

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), y-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried. The removal of the gamma-benzyl blocking groups from the glutamate residue is carried out by treating the protected copolymer-1 with 33% hydrobromic acid in glacial acetic acid at room temperature for 6–12 hours with stirring. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

EXAMPLE 4

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18g), alanine (50 g), τ-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried.

Protected copolymer-1 is treated with 33% HBr in acetic acid which removes the omega benzyl protecting group from the 5-carboxylate of the glutamate residue and cleaves the polymer to smaller polypeptides. The time needed for obtaining copolymer-1 of molecular weight 7,000±2,000 Da depends on the reaction temperature and the size of protected copolymer-1. At temperatures of between 20–28° C.

a test reaction is performed on every batch at different time periods for example, from 10–50 hours. The results concerning the molecular weights of these small scale reactions are calculated and a curve of molecular weight against time is drawn. The time needed for obtaining molecular weight 7,000±2,000 Da is calculated from the curve and performed on larger scale reaction. On average, working at 26° C. the time period is 17 hours. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

Preparation of Low-Toxicity Copolymer-1

20 g of trifluoroacetyl-copolymer-1 are dispersed in 1 liter of water to which 100 g piperidine are added. The mixture is stirred for 24 hours at room temperature and filtered. The solution of crude copolymer-1 is distributed into dialysis bags and dialyzed at 10°–20° C. against water until a pH=8 is attained. It is then dialyzed against about 0.3% acetic acid and again water until a pH=5.5–6.0 is obtained. This solution is then concentrated and lyophilized to dryness.

We claim:

1. Copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 kDa to about 20 kDa, prepared by a process comprising the steps of:

reacting protected copolymer-1 with hydrobromic acid to form trifluoroacetyl copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 kDa to about 20 kDa, wherein said reaction takes place for a time and at a temperature predetermined by test reaction, and

treating said trifluoroacetyl copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 kDa to about 20 kDa with aqueous piperidine solution to form copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2kDa to about 20kDa.

2. The copolymer-1 of claim 1 wherein said protected copolymer-1 is reacted with hydrobromic acid for about 10–50 hours at a temperature of about 20–28° C.

3. The copolymer-1 of claim 1, wherein said protected copolymer-1 is reacted with hydrobromic acid for about 17 hours at a temperature of about 26° C.

4. Trifluoroacetyl copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 kDa to about 20 kDa, produced by a process comprising the steps of reacting protected copolymer-1 with hydrobromic acid for a time and at a temperature predetermined by test reaction.

5. The trifluoroacetyl copolymer-1 of claim 4 wherein said protected copolymer-1 is reacted with hydrobromic acid for about 10–50 hours at a temperature of about 20–28° C.

6. The trifluoroacetyl copolymer-1 of claim 5 wherein said protected copolymer-1 is reacted with hydrobromic acid for about 17 hours at a temperature of about 26° C.

* * * * *

Exhibit D

US006620847B2

(12) **United States Patent**
Konfino et al.

(10) **Patent No.: US 6,620,847 B2**
(45) **Date of Patent: Sep. 16, 2003**

(54) **COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS**

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(73) Assignee: **Yeda Research and Development Co., Ltd.**, Rehovot (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/014,477**

(22) Filed: **Dec. 14, 2001**

(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation of application No. 09/510,466, filed on Feb. 22, 2000, now Pat. No. 6,362,161, which is a continuation of application No. 09/032,334, filed on Feb. 27, 1998, now Pat. No. 6,048,898, which is a continuation of application No. 08/447,146, filed on May 22, 1995, now Pat. No. 5,800,808, which is a continuation-in-part of application No. 08/344,248, filed on Nov. 23, 1994, now abandoned, which is a continuation of application No. 08/248,037, filed on May 24, 1994, now abandoned.

(51) **Int. Cl.**[7] **A61K 31/195**

(52) **U.S. Cl.** 514/561; 424/78.08; 424/78.26; 424/78.29; 525/420; 525/434; 525/435; 528/328

(58) **Field of Search** 514/561; 424/78.08, 424/78.26, 78.29; 525/420, 434, 435; 528/328

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(57) **ABSTRACT**

The present invention relates to an improved composition of copolymer-1 comprising copolymer-1 substantially free of species having a molecular weight of over 40 kilodaltons.

9 Claims, 2 Drawing Sheets

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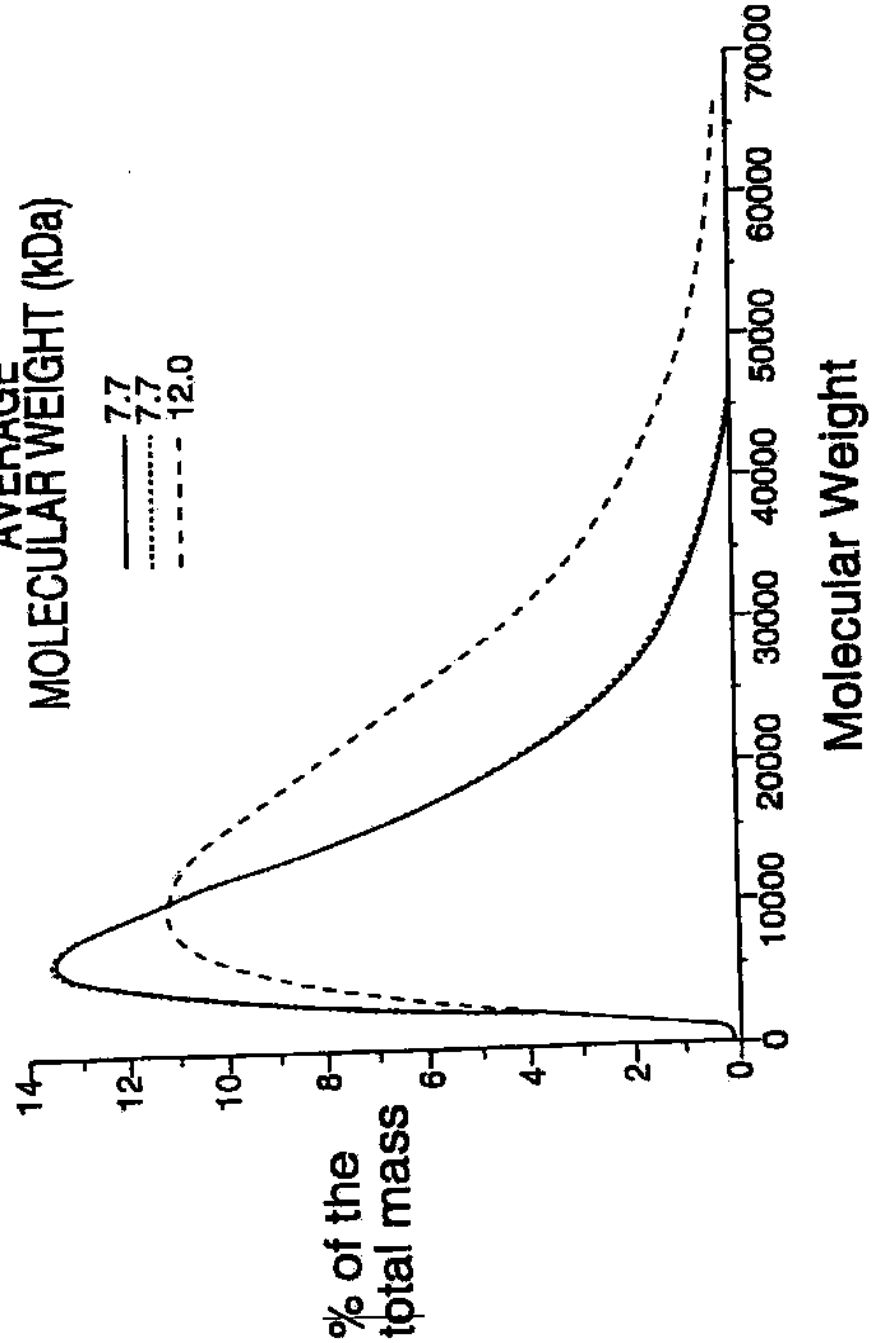
AVERAGE MOLECULAR WEIGHT (kDa)
7.7
7.7
12.0
% of the total mass
0
2
4
6
8
10
12
14
0
10000
20000
30000
40000
50000
60000
70000
Molecular Weight

FIG. 1

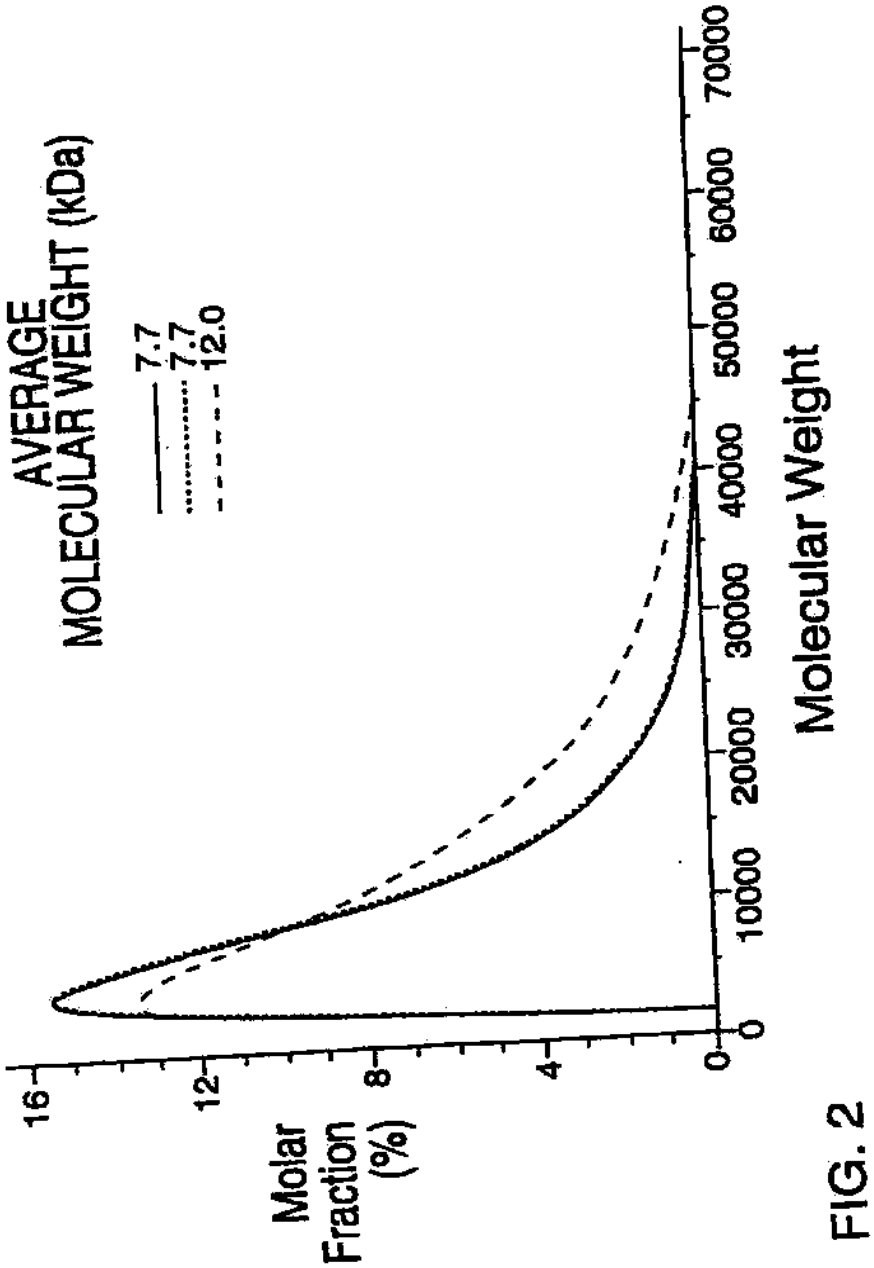
AVERAGE MOLECULAR WEIGHT (kDa)
7.7
7.7
12.0
Molar Fraction (%)
16
12
8
4
0
0
10000
20000
30000
40000
50000
60000
70000
Molecular Weight

FIG. 2

COPOLYMER-1 IMPROVEMENTS IN COMPOSITIONS OF COPOLYMERS

This application is a continuation of application Ser. No. 09/510,466, filed Feb. 22, 2000, now U.S. Pat. No. 6,362,161, which is a continuation of U.S. Ser. No. 09/032,334 filed Feb. 27, 1998, now U.S. Pat. No. 6,048,898, which is a continuation of U.S. Ser. No. 08/447,146, filed May 22, 1995, now U.S. Pat. No. 5,800,808, which is a continuation-in-part of U.S. Ser. No. 08/344,248, filed Nov. 23, 1994, now abandoned, which is a continuation of U.S. Ser. No. 08/248,037, filed May 24, 1994, now abandoned.

BACKGROUND OF THE INVENTION

Copolymer-1 is a synthetic polypeptide analog of myelin basic protein (MBP), which is a natural component of the myelin sheath. It has been suggested as a potential therapeutic agent for multiple sclerosis (Eur. J. Immunol. [1971] 1:242; and J. Neurol. Sci. [1977] 31:433). All references cited herein are hereby incorporated by reference in their entirety. Interest in copolymer-1 as an immunotherapy for multiple sclerosis stems from observations first made in the 1950's that myelin components such as MBP prevent or arrest experimental autoimmune encephalomyelitis (EAE). EAE is a disease resembling multiple sclerosis that can be induced in susceptible animals.

Copolymer-1 was developed by Drs. Sela, Arnon, and their co-workers at the Weizmann Institute (Rehovot, Israel). It was shown to suppress EAE (Eur. J. Immunol. [1971] 1:242; U.S. Pat. No. 3,849,550). More recently, copolymer-1 was shown to be beneficial for patients with the exacerbating-remitting form of multiple sclerosis (N. Engl. J. Med. [1987] 317:408). Patients treated with daily injections of copolymer-1 had fewer exacerbations and smaller increases in their disability status than the control patients.

Copolymer-1 is a mixture of polypeptides composed of alanine, glutamic acid, lysine, and tyrosine in a molar ratio of approximately 6:2.5:1, respectively. It is synthesized by chemically polymerizing the four amino acids forming products with average molecular weights of 23,000 daltons (U.S. Pat. No. 3,849,550).

It is an object of the present invention to provide an improved composition of copolymer-1.

SUMMARY OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa).

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa.

Moreover, the invention relates to a pharmaceutical composition and a method for the treatment of multiple sclerosis, using the above-discussed copolymer-1.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 displays the molecular weight distribution of three batches of copolymer-1, showing the proportion of species with molecular weight above 40 KDa. FIG. 2 shows similar data relating to the molar fraction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a composition of copolymer-1 substantially free of species of copolymer-1 having a molecular weight of over 40 kilodaltons (KDa). Preferably, the composition contains less than 5% of species of copolymer-1 having a molecular weight of 40 KDa or more. More preferably, the composition contains less than 2.5% of species of copolymer-1 having a molecular weight of 40 KDa, or more.

The invention further relates to a copolymer-1 having over 75% of its molar fraction within the molecular weight range from about 2 KDa to about 20 KDa.

In addition, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8.6 KDa. In particular, the invention relates to a copolymer-1 having an average molecular weight of about 4 to about 8 KDa and a copolymer-1 having an average molecular weight of about 6.25 to about 8.4 KDa.

Copolymer-1, according to the present invention, may be prepared by methods known in the art, for example, the process disclosed in U.S. Pat. No. 3,849,550, wherein the N-carboxyanhydrides of tyrosine, alanine, γ-benzyl glutamate and E-N-trifluoro-acetyllysine are polymerised at ambient temperature in anhydrous dioxane with diethylamine as initiator. The deblocking of the γ-carboxyl group of the glutamic acid is effected by hydrogen bromide in glacial acetic acid and is followed by the removal of the trifluoroacetyl groups from the lysine residues by 1M piperidine. For the purposes of the application, the terms "ambient temperature" and "room temperature" should be understood to mean a temperature ranging from about 20 to about 26° C.

The copolymer-1 with the required molecular weight profile can be obtained either by methods known per se. Such methods include chromatography of copolymer-1 containing high molecular weight species and collecting the fractions without the undesired species or by partial acid or enzymatic hydrolysis to remove the high molecular weight species with subsequent purification by dialysis or ultrafiltration. A further method to obtain copolymer-1 with the desired molecular weight profile is by preparing the desired species while the amino acids are still protected and then obtain the correct species directly upon removing the protection. The compositions of the present invention may be formulated by conventional methods known in the art. Preferably, the composition is lyophilized and formed into an aqueous solution suitable for sub-cutaneous injection. Alternatively, copolymer-1 may be formulated in any of the forms known in the art for preparing oral, nasal, buccal, or rectal formulations of peptide drugs.

Typically, copolymer-1 is administered daily to patients suffering from multiple sclerosis at a dosage of 20mg.

The invention will be exemplified but not necessarily limited by the following Examples.

EXAMPLE 1

Chromatographic method of preparation of low-toxicity copolymer-1 Two batches of copolymer-1 were prepared according to the methods known in the art, for example, U.S. Pat. No. 3,849,550.

One batch was then subjected to chromatographic separation, as described below.

A column for gel filtration, FRACTOGEL TSK HW55 (600×26 mm) was prepared in a Superformance 26 Merck cartridge according to the manufacturer's instructions. The column was equilibrated with water and acetone solution was injected for total volume determination. The column was equilibrated with 0.2M ammonium acetate buffer pH

5.0. 30 ml copolymer-1 samples (20 mg/ml, in 0.2M ammonium acetate pH 5.0) were loaded on the column and fractions were collected every 10 minutes. A fraction having an average molecular weight of 7–8 KDa was isolated between 120–130 minutes (Batch A)

Molecular Weight Analysis

UV absorbance at 275 nm was determined in a UVIKON 810 spectrophotometer. Samples were diluted to obtain a UV absorbance lower than 1 Absorption Unit. The molecular distribution of the 2 batches was determined on a calibrated gel filtration column (Superose 12).

Copolymer-1 batch A was found to have an average molecular weight of 7–8 KDa. 2.5% of this batch had a molecular weight above 32 KDa, but no copolymer-1 species present in this batch had a molecular weight of over 40 KDa.

The other batch of copolymer-1 which was not subjected to chromatography, had an average molecular weight of 12 KDa. 2.5% of the batch had a molecular weight above 42 KDa and 5% of the total copolymer-1 species in this batch had a molecular weight of over 40 KDa.

EXAMPLE 2

Toxicity Analsis

A: In Vivo

Three batches of copolymer-1 having an average molecular weight of 7.3 and 8.4 KDa (less than 2.5% copolymer-1 species over 40 KDa) and 22 KDa (more than 5% copolymer-1 species over 40 KDa) were subjected to the toxicity test described below. In each case 5 mice were used in each experimental group.

Method

Copolymer-1 was dissolved in distilled water to yield a solution of 2 mg/ml of the active ingredient. Each mouse was injected with 0.5 ml of the test solution into the lateral tail vein. Mice were observed for mortality and relevant clinical signs over a 48 hour period. Observations were recorded 10 minutes, 24 hours and 48 hours post-injection. If, at the end of 48 hours, all the animals were alive and no adverse signs had been observed, then the batch was designated "non-toxic". If, however, one or more of the mice had died or had shown adverse signs, then the batch was designated "toxic".

The batches with the average molecular weight of 7.3 and 8.4 KDa were both designated "non-toxic", whereas in the batch with the average molecular weight of 22 KDa, 3 out of 5 mice had died at the end of 48 hours, and it was consequently designated "toxic".

B: In Vitro

RBL-Degranulation Test

I. Introduction

Histamine (or serotonin) release from basophile is an in vitro model for immediate hypersensitivity. The Rat Basophilic Leukemia cell line (RBL-2H$_3$) was developed and characterized as a highly sensitive, uniform, easy to maintain in culture and reproducible system (E. L. Basumian, C. Isersky, M. G. Petrino and R. P. Siraganian. Eur. J. Immunol. 11, 317 (1981)). The physiological stimulus for histamine release involves binding of the antigen to membrane-bound IgE molecules, resulting in the latter's cross-linking and the consequent triggering of an intricate biochemical cascade. Beside these physiological, immunoglobulin-mediated triggers, degranulation can be induced by different non-IgE-mediated stimuli. Among these are various peptides and synthetic polymers, e.g. polylysine (R. P. Siraganian. Trends in Pharmacological Sciences, October 432 (1983)). The RBL degranulation test is, therefore, used in order to screen out those batches of copolymer-1 which evoke substantial degranulation and thus might elicit undesirable local and/or systemic side effects.

II. Principle of the Test Method

Rat Basophilic Leukemia cells (RBL-2H$_3$), are loaded with [^{3}H]-serotonin, followed by incubation with 100 μg of the copolymer-1 to be tested. Batches of copolymer-1 which induce non-specific degranulation, release [^{3}H]-serotonin into the medium. The radioactivity in the medium is counted by a scintillation counter and the total radiolabeled serotonin incorporated into the cells is determined in the pelleted cells. Percent degranulation is calculated as the percentage of serotonin released out of the total incorporated.

III. Results

Four batches of copolymer-1, with average molecular weight between 6,250–14,500 were analyzed for both % of the species with molecular weight over 40 KDa and for degranulation of RBL's. Results are summarized in the following table.

Average M.W. (Daltons)	% of species with M.W. over 40 KDa	% Serotonin Release
6,250	<2.5	12.4
7,300	<2.5	21.0
13,000	>5	66.9
14,500	>5	67.8

As can be seen, when the % of high molecular weight species is low (<2.5), the % release of serotonin, indicative of toxicity, is low, and vice versa.

EXAMPLE 3

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), γ-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried. The removal of the gamma-benzyl blocking groups from the glutamate residue is carried out by treating the protected copolymer-1 with 33% hydrobromic acid in glacial acetic acid at room temperature for 6–12 hours with stirring. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

EXAMPLE 4

Preparation of Trifluoroacetyl-Copolymer-1

Protected copolymer-1 is prepared as described by Teitelbaum et al. Eur. J. Immun. Vol. 1 p. 242 (1971) from the N-carboxyanhydrides of tyrosine (18 g), alanine (50 g), T-benzyl glutamate (35 g) and trifluoroacetyllysine (83 g) dissolved in 3.5 liters of dioxane.

The polymerization process is initiated by the addition of 0.01–0.02% diethylamine. The reaction mixture is stirred at room temperature for 24 hours and then poured into 10 liters water. The product (protected copolymer-1) is filtered, washed with water and dried.

Protected copolymer-1 is treated with 33% HBr in acetic acid which removes the omega benzyl protecting group from the 5-carboxylate of the glutamate residue and cleaves the polymer to smaller polypeptides. The time needed for obtaining copolymer-1 of molecular weight 7,000±2,000 Da

depends on the reaction temperature and the size of protected copolymer-1. At temperatures of between 20–28° C. a test reaction is performed on every batch at different time periods for example, from 10–50 hours.

The results concerning the molecular weights of these small scale reactions are calculated and a curve of molecular weight against time is drawn. The time needed for obtaining molecular weight 7,000±2,000 Da is calculated from the curve and performed on larger scale reaction. On average, working at 26° C. the time period is 17 hours. The product is poured into excess water, filtered, washed and dried, yielding the trifluoroacetyl-copolymer-1.

Preparation of Low-Toxicity Copolymer-1

20 g of trifluoroacetyl-copolymer-1 are dispersed in 1 liter of water to which 100 g piperidine are added. The mixture is stirred for 24 hours at room temperature and filtered. The solution of crude copolymer-1 is distributed into dialysis bags and dialyzed at 10°–20° C. against water until a pH=8 is attained. It is then dialyzed against about 0.3% acetic acid and again water until a pH=5.5–6.0 is obtained. This solution is then concentrated and lyophilized to dryness.

What is claimed is:

1. Copolymer-1 having a molecular weight of about 4 to about 9 kilodaltons, made by a process comprising:

treating trifluoroacetyl copolymer-1 with aqueous piperidine to form a solution of copolymer-1; and

purifying copolymer-1, thereby producing copolymer-1 having a molecular weight of about 4 to about 9 kilodaltons.

2. Copolymer-1 made by the process of claim 1, wherein the process further comprises reacting protected copolymer-1 with hydrobromic acid to form trifluoroacetyl copolymer-1.

3. Copolymer-1 made by the process of claim 2, wherein the hydrobromic acid comprises about 33% hydrogen bromide in acetic acid.

4. Copolymer-1 made by the process of claim 2, wherein the process further comprises polymerizing N-carboxyanhydride derivatives to form protected copolymer-1.

5. Copolymer-1 made by the process of claim 4, wherein the polymerizing step comprises providing a diethylamine initiator.

6. Copolymer-1 made by the process of claim 1, wherein the process further comprises adding acetic acid subsequent to the treating step.

7. Copolymer-1 made by the process of claim 1, wherein the purfying step comprises filtration.

8. A method of manufacturing trifluoroacetyl copolymer-1 having a predetermined molecular weight profile, comprising the steps of:

selecting a predetermined molecular weight profile, and then

reacting protected copolymer-1 with hydrobromic acid at about 26° C. for a time predetermined by test reaction to provide trifluoroacetyl copolymer-1 having the predetermined molecular weight profile.

9. The method of claim 7, further comprising a step of treating the trifluoroacetyl copolymer-1 having the predetermined molecular weight profile with aqueous peperidine solution to form copolymer-1 having the predetermined molecular weight profile.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,620,847 B2
DATED : September 16, 2003
INVENTOR(S) : Konfino et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page, Item [54] and Column 1, line 1,
Title, change "**COPOLYMER-1 IMPROVEMENTS**" to – **COPOLYMER-1: IMPROVEMENTS** –

Column 1,
Line 5, change "flied" to – filed –.

Column 2,
Lines 56 and 57, "chromatographic .. copolymer-1 ....." place on separate line as subheading for Example 1.

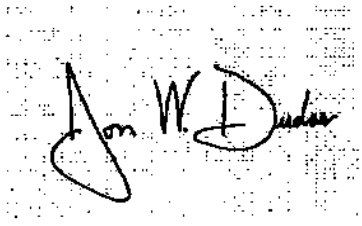
Column 3,
Line 23, change "Analsis" to -- Analysis –

Column 4,
Line 37, change "trifluoroacetyllysine" to – trifluoroacetyllysine –

Column 6,
Lines 1, 12 and 15, change "Copoloymer" to – Copolymer –
Line 16, change "purfying" to – purifying –
Line 28, change "copoloymer" to – copolymer –
Line 29, change "peperidine" to -- piperidine –

Signed and Sealed this

Ninth Day of November, 2004



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Director of the United States Patent and Trademark Office